A-VAX: Applying Quality by Design to Vaccines

CMC-Vaccines Working Group

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Preface

The A-VAX Case Study involved the efforts of many individuals and would not have been made possible if it were not for the countless number of hours spent by the 5 participating companies (GlaxosmithKline, MedImmune, Merck, Pfizer, and sanofi pasteur).

To that end, the Facilitation Team from Pricewaterhouse Coopers would like to thank the following participants from each company for their energy and dedication.

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1.   Introduction to CMC-Vaccine Working Group (CMC-VWG) QbD Case Study

1.1.   Background

Following the publication of the A-Mab case study in 2009 that applied Quality by Design (QbD) principles to the production of an example monoclonal antibody, (http://www.casss.org/associations/9165/files/Case_Study_Press_Release.pdf and http://www.ispe.org/index.php/ci_id/20555/la_id/1.htm), suggestions were made to do a vaccine case study. Considering the differences in development strategies between a monoclonal antibody and a vaccine, the rationale was clear for creating a new case study.

In early 2010, key industry and regulatory agency thought leaders were consulted to consider the feasibility of such a case study. Based on the feedback, some of these thought leaders engaged a consulting group (PRTM, now Pricewaterhouse Coopers) to further develop the feasibility package and solicit participation from the industry and regulators.

Five companies — GSK, MedImmune, Merck, Pfizer, and sanofi pasteur — responded to the solicitation and committed to participate in the Vaccine Working Group (VWG). The main objective of the VWG was to work together to see if and how QbD could be applied to vaccine development and manufacturing.

1.2.   Differences in Development Strategies

Although a vaccine case study would likely emphasize some of the same QbD principles as the A-Mab case study, applying the QbD principles to a vaccine and emphasizing the differences may broaden the scope and enhance the value of the discussions.

One major difference between the A-Mab case study and a vaccine case study would be a focus on the value of QbD for non-platform products/processes typical of vaccines, rather than the platform Chinese Hamster Ovary (CHO)-based, stirred tank cell culture and column purification process typical of monoclonal antibodies). The ultimate ability to define a multivariate design space, then generate the associated process/product understanding, would be of interest for a vaccine product in light of historical challenges to develop potency assays and establish the clinical relevance of quality attributes to specifications.

Other differences for the vaccine case study arise from the fact that most vaccines are given to healthy patients prophylactically. Feedback from ongoing pharmacovigilance and the question of whether QbD can improve a manufacturer's quality management systems to lessen oversight by Health Authorities (e.g., lot releases by regulatory agencies) are also important topics for discussion. The need for consistent availability of high-quality vaccines often made from complex raw materials leads to an emphasis on the raw material attribute identification, risk

40 analysis, and control strategy. The final difference arises from the availability of key guidance,
41 such as International Conference on Harmonization (ICH's) Q11 and FDA's process validation
42 (PV) documents, that was not as fully developed at the time of the A-Mab case study.
43
44 There are some key differences between monoclonal antibodies and vaccines that influence the
45 development and manufacturing strategy:
46

Monoclonal antibodies	Vaccines	Implications
Often well-characterized	*Often difficult to characterize*	*Less definitive analytical comparability pathways* *Less ability to monitor product quality in mid-process*
Clear link to mechanism of action (MoA) and/or biomarker surrogate for clinical performance	*Difficult to establish clinical potency surrogates*	*Challenging to improve process post-licensure*
Consistent process and product	*Sometimes more complex, less predictable process/product*	*Variability over product/process life cycle*
Therapeutic patient population	*Prophylactic patient population*	*"Process is product" philosophy to assure quality*
Well-understood process; good detectability for test methods	*Less understood process; difficult to measure attribute changes*	*Empirical process models for linking parameter inputs to quality outputs* *More stringent threshold for reporting manufacturing changes*

47
48 Certain differences between monoclonal antibodies and vaccines result in differences in
49 development strategy. The aim of the case study has been to demonstrate how QbD can be
50 applied to vaccines, emphasizing these differences.
51

52 ## 1.3.   Goals of Case Study

53 The goals of the case study are to present potential approaches and stimulate discussion about
54 how to:
55 • Apply QbD to develop a robust vaccine manufacturing process that meets the public health
56   need. It includes:
57   – Risk-based approaches to vaccine development
58   – Leveraging of science to gain process and product understanding
59   – Continual improvement
60   – Merging of process and analytical controls for vaccine manufacturing
61 • Make the rationale for development more transparent in regulatory submissions.

62   • Document techniques to bring safe and effective vaccines to the market more quickly.

63   • Strive to make reviews more efficient; decrease the number of post-approval supplements
64   that are needed.

65   • Develop realistic examples to better illustrate how QbD can be applied within the
66   development space and overall product quality system.

67   • Highlight and/or develop tools, frameworks, etc., to enable ICH Q8, Q9, Q10, and Q11
68   implementation strategies.

69   • Tie key benefits with the strategies illustrated in the case study.

70

71   It should be noted that this case study examines key aspects of applying QbD to vaccines. The
72   ideas and concepts described are examples of potential strategies, but other approaches may
73   also be appropriate. Specifically, substantial changes in manufacturing quality systems and/or
74   regulatory approaches may be needed to fully enable application of QbD to vaccines.

75

76   ## 1.4.   Potential Benefits

77   The hope is that the case study may lead to a better understanding of QbD principles and their
78   potential application to vaccine development. This may encourage promotion of QbD concepts
79   and benefits to industry and regulatory agency management. In addition, incorporating
80   examples of QbD applications for vaccines may challenge traditional thinking about
81   vaccine development.

82

83   The case study will also identify the value created (e.g., business and regulatory drivers) through
84   implementing a QbD approach to development. The value includes:

85   • Better understanding of the product and process, considering the different implementation
86   tools and approaches available to attain this understanding

87   • Robust and consistent processes with clear understanding of the impact of future
88   process changes

89   • Expedited development and regulatory review

90   • Cost- benefit analysis framework

91

92   The QbD approaches presented support the development of the systematic accumulation of
93   product and process understanding that is a major pillar of the vaccine product life cycle.

94

95   ## 1.5.   Publication and Use for Educational Purposes

96   The case study will be published and publically publicly available through the Parenteral Drug
97   Association (PDA) (Website: http://www.pda.org/) for use in stimulating further discussions
98   about QbD implementation. It should be understood that that this document does not
99   represent new regulatory policy, nor does it define a new "Gold" standard for future regulatory
100   submissions. However, it is aligned with the available guidances available from of ICH and other
101   sources guidances, where available. Individual companies will interpret and apply the principles
102   differently. The extent of application applicability will vary for each development effort.
103

104   The case study is composed of thought-provoking options. The point of executing doing the case
105   study was to push boundaries and explore scenarios, and this has been accomplished in several
106   instances. It is critical to avoid the case study examples becoming regulatory expectations
107   and/or standards. Vaccine development has been and continues to be an area of tremendous
108   success and challenges. Day-to-day options differ for every project based on project its needs,
109   timing, and markets under consideration. Although risk assessment and design of experiment
110   (DOE) -driven development is an excellent approach, it is only one of many alternatives.
111

112   The case study is not a consensus opinion document. Working group members expressed
113   diverse opinions regarding risk assessment tools, critical quality attribute (CQA) determination,
114   process performance, and depth of data presented. To complete the case study, some topics
115   were not addressed and positions were not taken even though one or more companies may
116   have advocated for the positions.
117

118   The case study may suggest some areas where future changes to regulatory policy would benefit
119   QbD implementation. In addition, the examples cited are meant to be illustrative of possible
120   approaches to QbD and may not fully represent "real-life" situations. There were multiple
121   simplifying assumptions that the case study was based on. One such simplification is that the
122   case study does not represent the impact of collective changes across several units' operations.
123   There are multiple options for risk assessment, statistical analysis and establishment of a design
124   space. It is also assumed that the manufacturer's quality management system is augmented as
125   needed to be able to fully support reliable QbD implementation post-licensure.
126

127   1.6.   Case study focus and structure

128   There are many types of vaccines, including: live/attenuated/killed virus vaccines, protein
129   conjugate vaccines, protein subunit vaccines, and DNA vaccines. Because it would be impractical
130   to cover all vaccine types, the VWG chose to focus this case study on a fictional carbohydrate-
131   /protein conjugate vaccine as an example of a more complex process. Also included in the case
132   study is another example of viral vaccine production and harvest that is unrelated to the protein
133   conjugate vaccine example but is provided to extend the concepts to more than one type of
134   vaccine. The specific concepts and examples were selected to be complementary to those
135   presented in the A-Mab case study, as well as illustrative of "real -world" "real-world" vaccine
136   applications.
137

138   The case study is structured into two types of sections: general topics and process- specific. For
139   each general topic section, the enhanced QbD approach was applied to several aspects of the
140   selected vaccine in the case study. Within each of the process-specific sections, the enhanced
141   QbD is approach to process development is demonstrated for process development of a single
142   step or several steps. Example steps have been studied from upstream, downstream, and drug
143   product functions. It is beyond the scope of this case study to demonstrate linkage of the
144   enhanced approach across steps described in two or more of these process development
145   sections. As such, changes proposed in one step would still be subject to downstream
146   confirmation of no adverse impact on other steps. This document can serve as a foundational
147   tool for further discussion leading toward that aspirational goal.
148

149 ## 1.7.   Section summaries

150 An executive summary of each section of the case study is included below.
151
152 ### 1.7.1.   Target Product Profile, Critical Quality Attributes, and Product Risk Assessment

153 A-VAX is the name of the case study vaccine. It is a pentavalent polysaccharide-VLP conjugate
154 vaccine that has successfully completed a Phase 2 clinical trial for the prevention of cooties, an
155 infectious disease inflicted by the organism X. horrificus in children.
156
157 The vaccine consists of five serotypes of polysaccharide that have been demonstrated to
158 account for 80% of the disease. The exact mechanism of protection is not known. However,
159 when conjugated to a carrier protein (VLP) and adsorbed to an adjuvant (aluminum salt), the
160 vaccine elicits enhanced cellular and humoral responses in animals and in adult populations.
161 These responses are similar to those observed in surviving individuals as measured after disease
162 outbreaks. The biopharmaceutical development and manufacturing strategy for A-VAX are
163 guided by the product's quality target product profile (QTPP).
164
165 Quality by Design (QbD) principles are applied from the onset of product definition and
166 development and are intended to ensure the following:

167 •   Product is designed to meet patient needs and efficacy requirements

168 •   Critical sources of variability are identified and controlled through appropriate strategies

169 •   Process is designed to consistently meet product critical quality attributes (CQAs)

170 •   Process is continually monitored, evaluated, and updated to ensure that product quality is
171   maintained throughout the product life cycle
172
173 Potential CQAs are selected on the basis of prior knowledge and current understanding of
174 structure-function relationships, and a risk-assessment tool is developed and applied to each
175 quality attribute. CMC-related activities focus on refining structure-function relationships and
176 their impact on safety and efficacy through the addition of knowledge from internal studies or
177 external publications; this information is used to iteratively update the CQA risk assessments
178 throughout the product life cycle as it becomes available.
179
180 ### 1.7.2.   Process Development Sections (Upstream, Downstream, and Drug Product)

181 The process development sections are structured to illustrate different QbD elements across
182 three categories of unit operations (Upstream, Downstream, and Drug Product). Within these
183 categories, a number of areas are explored. These include:

184 •   Prior knowledge and/or initial development for process definition

185 •   Early stage process risk assessment (e.g., cause and effect (C&E) analysis)

186 •   Identification of high-risk parameters (e.g., screening DOE, one factor at a time)

187 •   Later stage (as well as scale-up) risk assessment (e.g., failure mode and effects analysis,
188   or FMEA)

189 • DOE for understanding high-risk steps and their associated high-risk parameters (e.g.,
190   optimization DOE, design space ranging experiments, modeling simulations for
191   defect rates)

192 • Scale-up confirmation

193 • Control strategy, process validation, and continuous improvement implications (i.e.,
194   remaining areas of high variability and high risk)

195 **1.7.2.1. Upstream Section**

196 The Upstream Section covers three key areas of development:

197 • Expression and production of both the polysaccharide and virus-like particle (VLP).

198 • Development of a live vaccine. (The team felt that exploring how QbD can be applied to a
199   live vaccine could add depth to the case study.) This is included as a special section in the
200   case study.

201

202 **Polysaccharide**

203

204 In the manufacturing process for polysaccharide, a well-defined upstream process is required to
205 provide sufficient material (bulk volume) with well-defined quality attributes for the
206 downstream processing.

207

208 The polysaccharide section describes the polysaccharide fermentation process and the effects of
209 the complex raw materials, fermentor operating parameters, and inactivation parameters. Prior
210 knowledge from published literature and process risk assessments are used to ascertain the
211 factors that will be evaluated further. Ishikawa diagrams and cause-and-effect matrices facilitate
212 the identification of process steps for further exploration via design of experiments (DOEs) or
213 one factor at a time (OFAT) evaluations. Failure modes and effects analysis is used to assess the
214 process risks and to develop appropriate strategies for managing critical process attributes.

215

216 *VLP Example*

217

218 The VLP section assesses the contribution of the upstream process in E. coli VLP production and
219 the potential impact of the quality attributes of the upstream material to the critical attributes
220 of the bulk VLP. The harvest step of the upstream VLP production step was selected as an
221 example of the application of tools that provide operational confidence in selecting input
222 parameters that may affect the quality attributes of the VLP.

223

224 *Key Points from VLP Example*
225 1. Several commonly used tools are explored throughout the document to illustrate the QbD
226   approach for selection of critical process parameters and the design space to support the
227   operational ranges for continuous production post validation.
228 2. In addition, examples of changes post validation that may or may not have any impact on
229   the quality attributes are shown.
230 3. A rational approach is used to evaluate the risk of process changes associated with vaccine
231   production with commonly used tools such as cause and effect (C&E) matrices and failure
232   mode and effects analysis (FMEA). They assess the risk of individual process parameter
233   changes, while taking a DOE-based approach to analyze the effects of these process
234   parameters on the product quality attributes.

235   4. Scale-down models are used to reduce the number of parameters in series of fractional and
236   full factorial designs as well as justify the design space that is defined.

237   ***Live Vaccine Example***
238

239   Vaccines based on viral components represent an important segment of the vaccines available
240   on the market including influenza, poliovirus, and hepatitis A. Because of their viral composition,
241   these vaccines present some process requirements that must be taken into account during their
242   development to establish robust manufacturing processes. These process constraints make it
243   more challenging to establish a process platform than for monoclonal antibodies (mAb)
244   processes, with a potential consequence of having less process history data and less prior
245   knowledge in some cases.
246

247   Having these specificities in mind, the section of this case study dedicated to viral-based
248   vaccines illustrates how Quality by Design methodology can be applied to the development of
249   such vaccines.
250

251   ***Key Points from Live Vaccine Example***
252   1. Illustrate how to consider in parallel critical quality attributes (CQAs) and key process
253   attributes (KPAs) during the development of a viral vaccine. A specific risk assessment
254   methodology considering CQAs as well as KPAs is proposed.
255   2. A methodology is proposed to ensure the definition of an efficient way to perform the
256   scaling-up of the bioreactor size with the establishment of scale-down bioreactor model,
257   taking into account specific aspects of micro-carrier-based cell culture (i.e., impact on mixing
258   and shear stress).
259   3. The design space is built by taking into account the variability of the analytical tools used
260   during the development of such vaccine.

261   **1.7.2.2. Downstream Processing**

262   The Downstream Manufacturing Process Development Section has three parts. Two parts cover
263   the purification of the polysaccharides (PSs) and virus-like particles (VLPs) produced by the
264   upstream processes, and the remaining part addresses the process for conjugating the PSs and
265   VLPs. These processes are "platform-like" in that a common set of unit operations typically can
266   be employed to purify PSs and VLPs and conjugate them. Therefore, prior knowledge is available
267   to inform process development based on experiences with similar processes and products.
268   However, the processes are not considered "platform" because of differences specific to the PSs
269   and VLPs involved, which may require unique bioprocess conditions.
270

271   As with the Upstream Section, the Downstream Section uses select unit operations for the three
272   processes to illustrate how Quality by Design principles can be applied to vaccine process
273   development. The three parts of the Downstream Section are similarly composed for each
274   process (PS purification, VLP purification, and PS-VLP conjugation). First, there is a description of
275   the overall process with an explanation for the selection of the representative process step used
276   as an example. Then, for each representative process step, there is a summary of prior process
277   knowledge, an initial process risk assessment, and early stage process development. A late
278   development stage process risk assessment is then presented followed by the development of a
279   design space. This knowledge is used to demonstrate two types of post-licensure changes that
280   can be justified, building on the design space that is defined:

281   • Replacement of non-recombinant enzyme (horrificase) that is purified from the bacterium
282   *X. lyticus* with a new recombinant horrificase that is expressed in E. coli as part of a post-
283   launch change.
284   • Increase in capacity in the manufacturing facility by reducing the incubation time during the
285   conjugation step.
286

287   ***Key Points from Downstream Section***
288   1. Multiple approaches for conducting risk assessments are applicable for evaluating
289   vaccine processes
290   2. Definition of design space can ensure robust process operation (PS extraction)
291   3. Enhanced process understanding is possible regarding linkages between process parameters
292   and both vaccine quality attributes and vaccine process performance
293   4. Post-licensure changes benefit from a defined design space and enhanced process
294   knowledge achieved by using QbD development.

295   **1.7.2.3. Drug Product**
296   Three main processes associated with the drug product development are investigated utilizing
297   various elements of Quality by Design. These processes are formulation development of an
298   aluminum adjuvant vaccine development of a lyophilized formulation, and development
299   of a sterilization process for an aluminum adjuvant diluent to ensure a homogenous product
300   is achieved.
301

302   For formulation development efforts, understanding the optimal solution conditions that
303   provide rapid adsorption of antigens to the aluminum adjuvant is critical since a lyophilization
304   step is included in the process development to ensure antigen stability. Because of limited prior
305   knowledge, a single lyophilized product containing antigens along with aluminum is not
306   developed. Thus, it is important to clearly understand the adsorption kinetics of antigens to an
307   aluminum adjuvant so that upon reconstitution, antigens are adsorbed quickly to the adjuvant
308   and the administered vaccine is consistent from lot to lot.
309

310   Lyophilization cycle development is initially investigated at the laboratory scale; scalability and
311   applicability of lyophilization are discussed in moving from laboratory to pilot to commercial
312   scale. Prior knowledge plays a critical role in scalability aspects of lyophilization because
313   key factors that should be investigated are very well understood to ensure a robust, fully
314   scalable process.
315

316   The final area in the drug product section evaluates the sterilization and mixing processes
317   associated with an aluminum adjuvant diluent. It is necessary that the aluminum adjuvant
318   diluent is homogenous in nature and sterilized appropriately so that upon reconstitution of the
319   drug product with diluent, proper adsorption and homogeneity are achieved in the final drug
320   product. This ensures that, once reconstituted, an administered vaccine product is consistent
321   from lot to lot.
322

323   Similar to the Upstream and Downstream sections, specific unit operations associated with
324   formulation, lyophilization, and aluminum sterilization are selected to be examined using both
325   traditional and Quality by Design approaches. An initial, early stage risk assessment (cause and
326   effect matrix) is performed to identify process parameters where additional experiments may

327   have to be performed to obtain process understanding. Since the drug product processes
328   examined are common unit operations associated with multiple vaccine drug products, the prior
329   knowledge needed to make an informed assessment is vast.
330

331   ***Key Points from Drug Product Section***
332   1. It outlines the entire drug product formulation process and indicates places where QbD can
333   be applied.
334   2. It demonstrates the effective use of prior knowledge and initial risk assessment tools
335   (multiple tools and approaches can be used) to determine where development should be
336   focused for a robust process.
337   3. Development of a robust process requires multiple iterations of risk assessments, and
338   defining the design space is critical.
339   4. It uses process risk assessment to link parameter risks to their respective CQAs and confirm
340   the design space that has been defined based on the early development studies
341   5. The scale-up process uses a small-scale model during lyophilization development to confirm
342   that laboratory- and pilot-scale results align with the final commercial-scale process.
343   6. For site to site transfer, knowledge is used to demonstrate understanding of key equipment
344   attributes that are used to ensure proper modeling (i.e. choke flow, rate of heat transfer,
345   freezing processes and parameters) and provide confidence that the transfer is acceptable.
346   (It is supplemented with comparability protocols to ensure process transfer between sites is
347   successful either before or after licensure.)

348   ## 1.7.3. Control Strategy

349   The control strategy for A-VAX is written from a life-cycle management point of view. Early
350   development experience, such as identification of potential critical quality attributes, and prior
351   knowledge are built on throughout development. Nonclinical and clinical experiences are
352   combined and are used to identify analytical attributes and process control parameters and
353   their appropriate specifications and operating ranges.
354

355   Unique properties of some vaccines are acknowledged in development of the control strategy.
356   Vaccine release is coupled with quality requirements to help assure acceptable vaccine
357   properties throughout product shelf life. Key assays such as potency assays are developed to the
358   suitable standards, employing Quality by Design principles to assure reliable measures of
359   vaccine quality. Because of the nature of vaccine quality measurements, the case study
360   emphasizes the roles and distinctions between specifications and control limits, as well as
361   proper analysis of the measurements.
362

363   Critical quality attributes and their specifications are the foundation to identify and set ranges
364   for critical process parameters. Vaccine unit operations are evaluated, both scientifically and
365   experimentally, throughout the process to optimize it and identify the regions that yield
366   acceptable product performance. Thus experiments are performed on a small scale to link
367   process parameters to process performance, revealing the region where the product meets its
368   quality specifications (the "design space"). The robustness of the control strategy is monitored,
369   and adapted as necessary, when operated at a large scale through continuous verification. Thus
370   the control strategy is a living plan, which is modified and improved throughout the lifetime of
371   a vaccine.
372

373 Example scenarios are provided for assessments of quality attributes throughout development,
374 leading to a final control strategy. Manufacturing modeling is used to inform development of
375 nonclinical and clinical studies, which must be performed to support the control strategy.
376 Conventional thinking is augmented by sound scientific development and documentation,
377 which serves to communicate the control strategy and react to unexpected process and
378 product events.
379

Key Points from Control Strategy Section

381 1. The final control strategy is the synthesis of early through late process, analytical,
382   preclinical, and clinical experiences.
383 2. A sound scientific and risk-based approach to the evolution of the vaccine control strategy
384   yields greater confidence in product quality and process control.
385 3. Strategic development experiments may be used to gain and communicate understanding,
386   and to serve as a foundation for continuous process verification and improvement.

1.7.4. Regulatory Section

388 The environment for incorporating design space into regulatory filings for vaccines is expected
389 to evolve in the coming years as regulators as well as vaccine companies gain more experience
390 in application of these enhanced methodologies and they are applied earlier in the development
391 life cycle.
392

393 With this in mind, this section of the case study explores the application of QbD concepts to the
394 content of regulatory filings. Its purpose was to review the strategies offered in the other
395 sections of the case study and give guidance on how best to illustrate these strategies in various
396 types of regulatory filings. While the intent was not to "approve" a specific strategy, it did offer
397 guidance regarding the level of data and/or justification appropriate to pursue a specific
398 strategy. Structuring the case study in this manner generated and captured the dialog
399 needed to better understand the challenges associated with implementing QbD within
400 vaccine development.
401

402 The case study is a scientific document addressing the application of Quality by Design to
403 vaccine development and product life cycle management. It is intended to serve as an example
404 of potential ways that scientific principles and tools described under ICH documents Q8, Q9,
405 Q10, and Q11 could be applied seamlessly during vaccine development and through post-
406 approval life cycle management.
407

408 The examples are created as a teaching tool and as an opportunity to encourage stakeholder
409 discussions on the application of these concepts. These examples are not presented as a mock
410 submission, nor is there any expectation that the combination of illustrative examples would
411 represent a realistic filing. The scientific principles are discussed and data is provided to
412 demonstrate how the assignment of quality attributes, conduct of risk assessments,
413 performance of experiments, and development of design space and control strategy could be
414 utilized in regulatory filings to enhance the depth of product knowledge, increase the
415 robustness of process control, and facilitate continuous improvement. We indicate what data
416 could be presented to support the analysis, where summary information is appropriate, and
417 how the data would be analyzed in each of the process sections:

418 • Industry will generally implement QbD for vaccines in certain process steps ("targeted QbD
419   implementation" for vaccines), and hybrid QbD filings will be standard.

420 • QbD implementation for vaccines may be limited to areas that would benefit most from
421   QbD, most likely the areas that require most of the changes post licensure (e.g., equipment
422   changes, process changes, site changes).

423 • Comparability protocols, such as post-approval change management and expanded change
424   protocols, provide a flexible mechanism to implement Quality by Design across the product
425   life cycle (e.g., by including comparability protocols in initial marketing authorization or
426   submitting them post approval).

427

428 *Key Points from Regulatory Section*

429

430 Although a few examples of vaccines developed using QbD exist, integration of key Quality by
431 Design concepts, specifically the increased product knowledge that can be gained, will yield the
432 following benefits:

433 • Provide additional strength to the data set supporting operational ranges and control
434   strategy elements described for the product

435 • Justify management of change in a manner that increases the assurance of maintaining
436   product quality. This ensures appropriate assessment across the spectrum, from full prior
437   approval, board of health review to the firm's quality systems that oversee changes.

438

439 A summary of the type of guidance offered includes the following:

440 • To take advantage of the increased product and/or process knowledge that is generated it
441   was required to capture and document the defined design space in the regulatory filings.

442 • Given the limited experience to date in managing change in a design space, it was
443   recognized that to accomplish this in the EU and US filings today, a change management
444   plan could be submitted. It would clarify the anticipated treatment of changes envisioned
445   for the product life cycle.

446

447 The regulatory section concludes with a section on future challenges. The section introduces
448 topics with tremendous potential value from applying the principles. However, there are also
449 enough unanswered questions that it is important to emphasize the fluid and exploratory nature
450 of the discussion.

451

452 One example is possible secondary or adaptive acceptance criteria in a CMP. In the development
453 of a CMP, an acceptance criterion for CQA/CPP is required to build the control strategy. During
454 manufacturing, a result for a CQA may be at the limit for a particular lot. This could be handled
455 as a deviation in the usual way. Alternatively, secondary or adaptive criteria could be developed
456 in advance and incorporated into the CMP that justify the maintained acceptability of the
457 CQA result.

458

459   1.7.5.   Implementation Section

460   In this section of the case study we present considerations for evaluating the business case of
461   applying QbD to vaccine process development. The focus of this section is to present potential
462   value drivers and evaluation tools for a step-by-step investigation of the business case
463   development. This discussion may lead to a better understanding of the value drivers applying
464   QbD principles in vaccine development. Also, it may encourage promotion of the concepts and
465   benefits of QbD to industry management in situations where additional potential value is
466   suggested. The traditional approach to vaccine process development has provided the industry
467   with safe, effective, and reliable manufacturing processes, so the focus of evaluating the
468   business case for QbD is to determine the specific additional value returned for the investment.
469   The decision to apply QbD to a unit operation or step in the process is often made as a means to
470   mitigate a risk identified in a process risk assessment. In this case study, we evaluate the
471   potential value from the specific examples chosen in the downstream and drug product
472   development sections.
473
474   The approach used for determining costs and benefits for these examples is a value stream
475   measure of improved efficiency. This measure is defined in terms of the organization's "ability to
476   predict":
477   •   Safety and efficacy
478   •   Product availability (robustness)
479   •   Cost effectiveness
480
481   The business case for the QbD approach is unequivocal if this method eliminates all uncertainty
482   and risk. However, neither the traditional nor enhanced approach is expected to produce
483   perfectly comprehensive process and product knowledge. Thus, the key differentiator between
484   the approaches is the value of additional process knowledge and how that information is used.
485
486   The process development risk assessment often determines where QbD will deliver the most
487   benefit when applied. Both the traditional and QbD strategies can be applied successfully.
488   However, in some situations the additional process knowledge gained through QbD proves
489   useful for establishing robust control strategies and making risk-based decisions. In high-risk
490   situations where this additional knowledge provides value to key stakeholders, the business
491   case supports the enhanced approach. In many low-risk situations, however, the traditional
492   approaches are very effective so there is limited value returned for the additional efforts
493   required for QbD.
494
495   Applying this additional effort in these low-risk situations is not valuable to stakeholders and
496   might hinder the process of delivering safe and effective drugs because of the significant
497   increase in investment and resources required from both manufacturer and regulators.
498   Consequently, a clear understanding of the stakeholders and value drivers for the QbD .
499   approach improves manufacturers' and regulators' effectiveness by focusing resources where
500   substantial value can be gained.
501

502   *Key Points from Implementation Section*

503   1.   Multiple stakeholders (patients, manufacturers, and regulators) benefit from the
504   enhanced approach to vaccine process development. (See ICH Q8 and Q11 for concepts
505   and definitions.)
506   2.   The enhanced approach improves the ability to predict the value stream measures of safety,
507   efficacy, availability, and cost effectiveness.
508   3.   A value stream approach can be used to successfully prioritize business and regulatory
509   drivers, which supports investment in the enhanced approach.
510   4.   ROI analysis for the enhanced approach needs to be specific to the company, regulatory
511   agency and product because these factors drive the value stream and each situation may
512   have unique considerations. In this case study we have provided an example framework,
513   which can be used to develop an individualized approach.

514

2.   Target Product Profile, Critical Quality Attributes, and Product Risk Assessment

2.1.   Executive Summary

A-VAX is a pentavalent polysaccharide- virus-like particle (VLP) conjugate vaccine that has successfully completed a Phase 2 clinical trial for the prevention of cooties, a fictional infectious disease inflicted by the organism *X. horrificus* in children. The vaccine consists of five serotypes of polysaccharide that have been demonstrated to account for 80% of the disease. The exact mechanism of protection is not known; however, when conjugated to a carrier protein (VLP) and adsorbed to an adjuvant (aluminum salt), the vaccine elicits enhanced cellular and humoral responses in animals and in adult populations. These responses are similar to those observed in surviving individuals as measured after disease outbreaks.

The biopharmaceutical development and manufacturing strategies for A-VAX were guided by the product's quality target product profile (QTPP). Quality by Design (QbD) principles were applied from the onset of product definition and development and were intended to ensure that:
i.   The product would be designed to meet patient needs and efficacy requirements
ii.   Critical sources of variability were identified and controlled through appropriate control strategies
iii. The process was designed to consistently meet product critical quality attributes (CQAs)
iv.   The process would be continually monitored, evaluated, and updated to maintain product quality throughout its life cycle

Potential CQAs were selected on the basis of prior knowledge and current understanding of structure-function relationships for conjugate vaccines, and a risk-assessment tool was developed and applied to each quality attribute. Chemistry, Manufacturing and Controls (CMC)-related activities focused on refining structure-function relationships and their impact on safety and efficacy. As new information becomes available throughout the product life cycle, it is used to iteratively update the quality attribute risk assessments, CQA classifications, and acceptance criteria.

2.2.   Scientific Rationale and Disease Area Overview

In child lore, "cooties" is a fictional, widespread infectious disease. Infection with the fictional bacteria *X. horrificus* causes the rapid onset of a short-lived illness (usually lasting for a week or less) called cooties, which generally occurs in children. Cooties is typically a mild, self-limited illness manifest by fever and rash In some cases, however, cooties may be complicated with a bloodstream infection, pneumonia, or meningitis, thus requiring treatment with systemic antibiotics.

553
554 Once an episode of cooties has resolved, recidivism is rare (the bacteria is essentially eliminated
555 from the body by the immune response), and re-infection also is rare (protection via an adaptive
556 immune response to the natural infection). Cooties most commonly occurs in children aged 4 to
557 10 years as they enter school settings; however, it is also occasionally confirmed in those older
558 than 10.
559
560 A-VAX's target product profile (TPP), a prospective summary of the vaccine development
561 program described using labeling concepts, is located in Table 2-1: TPP for A-VAX.
562
563 **Table 2-1: TPP for A-VAX**

Mechanism of Action	• A-VAX (drug product) is a pentavalent vaccine containing the capsular Ps of *X. horrificus* serotypes 1-5, individually linked to a recombinant, non-infectious virus-like particle (VLP) and adjuvanted with an aluminum salt. • A-VAX is expected to provide an enhanced cellular (Th1) and humoral (Th2), antigen-specific, protective immune response when compared with a natural *X. horrificus* infection.
Indication	A-VAX is indicated for the active immunization of 2-month-old to 60-month-old infants for prevention of cooties-related illnesses caused by *X. horrificus*.
Primary Endpoints	• 70% reduction of *X. horrificus*-confirmed cooties disease within one year after dosing (below 60% is a no go) in the target population • Safe and tolerable as defined by solicited symptoms, adverse events, and serious adverse events (no evidence of enhanced *X. horrificus* disease)
Key Claims	• Has a favorable risk-benefit profile • Can be dosed with other pediatric vaccines • Universal recommendation except for premature infants (<36 weeks), immunocompromised infants, or infants with previous adverse reactions to A-VAX • Achieves World Health Organization (WHO) stability requirements
Secondary Endpoints	• Analysis supportive of primary endpoint in target population • Reduction in *X. horrificus*-specific hospitalizations and emergency-room visits • Reduction in *X. horrificus*-specific rates of bacteria-confirmed cooties disease • Reduction in antibiotic use for *X. horrificus*-confirmed cooties disease • Duration of protection >1 year (with/without booster)

Formulation/Dosing	• Antigen and adjuvant in pre-filled syringe or single-dose vial
	• Antigen and adjuvant containers are pre-mixed prior to injection
	• 3 doses administered 2 months apart (preferably 0-, 2-, and 4-month pediatric vaccine schedule)
Approvals and Recommendations	• Expecting Advisory Committee on Immunization Practices and other universal recommendations (i.e., United States, European Union, Canada, Japan, and WHO)

564

2.3. Biological Target and Its Role in the Disease Area

566 The exact mechanism by which *X. horrificus* bacteria causes cooties disease is not known, but
567 anticapsular polysaccharide (Ps) antibody levels (humoral response) and an enhanced cellular
568 response correlate with a significantly reduced incidence of invasive *X. horrificus* infection.
569 These humoral and cellular responses are similar to those observed in surviving individuals who
570 fully recovered from the disease.

571

572 Five *X. horrificus* strains, each composed of a unique polysaccharide serotype (1, 2, 3, 4, or 5),
573 account for about 80% of the total disease. A-VAX is indicated for the active immunization of 2-
574 month-old to 60-month-old babies for prevention of cooties-related illnesses caused by *X.*
575 *horrificus*, and the vaccine is designed to elicit antibodies to *X. horrificus* capsular Ps.

576

577 A-VAX is a pentavalent vaccine that has finished Phase 2 clinical trials and contains the capsular
578 Ps of *X. horrificus* serotypes 1-5, individually linked to a recombinant, non-infectious VLP and
579 adjuvanted with an aluminum salt. The mechanism by which A-VAX stimulates the cellular and
580 humoral immune response is not fully understood; however, prior knowledge supports the
581 assumption that only the Ps-VLP conjugate can initiate a protective immune response to Ps in
582 this age group. Ps 1-4 are more immunogenic than Ps 5 (no neutralizing monoclonal antibody
583 [Mab] is available for Ps 5). A murine challenge-protection model is available for each of the
584 serotypes. However, no *in vitro* model exists that can be correlated with human protection for
585 serotype 5.

586

587 The total pAb titer (Th2) and cytokine panel (Th1) show a dose response to each adjuvanted Ps-
588 VLP (either separately or in combination). No synergistic immune response is observed – the
589 immune response to each serotype is independent. Unconjugated Ps does not illicit an immune
590 response; for this reason, the level of free Ps and VLP, in addition to their extent of conjugation,
591 must be controlled. The immune response to the conjugate promotes phagocytosis and
592 microbial killing; the opsonophagocytic killing assay (OPA) is widely accepted as the reference
593 method for measuring the protective capacity of *X. horrificus* antibodies, and it is employed for
594 serotypes 1-4. An OPA level of 90% of subjects with 1:8 OPA titers is considered effective.

595

2.4.   Status of Clinical Development

The concept of clinical design space, the link between the clinic and CQAs, and approaches to quantify the clinical experience with a biotech product candidate have been reviewed (A.S. Rathore and H. Winkle, *Nature Biotechnology* 27, 26-34 [2009]).

The clinical development program for A-VAX has completed a Phase 2 study, with an 87% response rate for each serotype. Key assumptions in the clinical development program included:

i.   The "null hypothesis" was that at least one serogroup has a seroresponse rate with a lower bound of the 95% confidence interval (CI) being less than 70%.

ii. The 70% bound was selected on the basis of a sample-size estimation involving 90 participants in the study group providing 80% power to reject the null hypothesis

iii. Enrollment was, therefore, 100 subjects with an assumed 10% drop-out rate to have 90 subjects available for the assumed immunogenicity analysis (Table 2-2: Assumed Seroresponse Rates*) and reactogenicity profile (Table 2-3: Assumed Reactogenicity, Infant Stage*).

Table 2-2: Assumed Seroresponse Rates*

Serotype	Seroresponse Rate % (95% CI)
1	92 (84, 97)
2	96 (89, 99)
3	97 (91, 99)
4	94 (86, 98)
5	92 (84, 97)

* Adapted from: Immunogenicity of a Tetravalent Meningococcal Glycoconjugate Vaccine in Infants, A Randomized Controlled Trial. Matthew D. Snape, JAMA, January 9/16, 2008—Vol 299, No. 2, corrected on February 15, 2011

Table 2-3: Assumed Reactogenicity, Infant Stage*

Adverse Event	UK234 (n = 90)
Local Reactions	
Erythema	
Any	69 (77)
Grade 3	1 (1)
Pain	
Any	40 (44)
Grade 3	6 (7)
Induration	
Any	21 (23)

Adverse Event	UK234 (n = 90)
Grade 3	0
Systemic Reaction	
Irritability	63 (70)
Sleepiness	56 (62)
Diarrhea	29 (32)
Reduced Feeding	28 (32)
Vomiting	28 (31)
Persistent Crying	19 (21)
Axillary Temperature	
≥38 °C	7 (8)
≥40 °C	0
Analgesic/Antipyretic Use	43 (48)

617
618   * Adapted from: Immunogenicity of a Tetravalent Meningococcal Glycoconjugate Vaccine in Infants, A Randomized
619   Controlled Trial. Matthew D. Snape, JAMA, January 9/16, 2008—Vol 299, No. 2, corrected on February 15, 2011
620

621   ## 2.5.   Key Molecular Characteristics of A-VAX

622   Table 2-4: QTPP for A-VAX lists the vaccine's quality target product profile. The QTPP is a
623   prospective summary of the desired quality characteristics of the drug product that will ideally
624   be achieved, taking into account the safety and efficacy of A-VAX (ICH Q8):
625

626   **Table 2-4: QTPP for A-VAX**

Key Claims	• Easy to administer, 0.5-mL subcutaneous delivery in a healthcare (outpatient) setting using a 1-mL syringe (27G × ½ inch needle) • Stability: 2 years at room-temperature storage or 4 years at 2–8 °C, and 24 hours' physical and chemical stability following reconstitution at 2–8 °C or 8 hours at room temperature (achieves WHO stability requirements) • No animal- or human-derived products are used in the manufacture of A-VAX
Formulation/ Dosing	• Sterile product: the drug substance (Ps-VLP) can be sterile filtered • 3 doses (containing 5 mcg each of Ps 1-4 and 50 mcg Ps 5; adsorbed to 300 mcg aluminum adjuvant as Ps-VLPs) administered 2 months apart (preferably 2, 4, and 6 months pediatric vaccine schedule) • Lyophilized and reconstituted with standard diluents containing adjuvant: rapid reconstitution profile with viscosity of 1-3 cP • Composition: sugar, surfactant, buffer (isotonic pH), and Ps-VLP conjugate • Label volume 0.5 mL filled (actual fill volume will be greater than the

	label volume to account for losses)
	• Single-dose vial (ISO2R vial, clear, Type I glass), latex-free stopper (13-mm coated stopper) and seal (13-mm aluminum seal with flip-off plastic button)
	• Secondary packaging and shipping: allowed shipping-excursion temperature 2-40 °C for 3 days in a carton (10 vials/carton)

627

628 A-VAX consists of polysaccharides purified from fermentation of *X. horrificus* on a large scale,
629 conjugated to VLPs, and then adsorbed to an aluminum salt adjuvant. Each *X. horrificus* serotype
630 is fermented, and the individual Ps are purified by a series of chemical and physical methods.
631 The Ps are sized (average of 15 repeat units, each representing the critical epitope), chemically
632 activated to aldehydes, and directly conjugated to the VLP carrier protein through reductive
633 amination to form the Ps-VLP conjugate.

634

635 VLPs are composed of individual polypeptides of a recombinant protein. The VLP is produced in
636 *E. coli* and is purified by a series of chemical and physical methods. VLPs first assemble through
637 non-covalent forces (hydrogen bonding and hydrophobic interactions), followed by the
638 formation of inter-chain disulfide bonds. The fully assembled VLP ranges in diameter from 20 to
639 50 nm.

640

641 Individual Ps are conjugated to the VLP through the accessible amino groups on the exterior
642 of the VLP. The individual Ps-VLP conjugates (drug substance) are then formulated to create
643 a polyvalent drug product containing the five different Ps-VLPs, followed by vial filling
644 and lyophilization.

645

646 Candidate selection experiments established that A-VAX provides an enhanced cellular (Th1)
647 and humoral (Th2), antigen-specific, protective immune response, which is observed only for the
648 Ps-VLP conjugate. Non-conjugated Ps are unable to illicit an immune response in the target
649 population. Experience with other conjugated vaccines using the same VLP carrier identified T-
650 cell epitopes critical for obtaining a robust response and long-term immunity.

651

652 For the analytical development strategy, the initial focus was to support an Investigational New
653 Drug-application, enabling activities for the Phase 1 study. Particular focus was on lot-release
654 assays and characterization of key neutralizing epitopes during manufacture and storage. The
655 main emphasis was on developing and implementing analytics for monitoring clinically relevant
656 epitopes. This involved establishing antigenicity-immunogenicity correlates with the critical
657 structural attributes of the antigen:adjuvant complex.

658

659 To support later stages of development, the analytical strategy included assays for monitoring
660 potency, identity, purity, product- and process-related impurities, stability, and drug titer of the
661 soluble-protein antigen in the presence and absence of the adjuvant.

662

663 A key development tool for A-VAX was the availability of a murine-potency assay (with both
664 serology and neutralization readouts); it was used for establishing the important link between
665 immunogenicity (and its mechanistic relevance) in an animal model and antigenicity in ligand-
666 binding assays [in this case study, we assume enzyme-linked immunosorbent assays (ELISAs)] for
667 serotypes 1-4. Selection of neutralizing mAbs for use in the ligand-based assays for these
668 serotypes was confirmed using the murine-potency assay. Clinical results (human serology)
669 support the conclusions that:
670 i. The ELISA is predictive of human immunogenicity
671 ii. Antigenicity, as defined by the specific epitope, can be used as a surrogate
672 for immunogenicity
673 iii. The ELISA is suitable as the sole potency assay for serotypes 1-4 since a correlation with
674 animal model and human immunogenicity has been demonstrated for serotypes 1-4, but
675 not serotype 5

676 Serotype 5 potency was evaluated using the *in vivo* animal model only, though an antigen-
677 binding assay (rate nephelometry) was also performed in hopes of establishing a correlation and
678 replacing the animal model in the future.
679

680 ## 2.6.   Product Risk-Assessment Tool and Potential Critical
681 ## Quality Attributes

682 CQAs are the molecular and biological characteristics found to be critical in ensuring the safety
683 and efficacy of a drug product. Because of the complexity of vaccine products, defining their
684 CQAs often is difficult. Therefore, many attributes are explored during development.
685

686 For A-VAX, an initial list of quality attributes to be assessed included all product attributes that
687 could be characterized using existing technology and analytical methods. A risk-assessment tool
688 was developed and applied for each A-VAX quality attribute. Potential CQAs were identified on
689 the basis of prior knowledge and current understanding of structure-function relationships.
690 Then initial acceptance criteria were established for each CQA on the basis of prior knowledge,
691 as well as manufacturing experience, clinical or pre-clinical data, and stability. It is important to
692 note that knowledge gained from other conjugate products, in addition to polysaccharide
693 products, and relevant published literature articles were evaluated in the assessment of CQAs
694 and acceptance criteria.
695

696 Activities then focused on refining structure-function relationships and assessing the impact of
697 their ranges on safety and efficacy of the product. As new information is discovered during the
698 product's life cycle, it is used to iteratively update the CQA risk assessments (outlined in Figure
699 2-1: CQA and Control Strategy Information 'Decision Tree'*).
700

701 **Figure 2-1: CQA and Control Strategy Information 'Decision Tree'***
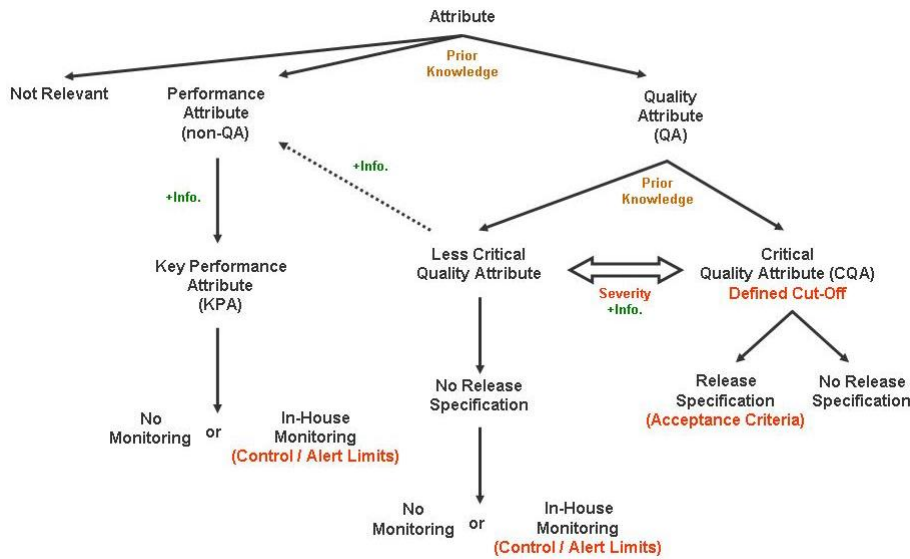
702
703 * The approach of using a criticality continuum (⇔) is a key aspect of the control strategy in the case study. The
704 exercise of classifying each attribute into quality attribute (QA) or performance attribute (non-QA) should have been
705 done prior to Phase 2. A less critical QA is a quality attribute that has a relatively lower risk of impacting safety and
706 efficacy of the product. Using the risk-assessment tool, the criticality continuum allows adjustments within the QA
707 "envelope" as new information is obtained. A performance attribute is designated as a key performance attribute
708 (KPA) if it affects process performance (e.g., yield or duration), but not product quality.
709
710 A questionnaire-based severity analysis was performed to identify potential CQAs. Each quality
711 attribute was assessed for:
712 i.   level of impact on clinical performance (safety and efficacy, see Table 2-5: Impact Scores)
713 ii. level of uncertainty associated with this prediction of the impact (see Table 2-6: Uncertainty
714   Scores)

715 In this case study, we define (very high) uncertainty as a situation where the current state of
716 knowledge about an attribute is such that the consequences, extent, or magnitude of a change
717 event is unpredictable, and credible probabilities cannot be assigned to possible outcomes.
718
719 The quality attributes that have "severity" scores ≥25 are initially categorized as "critical"
720 (Equation 2-1).
721
722 **Equation 2-1: Severity**

723 **Severity = Impact × Uncertainty**
724
725 Quality attributes slightly below the cutoff value are further evaluated and discussed to confirm
726 their level of criticality. The ≥25 cutoff limit is justified even if all the uncertainty is removed
727 from the evaluation, because any parameter with a potential high impact will still remain a
728 potential CQA. Furthermore, the quality attributes with only moderate impact can be
729 considered critical if there is high uncertainty.
730

731   This case study illustrates how different risk-assessment approaches and types of knowledge
732   (prior or platform knowledge, laboratory data, non-clinical data, and clinical data) may be used
733   to assess quality attributes. The case study used the risk-assessment tools to evaluate the
734   impact of quality attributes on safety and efficacy. It did not consider process or manufacturing
735   capabilities or ability to detect an important process attribute in the evaluation. Prior knowledge
736   gained from the protein carrier generated for other conjugate products, in addition to
737   polysaccharide products, was considered relevant.
738
739   The risk-assessment process is composed of several steps, including product definition (see
740   Table 2-1: TPP for A-VAX and Table 2-4: QTPP for A-VAX), the identification of relevant
741   stakeholders and subject matter experts for the exercise, and the evaluation of new and
742   previous knowledge. Rather than describing the assessment of all quality attributes for the case
743   study, a subset of quality attributes was selected. Each attribute has a different level of
744   criticality, varies in the impact on efficacy and safety, and varies in the amount and types of
745   information available to assess criticality:
746   i.   As part of the preparation work for the risk assessment exercise, all relevant quality
747   attributes should be identified (starting with the DP), taking into consideration the target
748   product profile (refer to Table 2-1: TPP for A-VAX and Table 2-4: QTPP for A-VAX).
749   ii.   Impact scores (Table 2-5: Impact Scores) were created that take into consideration the most
750   important attributes of a vaccine: safety and efficacy (refer to Table 2-2: Assumed
751   Seroresponse Rates* and Table 2-3: Assumed Reactogenicity, Infant Stage*).
752
753   In contrast to other biologics, issues such as unwanted immunogenicity and pharmacokinetics
754   do not normally apply to vaccines. Because the ultimate goal is to link product attributes either
755   directly or indirectly to clinical performance, the impact score is restricted to characteristics that
756   have the potential to impact clinical performance, as assessed by clinical, animal, or *in vitro*
757   studies. The impact score is also simplified compared with other biologics because *in vivo* data
758   tend to be highly variable. Studies conducted with similar products, including published journal
759   articles, also provide information to help assign the impact scoring for a product.

760   **Table 2-5: Impact Scores**

Impact Score	Efficacy	Safety and Tolerability (Adverse Events, AEs)
Very High 25	Significant Change	**Severe** AE prevents normal, everyday activities (e.g., prevent attendance at school/kindergarten/day-care center, requiring medical attention or advice). Significant increase in severity and/or frequency.
Moderate 8	Moderate Change	**Moderate** Sufficiently discomforting to interfere with normal everyday activities. Moderate but detectable increase severity and/or frequency over placebo.
Minimal 2	Minor to No Change	**Mild** Easily tolerated, causing minimal discomfort and not interfering with everyday activities. Similar to placebo.

761
762

763 Uncertainty scores (Table 2-6: Uncertainty Scores) were based on the availability of relevant
764 information about the quality attribute under evaluation. The level of uncertainty ranges from a
765 minimal value of 1 (little or no uncertainty) to a high of 5 (total lack of information). Supportive
766 data from small clinical studies provides some level of assurance, but may not be statistically
767 powered to detect minor changes. Pre-clinical data and data from similar vaccines require a
768 more extensive discussion with relevant experts to determine their applicability to A-VAX
769 assessments. Literature searches about related vaccines, although useful, may not fully
770 represent A-VAX characteristics (e.g. conjugation process, formulation).
771
772 One important feature of the scoring system is that if there is data confirming a high impact or
773 high risk for the attribute (e.g., impact score = 25), it will result in assigning a high severity score
774 (e.g., severity score will be ≥25). Such attributes should be automatically considered as critical
775 (CQA defined as any product attribute with severity score ≥25), no matter the level of
776 uncertainty. Thus, any product attribute with high impact is automatically considered a CQA.
777 The uncertainty score is based on availability of information that supports an acceptable change
778 to the attribute.
779
780 **Table 2-6: Uncertainty Scores**

Score	Uncertainty
Very High 5	No information available
High 4	*External* information available from literature on related vaccine(s)
Moderate 3	Data from *internal* laboratory or nonclinical studies with this antigen:adjuvant complex, or *internal* data extrapolated from related vaccine(s)
Low 2	Supportive data from *clinical studies* with this antigen:adjuvant complex
Minimal 1	Published limits widely accepted by regulatory and scientific community

781
782
783 Severity scores are summarized in **Table 2-7: Severity Scores***. Using equation 1 with the scores
784 for impact (Table 2-5: Impact Scores) and uncertainty (Table 2-6: Uncertainty Scores) assigned as
785 part of the risk assessment, a potential critical quality attribute was assumed to have a severity
786 score ≥25 and a less critical quality attribute was assumed to have a severity score ≤24. To score
787 using the definitions in **Table 2-7: Severity Scores***, the risk-assessment team evaluated the
788 potential impact of an attribute being outside its acceptable range. As a first pass, the team may
789 consider the potential effect of the attribute as if it cannot be controlled.
790
791 It is important to note that an "iterative triage" was applied to all attributes, with particular
792 attention paid to scores near the cut-off (indicated as yellow), which involved reassessment of
793 impact and uncertainty scores as updated information became available. Time points for
794 conducting iterative triage are not defined, but rather the triage is done when new information
795 on clinical, non-clinical, or manufacturing data becomes available. This iterative triage allowed
796 severity scores to be adjusted on the basis of new impact and uncertainty information.
797

798   It is particularly important that a rationale is provided for any adjustment and a record of
799   how severity scores evolve is available for product life-cycle management and justification of
800   control strategies.
801
802   **Table 2-7: Severity Scores***

803
804   * Severity scores are categorized as critical (≥25, red), borderline (10-24, yellow), and less critical (≤10, green). As
805   stated previously, those scores considered borderline (10-24, yellow) require further evaluation and discussion among
806   the relevant technical experts. Note that scoring granularity and severity scoring are provided as an example in this
807   case study. Manufacturers should score and granulate as they consider appropriate. For example, more granularity in
808   the ranking system could be considered for either uncertainty or impact.
809
810   Upon completion of the CQA-scoring process (summarized in Table 2-8: Initial CQAs and Risk
811   Assessment for Reconstituted A-VAX **(adjuvant + Ps-conjugate)**) and Table 2-9: Triage Round 1
812   CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)), the full list of
813   attributes should be reviewed to ensure that the output of the scoring system is realistic.
814
815   In particular, attributes that score as less critical (not listed in Table 2-8 and Table 2-9) should
816   be reviewed carefully with consideration of whether they may be important markers of
817   process consistency or have been shown to be essential for the efficacy/safety of other
818   vaccine products.
819
820   For example, product-specific data may suggest that completeness of adsorption is not linked to
821   clinical performance. However, if the literature for a previously licensed vaccine suggests a link
822   between completeness of adsorption and safety or clinical performance, then it may be
823   necessary to adjust the interpretation of the scoring for this parameter to address the
824   knowledge gained from the other vaccine.
825

826   **Table 2-8: Initial CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-conjugate)**

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Serotype 5 (no correlation)	Rate Nephelometry (desorbed)	8	2	16
Animal Model (confirms correlation)	Murine Serology (adsorbed)	25	2	50
Th1/Th2 Profile	Cytokine-panel ELISAs (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Fragments	Reducing CGE	8	3	24
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps, and free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness-of-Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

827   * Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).
828

829 **Table 2-9: Triage Round 1 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-**
830 **conjugate)**

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Serotype 5 (no correlation)	Rate Nephelometry (desorbed)	8	2	16
Animal Model (confirms correlation)	Murine Serology (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Fragments	Reducing CGE	8	3	24
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps & free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness-of-Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

831 * Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).
832
833 The quality attributes for the A-VAX final drug product, including severity scores from the risk
834 assessment, are summarized in Table 2-10: Triage Round 2 CQAs and Risk Assessment for
835 Reconstituted A-VAX (adjuvant + Ps-conjugate). Although only the reconstituted drug product
836 CQAs are presented and less critical QAs are not included, this assessment was done for each
837 drug substance and drug product and their intermediates. More detailed information on the
838 evolving potential CQAs, risk assessments, and specifications is provided in the Appendix

CMC-VWG

839   (hyperlink). This information was then used to update the risk assessments in an iterative
840   manner.
841
842   **Table 2-10: Triage Round 2 CQAs and Risk Assessment for Reconstituted A-VAX (adjuvant + Ps-**
843   **conjugate)**

Quality/Product Attribute	Method	I*	U*	S*
Potency				
Serotypes 1-4 (correlation)	mAb-based Competitive ELISA (adsorbed)	25	2	50
Animal Model for Type 5	Murine Serology (adsorbed)	25	2	50
Purity (desorbed Ps-VLP)				
Peptidoglycan Level	Calculated	8	3	24
Monomer	Reducing CGE	25	2	50
Complexes/Aggregates	Non-reducing CGE	25	2	50
Product-derived Impurity (desorbed Ps-VLP)				
Complexes/Aggregates	Non-reducing CGE	25	3	75
Process-derived Impurity				
Activation and Conjugation Reactants	Calculated	8	5	40
Structure/Function (Charac.) (adsorbed Ps-VLP unless indicated)				
VLP Structure	Cryo-TEM	8	5	40
Ps/VLP/Adjuvant Ratio	Calculated	8	5	40
VLP Linear and Conformational Epitopes	mAb-based ELISA (desorbed)	8	5	40
Ps Size Distribution	HPSEC-MALLS-RI	25	5	125
Size of Aggregates	DLS (desorbed)	25	5	125
Extent of Conjugation (as Ps-VLP, free Ps, and free VLP)	Reducing CGE	25	3	75
Other				
Quantity (as Protein Content)	Calculated	25	2	50
Quantity (as Ps Content)	Calculated	25	2	50
Fill Volume in Container	Compendial	25	1	25
Endotoxin	Compendial	25	1	25
Completeness of Adsorption (Adsorption to Al)	mAb-based ELISA (adsorbed)	25	5	125
Aluminum Content	ICP or AA	25	1	25

844   * Impact = I, Uncertainty = U, and Severity = S (see Equation 2-1 and Table 2-7).
845
846   It is recognized that use of the risk-ranking tool and the assessment of criticality can be
847   considered a subjective process. To effectively utilize the tool, manufacturers should do their
848   best to consider many types of information and rely on relevant experts in a variety of relevant
849   fields. Thus, the risk assessment is considered a tool to help prioritize efforts during
850   development and highlight risks that should be communicated both internally and to regulatory
851   agencies.

852   It is not anticipated that the risk assessments provide a final decision on the justification of
853   criticality for a product, but rather that the assessments assist in the justification of CQAs
854   selected by a manufacturer. In the end, the manufacturer and regulatory agency will need to
855   agree upon the determined CQAs for a product, so discussions with the agency are
856   recommended to begin early in development.
857
858   Acceptable ranges for a subset of these CQAs were established based on a combination of
859   clinical experience, non-clinical studies, laboratory studies, and prior knowledge. The acceptable
860   ranges were used to establish the boundaries for the design spaces in the Upstream,
861   Downstream, and Drug Product sections of this case study.
862
863   It is important to note that testing for an attribute considered critical for the vaccine drug
864   product may be moved upstream in the process when acceptable business or testing reasons
865   exist to routinely control and monitor the CQA. As an example, the size of the polysaccharides
866   was identified as a CQA since it is important in eliciting an appropriate immune response.
867   However, for analytical reasons, testing for Ps size cannot be performed on the final drug
868   product. Thus, size testing was moved upstream to the first potential chance to test, which is on
869   the activated polysaccharide following size reduction. In addition, residual host-cell protein
870   (HCP) or DNA levels would be evaluated on the drug substance, rather than the drug product,
871   for business-efficiency reasons.
872
873   The overall CQA/risk-assessment workstream and control strategy (as outlined above) that was
874   conducted for A-VAX is summarized in Figure 2-2: CQA/Risk-Assessment Workstream for A-VAX.
875   It includes connections to the phase of clinical development and the "iterative triage" of the
876   CQAs and specifications as new information becomes available. Note that it is expected that a
877   manufacturer will begin with a relatively large number of tests (with broad acceptance ranges)
878   and narrow the number of tests, acceptance ranges, and criticality on the basis of knowledge
879   gained during development.
880

881   **Figure 2-2: CQA/Risk-Assessment Workstream for A-VAX***
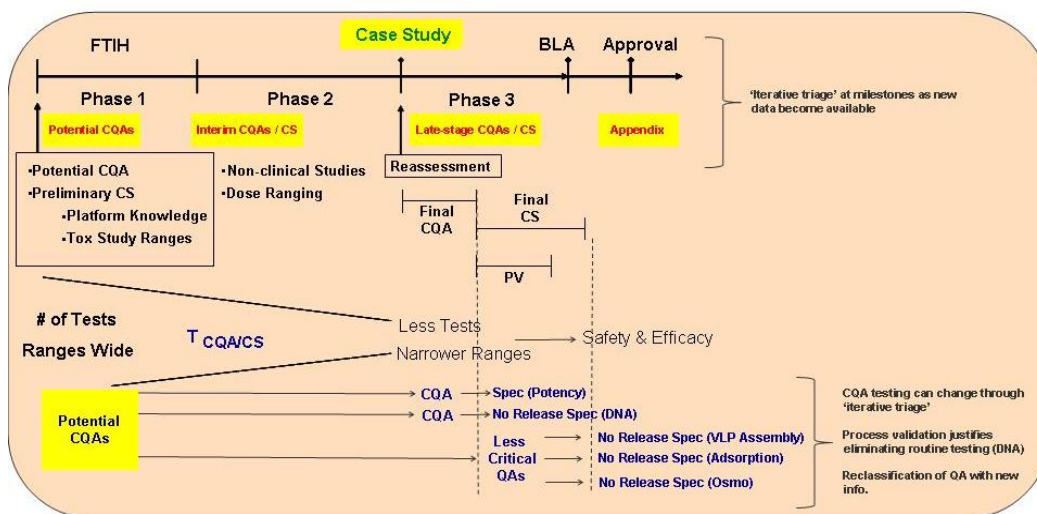
882
883   ***** CQA acceptance criteria generated from existing data – clinical data, non-clinical data, literature, and experience
884   with similar products. The abbreviation CS means control strategy, and $T_{CQA/CS}$ means triage of CQAs via the
885   control strategy.

886 CQAs, risk assessments, and specifications evolve with control strategy input as new information
887 is obtained with increasing biopharmaceutical development, manufacturing, and clinical
888 experience. Abbreviations and details are provided in the Appendix (hyperlink).
889
890 It is essential to document progression of quality attributes through the product's life cycle.
891 Quality attributes that are considered potential CQAs early in development may be further
892 defined as true CQAs later in development.
893
894 However, not all of these CQAs will be release specifications. For example, potency may be part
895 of the release specifications, but residual DNA may not be if the process routinely demonstrates
896 adequate clearance of the impurity, as demonstrated through process validation. Furthermore,
897 a quality attribute (VLP assembly in the example above) may be downgraded from a CQA to a
898 less critical QA during development. In addition, some QAs may be removed from the release
899 specification as they are confirmed to be non-essential for efficacy or safety (adsorption in the
900 example above).
901

902 ## 2.7.   Caveats and Limitations

903 "State-of-the-art" analytical methodology currently in practice is not advanced enough to allow
904 the classification of most vaccine candidates, including the conjugates described here. With
905 further advances in analytical methodology for vaccine candidates, QbD principles may be more
906 readily applied to provide for more meaningful specifications and improved understanding of
907 product design space.

908 # 3.   Control Strategy Section

909 ## 3.1.   Introduction

910 An integrated approach to a control strategy for a vaccine product includes elements which
911 impact both the process and the product. In addition to process and product controls at the
912 point of manufacture, the control strategy should include appropriate consideration of bulk and
913 final product stability, as well as strategies for addressing changes in manufacturing and
914 analytical methods.
915
916 A risk based approach should be taken in developing a vaccine control strategy. This commences
917 from the bottom up, in determining product quality attributes which are related to the safety or
918 efficacy of the vaccine. Also included are attributes which combine to affect those attributes
919 which impact safety or efficacy over the shelf life of the product. Thus while moisture of a
920 lyophilized product has no direct impact on safety and efficacy, it may impact the preservation
921 of potency throughout shelf life.
922
923 In conjunction with process development, preclinical and clinical development may be engaged
924 to explore vaccine quality attributes which may be related to clinical safety and efficacy, and
925 develop experimental plans which facilitate setting of specifications.
926
927 An iterative triage of potential critical quality attributes (CQAs) is undertaken during vaccine
928 development. Depending upon factors such as direct evidence of clinical impact, the ability to
929 manage the level of the CQA through the process, and others, the manufacturer will decide how
930 to incorporate the CQA into the vaccine control strategy. Thus while some CQAs will have
931 release and/or stability specifications (acceptance criteria) others will be managed as part of the
932 routine quality system. Testing of others may be eliminated after successful demonstration of
933 process control during validation.
934
935 Following the identification of attributes which are critical to quality, raw material, equipment,
936 and process factors may be explored to determine control points in the manufacturing process.
937 Prior knowledge combined with strategically designed experiments help identify those
938 parameters which will become a part of the vaccine control strategy, and the control levels
939 which must be maintained to ensure quality.
940
941 Stability studies are performed during development which helps reveal degradation pathways of
942 a vaccine product, which define optimal formulation, packaging, handling and shipping
943 conditions, and support vaccine shelf life. The information collected from development stability
944 studies is also valuable to support post licensure stability monitoring and comparability.
945
946 Given the importance of some vaccine assays, such as potency assays, a strategic approach to
947 analytical method development and maintenance may be undertaken and quality by design
948 principles can be employed during assay development to optimize assay performance. An assay
949 control strategy should utilize similar elements as a process control strategy, such as method

950   quality control, method change protocols and method change control which help ensure
951   continued quality of vaccine measurements.
952
953   The elements of a vaccine control strategy evolve over the course of development. Thus a
954   lifecycle approach should be taken in the development of a vaccine control strategy. This section
955   describes the evolution of the vaccine control strategy from early development when vaccine
956   quality attributes are identified for evaluation, through development studies to
957   establish specifications and process controls, to the final commercial control strategy which
958   will be used help ensure robust supply of safe and effective vaccines are administered to the
959   target population.
960
961   ### 3.1.1. Terminology
962   Wherever possible terminology has been used which is in accordance with regulatory guidance
963   and industry technical reports but new terminology has also been used in this case study to
964   introduce the concept of evolution of attributes throughout the product's life cycle and the
965   continuum of criticality of the attributes. The terminology also introduces the notion of process
966   performance attribute. As stated in the introduction to this case study, this approach is
967   illustrative of one possible approach to definition of terms and companies may or may not
968   adhere to this terminology. Companies should nevertheless consider including concepts related
969   to this terminology in the development practices and in their vaccine control strategy. The
970   terminology used throughout this section and other section of the case study follows.
971
972   **Table 3-1: Control Strategy Terminology**

Terminology	Definition
Quality attribute (QA)	A physical, chemical, biological, or microbiological property or characteristic of the product whose variability might have a potential impact on the safety and efficacy of the product. At early stages of development some of these quality attributes are likely to be equivalent to "potential CQA"
Critical quality attribute (CQA)	A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality - ICH Q8(R2)
Less critical quality attribute (less critical QA)	A quality attribute determined through risk analysis to be less critical to assurance of desired product quality, efficacy and safety.
Acceptance criteria	Numerical limits, ranges, or other suitable measures for acceptance which the drug substance or drug product or materials at other stages of their manufacture should meet to conform with the specification of the results of analytical procedures - ICH Q8(R1)
Performance attribute (PA)	A physical, chemical, biological, or microbiological property or characteristic whose variability might have a potential impact on process performance

Terminology	Definition
Key performance attribute (KPA)	A parameter than when controlled ensures optimal process performance
Critical process parameter (CPP)	A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality – ICH Q8(R1)
Key process parameter (KPP)	An adjustable process parameter (variable) of the process that, when maintained within a narrow range, ensures optimum process performance. A key process parameter does not meaningfully affect critical product quality attributes. Ranges for KPPs are established during process development, and changes to operating ranges will be managed within the Quality System – aMab
Design space	The multidimensional combination and interaction of input variables (eg, material attributes) and process parameters that have been demonstrated to provide assurance of quality – ICH Q8(R1)
Formal experimental design	A structured, organized method for determining the relationship between factors affecting a process and the output of that process. Also known as "Design of Experiments" – ICH Q8(R1)

973

974   ### 3.1.2.   Lifecycle approach to identifying and controlling critical quality attributes

975   **1.   Identification of critical quality attributes**

976   ICH Q1 (R2) defines a critical quality attribute (CQA) as "A physical, chemical, biological or
977   microbiological property or characteristic that should be within an appropriate limit, range, or
978   distribution to ensure the desired product quality." Quality is defined as "The suitability of either
979   a drug substance or a drug product for its intended use. This term includes such attributes as the
980   identity, strength, and purity." Thus vaccine critical quality attributes are properties which are
981   either directly or indirectly related to clinical safety or efficacy of
982   the vaccine.
983
984   A risk analysis is performed early in product development to identify quality attributes (QAs)
985   which may be related to the clinical safety and efficacy of a vaccine and considered as CQAs. The
986   factors which should be considered in earmarking a quality attribute as potentially
987   critical are:
988   1.   Local and worldwide compendial requirements;
989   2.   Pre-clinical data;
990   3.   Clinical experience;
991   4.   Requirements of a downstream process step;
992   5.   Assurance of stability; and
993   6.   Process capability (if known).

994   Prior knowledge as well as scientific understanding of the mechanism of action of the vaccine
995   are used to rank attributes according to impact on clinical safety or efficacy, as well as
996   uncertainty based on the strength of the evidence for a link to safety or efficacy. A threshold is
997   determined to provide guidance as to which CQAs should be further evaluated, to confirm their
998   impact on vaccine quality and as an aid in establishing acceptance criteria wherever relevant.
999
1000   In addition to QAs, performance attributes (PAs) may be identified which are potentially related
1001   to acceptable manufacturing throughput. A risk analysis is performed on the PAs to identify
1002   those which should be within an acceptable limit, range or distribution to ensure effective
1003   process performance and adequate product supply. These attributes are defined as KPAs (e.g.
1004   the viscosity or pH of an upstream material with impact on subsequent purification step, yield).
1005   The manufacturers may decide to include these KPAs in their control strategy.
1006
1007   The following scheme (Figure 3-1) depicts the classification of attributes into KPAs and CQAS.
1008
1009   **Figure 3-1: Classification of attributes into KPAs and CQAs**
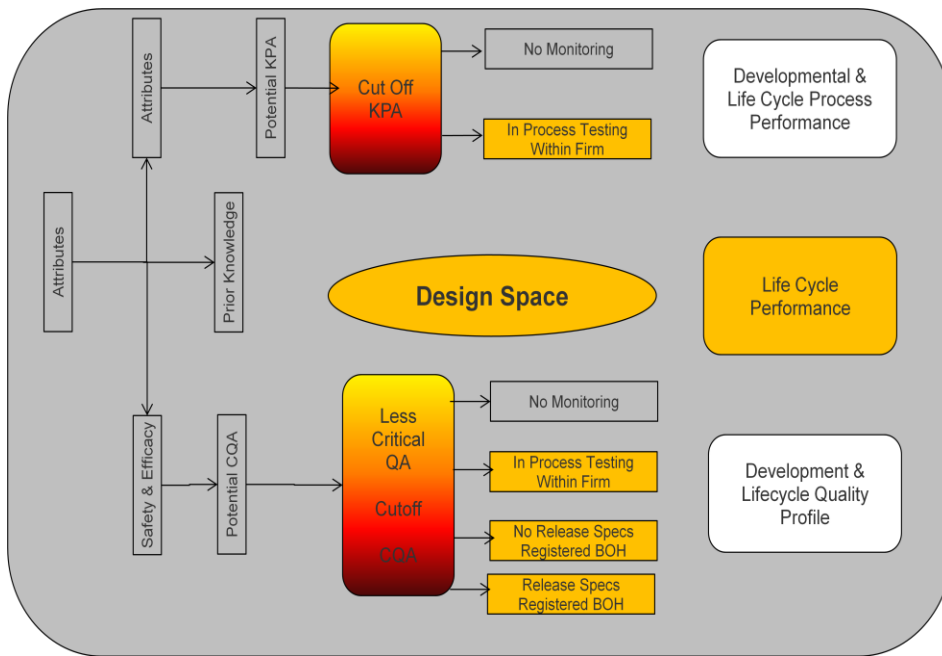
1010
1011
1012   **2.   Framework for identifying critical quality attributes**

1013   The vaccine manufacturer has multiple potential tools for further assessment of the "criticality"
1014   of quality attributes. In some instances, this may include *in vivo* studies in a suitable animal
1015   model. Routine safety assessment is performed on products throughout development, while
1016   vaccine efficacy can sometimes be forecast with the combination of an animal species which is
1017   sensitive to the target immunogen, and a readout which is linked to the vaccine effect. Thus, for
1018   example a murine model might be used in combination with immunogenicity readout to
1019   evaluate the impact of changes in level of a quality attribute. Likewise, *in vitro* systems may
1020   provide valuable information regarding impact on vaccine quality attributes. Infectivity in cell
1021   culture is a classical mechanism for determining changes in potency of formulations which may
1022   differ in their levels of a potentially significant quality attribute.

1023
1024   An additional consideration in the selection and use of an *in vivo* or *in vitro* model to assess
1025   "criticality" of a quality attribute is the variability of the model. The criticality of a quality
1026   attribute might be determined on the basis of changes in pre- clinical (*in vivo* response) or in
1027   clinical with changes in levels of the attribute. The useful model would be capable of detecting
1028   (or excluding) meaningful changes in response against the backdrop of uncertainty associated
1029   with model variability. Thus, experiments should be designed to address uncertainty, and
1030   control the risks associated with decisions made using these models.
1031
1032   The commercial control strategy for the vaccine will include acceptance criteria on critical
1033   quality attributes which help ensure that product is fit "for its intended use." Normal variability
1034   may have negligible impact on safety and efficacy of a vaccine in most quality attributes;
1035   however, excess variability in a critical quality attribute may lead to product, that when released
1036   is unsafe or ineffective. Experiments (*in vivo* or *in vitro*) which attempt to establish "criticality"
1037   should be performed in a range which is indicative of potential quality attribute variability.
1038   Manufacturing modeling can be utilized using mechanistic understanding, planned experiments,
1039   early development experience, and experience with platform technologies to determine the
1040   range of a quality attribute which must be supported in experiments to assess "criticality" of a
1041   quality attribute.
1042
1043   In instances where robust *in vivo* or *in vitro* models are not possible, evidence of immune
1044   responses and process consistency of CQA may be the primary factors considered in
1045   development of an appropriate control strategy.
1046
1047   Thus, some combination of these elements form the framework for a strategy to assess the
1048   "criticality" of quality attributes which have been identified through risk analysis:
1049   •   A sensitive model of product quality, performed *in vivo* or *in vitro*, and using a readout
1050   which forecasts safety or efficacy of the vaccine.
1051   •   A forecast of the range of quality attribute variability based on manufacturing modeling.
1052   •   Adequate model design, to assess "criticality" against the backdrop of model variability.
1053
1054   An experiment showing no impact on *in vivo* or *in vitro* response over a range spanning potential
1055   process capability could lead to either setting acceptance criteria on the basis of manufacturing
1056   variability or declaring the quality attribute as less critical (less critical QA). A quality attribute
1057   showing significant response across the range is a CQA. Acceptance criteria might then be set on
1058   some combination of the basis of "scalability" of laboratory limits or process capability to the
1059   clinical experience and prior knowledge.
1060
1061   **3. Lifecycle of critical quality attributes for A-VAX**
1062   A preliminary control strategy is established prior to first time in humans (FTIH). Potential CQAs
1063   are identified by risk analysis, and preliminary acceptance criteria are established and
1064   challenged in toxicology studies. The resulting list of CQAs, together with their associated tests,
1065   will be continuously evaluated throughout early development. In some cases a test might be
1066   eliminated or a criterion may be refined to reflect the evidence obtained in nonclinical studies,
1067   as well as strategic clinical studies. The total experience throughout Phase 1 and Phase 2 is
1068   utilized to reassess the list of potential CQAs. The list of final CQAs with associated acceptance

1069 criteria is determined prior to process validation and incorporated into the final control strategy.
1070 These limits are re-evaluated and re-defined, if necessary, prior to submission of the Biological
1071 License Application (BLA). On occasion, once new data become available, the CQAs and
1072 criteria will be re-evaluated yet again, as further understanding of the product and process
1073 become available.
1074
1075 A life cycle approach is considered in the framework of the overall clinical and nonclinical
1076 development program. This is depicted in Figure 3-2.
1077
1078 **Figure 3-2: Life cycle approach to management of critical quality attributes**
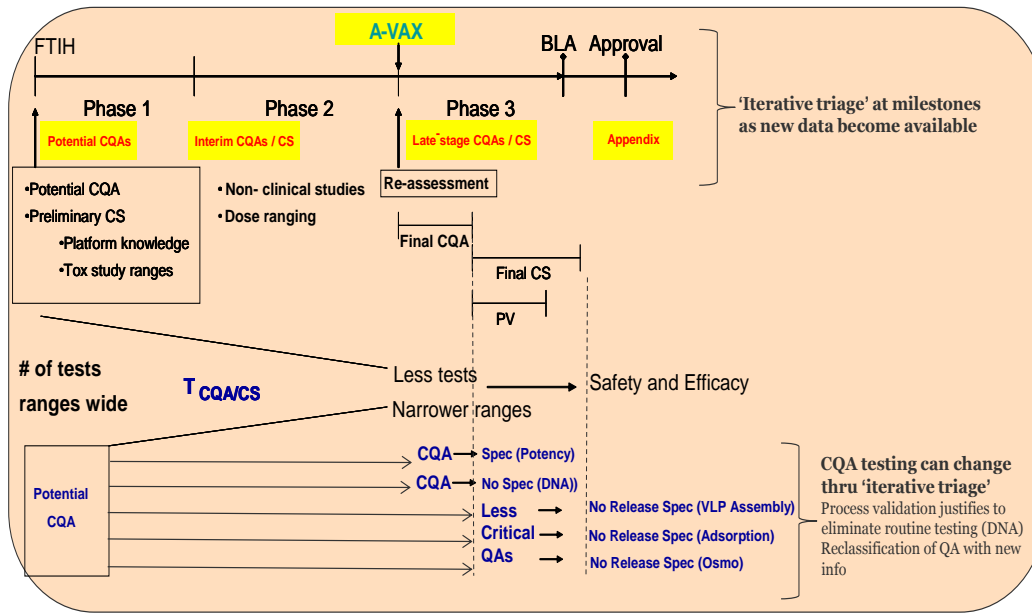
1079
1080
1081 The early risk analysis supporting A-VAX yielded a list of potential CQAs for the drug substances
1082 (PS and VLP), intermediate conjugated bulks (PS+VLP), and final drug product (PS+VLP+Alum).
1083
1084 A subset of potential CQAs and "less critical" QAs from the A-VAX early risk analysis are used to
1085 illustrate the lifecycle approach (Table 3-2).
1086
1087 **Table 3-2: Subset of critical quality attributes and less critical quality attributes from the early**
1088 **risk assessment**

Risk Analysis Category	Quality Attribute	Early Score	Process step	Preliminary Specification
Potential CQA	Potency	50	Ps+VLP and DP	0.5 – 2.00 (rel to ref std)
Potential CQA	Host Cell DNA	32	VLP	<100 ng/dose
Less Critical QA	Fragments	24	Ps+VLP and DP	<10%
Less Critical QA	Osmolality	8	DP	280-350 mOsm/kg

1089

1090   A combination of prior knowledge, and nonclinical and clinical studies were utilized to control
1091   substances throughout development, and to develop a final control strategy for commercial
1092   product.
1093

Potency

1094

1095   During early development, immunogenicity is measured in the conjugated bulk drug substance,
1096   and in the adjuvanted drug product. Potency of early development materials is measured both
1097   in a murine immunogenicity assay, and in a mAb-based competitive ELISA for 4 of the 5
1098   serotypes. An appropriate monoclonal antibody could not be identified for the 5th type which
1099   was tested instead by rate nephelometry with polyclonal antiserum. A standard was introduced
1100   into each assay, to calibrate potencies across time as well as across assays.
1101

1102   Experiments were performed throughout early development to establish a concordance
1103   between the clinically validated *in vivo* murine immunogenicity assay and the *in vitro* assays.
1104   Potency was modified in a series of samples by temperature inactivation, and the modified and
1105   unmodified samples were tested in both assays. Excellent concordance (equivalence of relative
1106   potency across modified levels) was observed between the *in viv*o murine assay and the mAb-
1107   based competitive ELISAs for serotypes 1-4. Concordance could not be established, however,
1108   between the murine assay and the rate nephelometry assay for serotype 5. Testing in both the
1109   *in vivo* and *in vitro* assays was carried forward throughout development.
1110

1111   Manufacturing modeling was used to establish a range of potencies which is forecast to support
1112   commercial product capability. The predicted range drives development to support commercial
1113   release and expiry acceptance criteria. Manufacturing modeling was performed to support the
1114   potency ranges required for A-VAX. The target potency range between maximum and minimum
1115   potencies in an ideal situation is depicted in Figure 3-3.
1116

1117   **Figure 3-3: Minimum and maximum potencies, release potencies, and process capability of A-**
1118   **VAX vaccine**
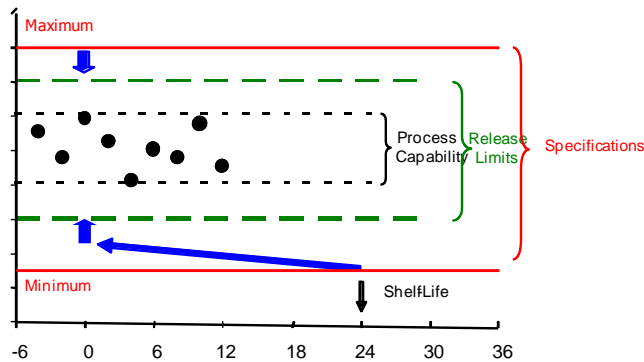
1119
1120

1121   It is recognized that complexity of manufacturing of many vaccines, and the balance required in
1122   setting limits for quality attributes that may be influenced in opposing ways by a specific change
1123   in process parameters, may result in relatively few situations where this ideal situation of
1124   release limits significantly wider than processs capability and comfortably nested within legal
1125   specifications. Routine manufacturing data for a licensed product which is manufactured and
1126   controlled similarly to A-VAX was obtained to forecast process capability of A-VAX. Accelerated
1127   stability studies show that A-VAX has similar stability as the licensed vaccine. The range in

1128   maximum to minimum potencies was determined through a process capability analysis (See
1129   Formula in Annex 1).

1130

1131   The final commercial lot control strategy for potency was based on the compiled experience
1132   throughout development. Based upon the excellent concordance observed between the *in vivo*
1133   murine immunogenicity assay and the *in vitro* mAb-based competitive ELISAs for the 4
1134   serotypes, and due to the ethical implications of using experimental laboratory animals in
1135   routine batch release, *in vitro* potency testing will be performed for commercial materials for
1136   these types. The *in vivo* assays will only be used as characterization assays to support major
1137   process and facility changes. Potency testing for the 5th type will be carried using the rate
1138   nephelometry testing out on every lot as part of the in-house management system. Due to the
1139   use of state-of-the-art production processes and intensive in-process monitoring of both process
1140   parameters and quality attributes through the use of state-of-the-art analytical tools and of
1141   strict quality systems such as GMP and QA, once confidence in the consistency of the production
1142   process has been demonstrated through validation of every step of the manufacturing process,
1143   the murine *in vivo* test will be omitted and replaced by the rate nephelometry test for routine
1144   commercial release . The final control strategy for potency of the vaccine is summarized in Table
1145   3-3.

1146

1147   **Table 3-3: Final Control Strategy for potency of A-VAX**

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	Ps+VLP and DP	All	All	0.50 – 2.00
Final CS	CQA	Ps+VLP	A-VAX$_1$-A-VAX$_4$	Release	0.77 – 1.30
			A-VAX$_1$-A-VAX$_4$	Expiry	0.50
			A-VAX$_5$	Release	0.50 – 2.00
			A-VAX$_5$	Expiry	0.35

1148

1149   **Host cell DNA**

1150   Host cell DNA is an impurity that originates from fermentation of X. horrificus (polysaccharides)
1151   and E. coli (VLP). Each polysaccharide serotype is purified by a series of chemical and physical
1152   methods, while the VLP is purified by a series of physical methods only. Host cell DNA was
1153   identified as a potential CQA in an early risk analysis due to a combination of a moderate impact
1154   score, and high uncertainty of the impact.

1155

1156   Based on this, downstream process development was undertaken to remove host cell DNA.
1157   Process development was successful in that spiking experiments were performed at small scale
1158   demonstrate the removal of host cell DNA to levels below the limit of detection of the assay.
1159   Continued testing of small and large scale batches, including process validation batches
1160   manufactured at commercial scale, showed successful clearance of even high levels of
1161   the residual.

1162

1163   Based on the implementation of a purification process which was demonstrated to successfully
1164   eliminate host cell DNA from purified batches of VLP and polysaccharides, the specification on

1165   host cell DNA was eliminated. In the control strategy VLP will to be tested for host cell DNA in
1166   process validation batches to verify clearance at manufacturing scale. However, in the final
1167   control strategy, the test will be eliminated as a routine test following demonstration of
1168   clearance during process validation. Host cell DNA testing will be used to characterize major
1169   process and facility changes thereafter.
1170
1171   **Table 3-4: Final control strategy for host cell DNA**

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	VLP	All	Release	<100 ng/dose
Final CS	CQA	VLP	All	Not required	Non detectable[*]

[*] Release testing eliminated after confirmation of clearance during process validation and small scale spiking experiments

1172

1173   **Fragments**

1174   VLP fragments were identified as a less critical quality attribute due to uncertainty in the impact
1175   of a high level of unassembled fragments. Percent of unassembled fragments was judged a
1176   potential efficacy concern, and was not believed to be a potential safety concern.
1177

1178   Phase 1 clinical studies were performed with materials with high amounts of unassembled
1179   fragments. Further development of the VLP process resulted in considerable improvement in
1180   the assembly process, resulting in an insignificant residual of unassembled fragments. Clinical
1181   studies performed with VLP materials with fully assembled particles yielded similar responses as
1182   early development experience with high levels of unassembled fragments.
1183

1184   On the basis of the lack of impact of unassembled fragments on clinical response, and a robust
1185   final reassembly process, the final control strategy does not include a specification for
1186   fragments. However, data will continue to be reported and maintained in the quality system as a
1187   means to evaluate excursions in the level of fragments during commercial manufacturing.
1188

1189   **Table 3-5: Final control strategy for fragments**

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Less Critical QA	VLP & Ps	All	Release	<10%
Final CS	Less critical QA	VLP	All	Report	NA

1190

1191   **Free polysaccharide**

1192   The level of free polysaccharide after conjugation was identified as a potential CQA. Drug
1193   product development was able to achieve >80% conjugation in early small scale formulations of
1194   the vaccine. Similar high levels of conjugation were sustained throughout development, and into
1195   process validation lots (>90% conjugation in full scale PV lots).

1196
1197   While published literature shows a negligible impact due to lower conjugation in animal studies
1198   for a similar vaccine utilizing materials which were artificially formulated to span 20% to 95%
1199   conjugation, prior knowledge with other similar vaccines indicates an impact at higher % free
1200   polysaccharide levels. Animal studies were therefore performed in a similar manner as
1201   described in the literature, on artificially formulated batches of the A-VAX polysaccharide
1202   conjugates with levels of 5-40% free polysaccharide and only a modest effect over this range
1203   was observed with immunogenicity endpoints met in each instance.
1204
1205   On the basis of the prior knowledge and confirmation of a modest effect over the expected
1206   range defined by the conjugation properties of A-VAX extent of conjugation by reducing CGE
1207   was retained as a release test in the commercial lot control strategy. The final control strategy
1208   does include a specification for % free polysaccharide. However, it is based upon the broadest
1209   ranges demonstrated to generate an adequate immune response. Additionally, data will
1210   continue to be reviewed in the quality system against tighter internal limits as a means to
1211   evaluate excursions during commercial manufacturing.
1212
1213   **Table 3-6: Final control strategy for free polysaccharide**

Stage	Risk Analysis Category	Process Component	Serotype	Test	Specification
Early	Potential CQA	DP	All	Release	<=20%
Final CS	CQA	DP	All	Release	<=40%

1214
1215   **Osmolality**
1216   The osmolality of the final adjuvanted vaccine was identified as a less critical QA due to
1217   publications identified early in development that show no impact on local tolerance or pain at
1218   the vaccine injection site, in addition to the small volume of A-VAX administration (0.5mL)
1219   versus other products administered by IV infusion.
1220
1221   The final adjuvanted drug product vaccine was tested for osmolality during development and
1222   results were consistently within the range of 280-350 mOsm/kg water, which is similar to the
1223   osmolality of serum.
1224
1225   On the basis this information, osmolality was classified as a less critical QA in early development
1226   and later eliminated from the specification and testing strategy for commercial manufacturing.
1227
1228   **Table 3-7: Final control strategy for osmolality**

Stage	Risk Analysis Category	Process Component	Test	Specification
Early	Less Critical QA	Adjuvanted DP	Report	NA
Final CS	Less Critical QA	Adjuvanted DP	Not Required	NA

1229

1230 ### 3.1.3.   Specifications versus control limits on quality attributes

1231 Specifications (acceptance criteria) should be contrasted with control limits, which are typically
1232 based on process performance and used to monitor a manufacturing process for potential shifts
1233 and trends in a quality attribute, as described above for %FS, where both types of limits are
1234 utilized. While the manufacturer may set acceptance criteria based on process performance,
1235 there are several advantages for considering alternatives.
1236
1237 Key among the advantages is the opportunity to develop a more flexible control strategy, which
1238 is responsive to both manufacturing drift as well as quality excursions. Using control limits as
1239 specifications may hinder a manufacturer's ability to monitor product and to make process
1240 improvements. This was highlighted in a PhRMA paper on *A Rational Approach for Setting and*
1241 *Maintaining Specifications for Biological and Biotechnology-Derived Products*. Separating
1242 specifications from control limits provides protection to the patient from receiving a product
1243 which is not fit for use, and protection for the manufacturer of potentially discarding acceptable
1244 product.
1245
1246 Furthermore, manufacturing flexibility and even improvement is difficult to achieve when
1247 specifications are based primarily on normal manufacturing variability. The experimental
1248 paradigm for defining the "design space" for a manufacturing process is the intersection of
1249 responses across a range of process parameters, with the product acceptance criteria. A design
1250 space which has been constrained by the normal performance of the process is the normal
1251 operating ranges of the process. Thus there is no opportunity to move outside the normal
1252 operating range, and thus limited opportunity to change or improve the process without
1253 significant effort.
1254
1255 When acceptance criteria are based upon normal manufacturing variability, special
1256 consideration should be given the risks associated with the proposed limits. Inherent in the
1257 approach are the following considerations:
1258
1259 1. The only risk which can be controlled using limits based on manufacturing variability is the
1260   manufacturer's risk of an out of specification (OOS) result.
1261 2. The risk of a product batch failure is the compound risk of not meeting one or more of the
1262   batch acceptance criteria.
1263 3. The manufacturer's risk can be controlled through consideration of the number of
1264   batches utilized to calculate the process limits, and the maturity of the process including
1265   normal process events such as variation in raw material inputs as well as other
1266   operational parameters.
1267
1268 Based upon these considerations, the manufacturer must develop a strategy for setting
1269 acceptance criteria which provides an adequate system of control, while assuring satisfactory
1270 product supply.
1271

1272   ## 3.2.   Framework for identifying critical process parameters, and definition
1273   of design space

1274   A key element of the vaccine control strategy is management of critical process parameters. ICH
1275   Q8(R2) defines a critical process parameter (CPP) as "A process parameter whose variability has
1276   an impact on a critical quality attribute and therefore should be monitored or controlled to
1277   ensure the process produces the desired quality." Additionally, key process parameters (KPPs)
1278   which do not meaningfully affect critical quality attributes but ensure optimum process
1279   performance are identified during development. CPPs and KPPs are identified through a
1280   process of risk analysis, followed by univariate or multivariate experiments. Subsequent
1281   experiments may be performed on confirmed CPPs and KPPs to define the "design space" for
1282   the process step.

1283
1284   As noted in the ICH definition, key to the identification of critical process parameters is their
1285   association with critical quality attributes and their acceptance criteria. In fact acceptance
1286   criteria are the basis for development of a control strategy across process steps.

1287
1288   The vaccine process can be conceptualized as a series of contiguous unit operations. The major
1289   operations are: (1) upstream synthesis of the API; (2) downstream purification; and (3) drug
1290   product formulation. Each of these may have multiple steps or sub-processes. Thus purification
1291   may be a series of steps, each expected to purify away one or several components of the input
1292   material. A schematic of the overall process might be depicted in Figure 3-4.

1293
1294   **Figure 3-4: Schematic of overall A-VAX process**
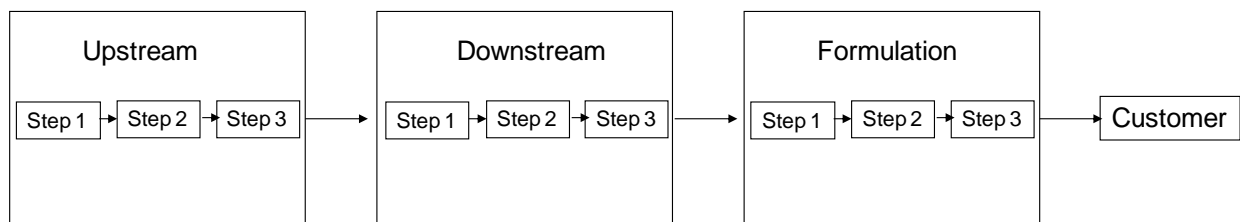
1295
1296
1297   In this scheme the arrows represent the quality attributes which are known to impact a
1298   subsequent step in the process. These may affect the next immediate step, or a step further
1299   downstream in the process. For simplicity these are shown as impacting the next immediate
1300   step. Limits on a quality attribute which ensure satisfactory performance in a step are an
1301   acceptance criterion that must be met by the previous step. Thus step k must output product
1302   with a quality attribute which meets specifications on the attribute defined by step k+1.

1303
1304   With such linkages between process steps and unit operations, it's possible to establish the
1305   design space for each process step. The design space is the "established range of process
1306   parameters that has been demonstrated to provide assurance of quality." Said otherwise, the
1307   design space for a process step is the ranges on critical process parameters which have been
1308   demonstrated to deliver output with quality attributes which meet the acceptance criteria
1309   defined by subsequent steps of the process.

1310

1311   The course of demonstrating satisfactory performance begins with a risk analysis of the process
1312   factors. That risk analysis can be carried out in a number of ways, and may use various sources
1313   of process information. It should begin, however, with a thorough understanding of the factors
1314   that could impact the process. A process map might be developed utilizing a "fishbone" or
1315   cause-and-effect diagram (Figure 3-5).
1316
1317   **Figure 3-5: Example of a process map (fishbone or Ishikawa diagram)**
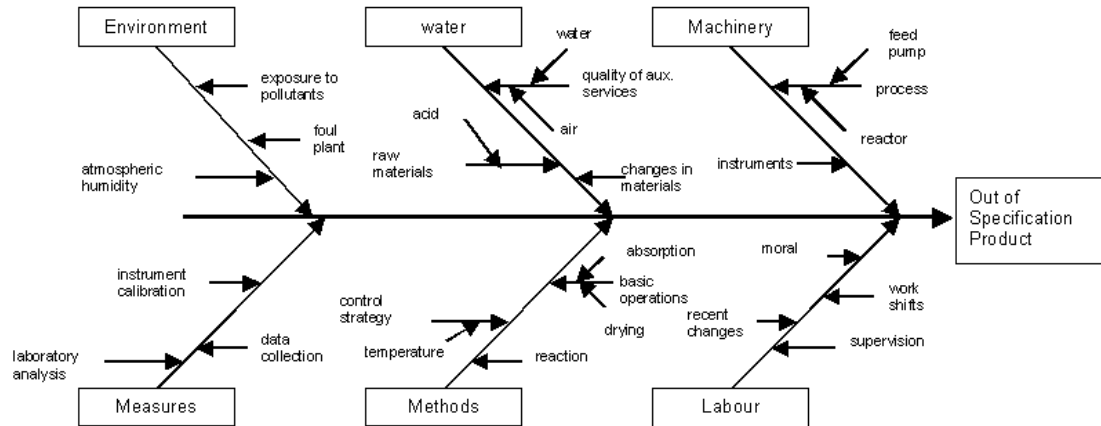
1318
1319
1320   Scientific understanding and historical information can be utilized to eliminate or select process
1321   parameters which may impact the quality attributes that have been identified to be important
1322   to a subsequent process step. One tool that is useful for documenting factor risks is Cause and
1323   Effects analysis, which scores process parameters and quality attributes in a matrix fashion. A
1324   rigorous scoring system utilizes mechanistic or empirical understanding of the parameter or the
1325   attribute, prior knowledge from other vaccine programs which follow a similar process, or early
1326   development experience with the process. A thorough analysis of the matrix scores, including a
1327   scientifically justifiable threshold will earmark factors which should be studied in subsequent
1328   development.
1329
1330   Process factors which have been identified by risk analysis to have a potential impact on
1331   subsequent process steps may be studied using multifactor design of experiments (DOE). The
1332   purpose of early studies are to "screen" out factors which have limited or no impact on a
1333   process step, and identify potential critical process parameters (CPPs) for further evaluation.
1334   DOE has the advantages over traditional "one-factor-at-a-time" (OFAT) experiments of being
1335   more efficient as well as more effective than OFAT strategies. DOE is more efficient in (1)
1336   requiring fewer numbers of experimental runs, and (2) in covering a broader "knowledge space"
1337   than OFAT experimentation. It is more effective in (1) addressing potential interactions among
1338   process factors, (2) in addressing artifacts such as experimental clustering and run order through
1339   randomization, and (3) in making use of "hidden replication," and thus in having better
1340   sensitivity for detecting important effects due to process factors or interactions.
1341
1342   For screening purposes, highly fractionated designs can be used to screen large numbers of
1343   factors simultaneously. Care must be taken to use sound scientific justification for the selection
1344   of a design, as highly fractionated designs lose their resolution to identify interactions among
1345   process factors. Thus scientific judgment and prior knowledge should be utilized to select a

1346   design which preserves the ability to discover significant factors and potential interactions. The
1347   levels which are set for the factors should also be varied according to sound scientific and
1348   statistical principles. These should vary far enough outside the expected normal operating range
1349   of the factor to establish an impact, if present, and thus help guide the future control strategy as
1350   necessary.
1351
1352   An additional consideration in design of a screening study is the approach which will be taken to
1353   identify "significant" effects (factors and interactions). Some approaches use statistical graphics,
1354   such as Pareto plots or normal plots (Figure 3-6), to highlight "unusual" effects.
1355
1356   **Figure 3-6: Pareto plot and half-normal plot for experimental effects**
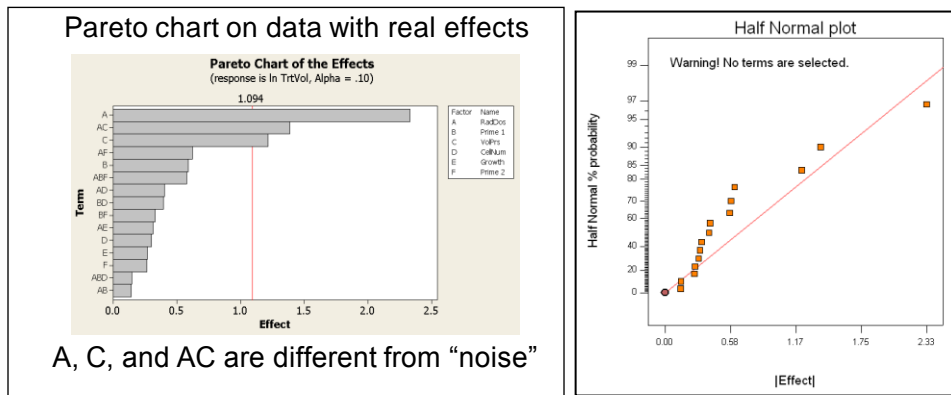
1357
1358
1359   A more rigorous statistical approach involves determining the P-value for effects which are
1360   estimated from the statistical model (ANOVA approach), or estimating the effects and declaring
1361   the effect non-significant if the estimate or a confidence interval on the effect falls within some
1362   margin which is determined to be an important variation in a quality attribute.
1363
1364   Both approaches require some consideration of the number of experimental runs which will
1365   need to be performed to mitigate study risks. There are two types of risks associated with factor
1366   screening: (1) the risk of missing a potentially important factor; and (2) the risk of detecting a
1367   practically insignificant factor. Screening should err on the side of minimizing the risk of missing
1368   an important factor which should be controlled to ensure acceptable process performance.
1369   Statistical support of these considerations should be sought to properly balance the risks against
1370   the number of runs which will be performed in the study.
1371
1372   Continuous process verification is another resource for identifying critical process parameters.
1373   While all parameters may not be evaluated in development studies, some of these may assert
1374   influence during routine manufacture. For example if the process monitoring shows that a
1375   quality attribute is OOT yet all of the identified CPPs are within their control ranges, then there
1376   most likely is a parameter not identified as critical that has a significant impact on the process.
1377   An investigation may reveal additional process parameters which must be controlled to ensure
1378   product quality and optimal process performance.
1379
1380   Those process parameters which have been identified in screening experiments to have impact
1381   on one or more quality attributes may be further studied using enhanced experimental designs,
1382   such as response surface designs. Response surface designs are carried out to derive a

1383 mathematical model of the responses in a quality attribute with changes in the process
1384 parameters. These are approximations to the true mathematic relationships. Mechanistic
1385 modeling can also be utilized when the relationship is known.
1386
1387 The mathematical model which is derived from DOE can be used together with acceptance
1388 criteria on the measured attributes to define the design space for the process step. This is
1389 depicted in Figure 3-7. Two process parameters (X1 and X2) are studied across the knowledge
1390 space defined by the multifactor DOE and yield a response surface in a critical quality attribute
1391 (Panel 1). The response surface intersects the lower (Panel 2) and upper (Panel 3) specification
1392 limits (USL and LSL) for a subsequent process step to yield its design space (Panel 4). The control
1393 space represents the normal operating ranges for the factors, falling well within the design
1394 space (Panel 5). Operating within this control space will yields quality attribute measurements
1395 falling within the upper and lower control limits (UCL and LCL in Panel 6). Since LCL and UCL fall
1396 well within LSL and USL, the process step is predicted to be highly capable of delivering product
1397 which meets the requirements of subsequent steps in the process.
1398
1399 **Figure 3-7: Schematic illustrating determination of design space**
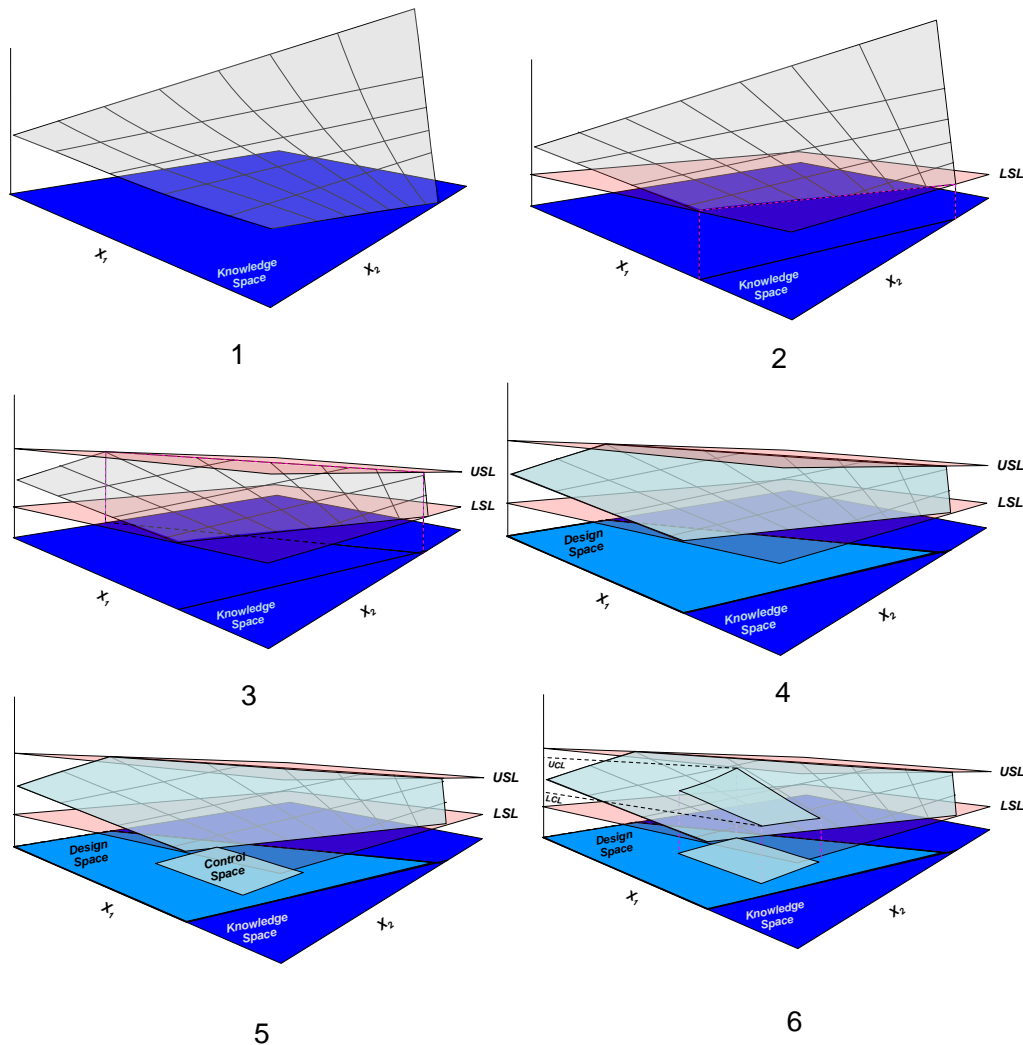
1400
1401

1402   Excursions outside the control space are predicted to deliver product with quality attributes
1403   which fall within the specification limits for the next step of the process, as long as the operating
1404   parameters are held to limits defined by the design space.
1405
1406   A risk based approach may be taken in the definition of design space. Mathematical modeling
1407   can be used together with simulations, to forecast the probability of out-of-specification (OOS)
1408   results within the experimental region. An example of a design space defined through the
1409   probability of OOS is illustrated in Figure 3-8.
1410
1411   **Figure 3-8: 3-D and contour plots of experimental results for enzyme kinetics**
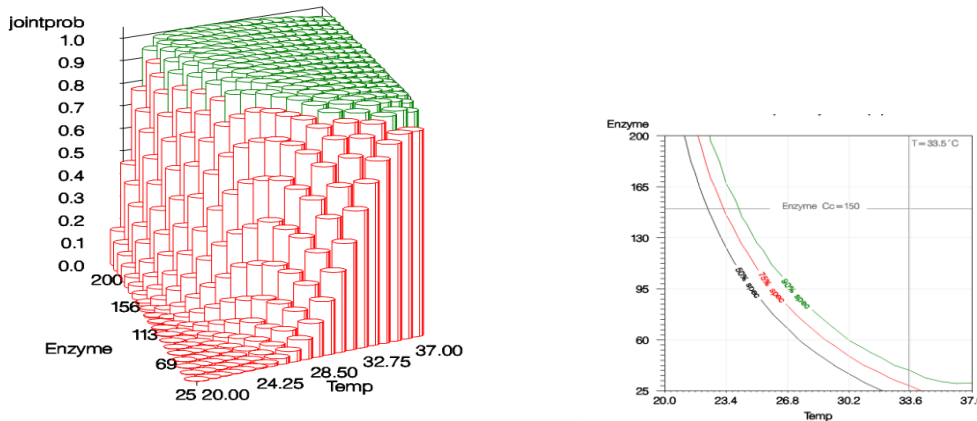
1412
1413   Here the region where the joint probability of an OOS among multiple quality attributes is
1414   depicted in green in the tower plot, and shown together with regions of 75% and 50%
1415   probability in the associated contour plot. The contour plots are useful to assess the "steepness"
1416   of the region associated with acceptable capability.
1417
1418   One consideration in applying this approach to definition of design space is the following. The
1419   design space defined by placing a limit on the probability of an OOS result provides protection to
1420   the manufacturer (or an upstream process step) of failing to meet the acceptance criterion for a
1421   quality attribute. Adequate protection should be built into the acceptance criterion to protect
1422   the customer (or the downstream step) of receiving material which has unacceptable quality.
1423
1424

1425   The design space for a process has traditionally been reported as a set of ranges on the relevant
1426   CPPs. Issues related to using ranges are the following:
1427
1428   1.   ICH Q8(R2) has depicted the ranges based on an inscribed rectangle within the design space.
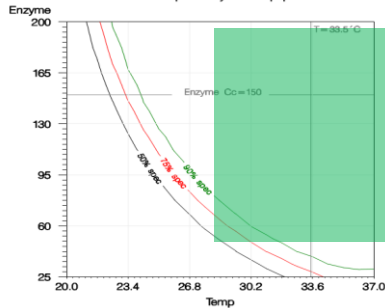
1429
1430
1431   This has the advantage of ensuring product quality within the design space (here >90%
1432   process capability). However, it is conservative because it doesn't capture the entire design
1433   space. In addition, here is no unique solution as an infinite number of rectangles can be
1434   inscribed in the non-rectangular region.
1435
1436   2.   Manufacturers might set the limits of design space to the extremes of the CPP ranges.
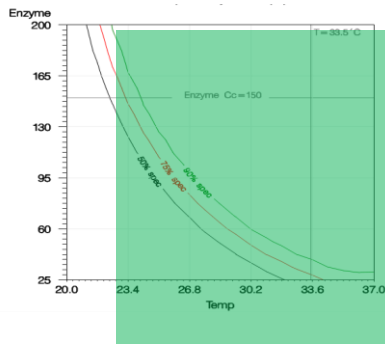
1437
1438
1439   This generates a larger design space, but the probability of OOS ranges from >90% to <50%
1440   across the ranges.
1441
1442   Based on these limitations, design space should not be defined using ranges. Design space
1443   might be reported as a multivariate function of CPPs, or more reasonably as an algorithm
1444   which is maintained as part of the control strategy for the product.
1445
1446   The design space for a manufacturing step need not be defined as limits on process
1447   parameters which ensure satisfactory performance (i.e., ensure specifications are met). This
1448   might be called the "edge of failure" approach. Alternatively experiments may be performed
1449   at ranges of process parameters that the manufacturer is comfortable can be maintained, to
1450   demonstrate "robustness"of the process step across these ranges.
1451
1452

3.3. Manufacturing Control Strategy

Once the Critical Quality Attributes and Critical Process Parameters have been identified a control strategy must be put in place to ensure the process meets each of the elements of control. That strategy will be comprised of:

- Input Materials Controls

  − Input materials can have significant effects on a manufacturing process. Challenges such as undefined media components to subtle vendor changes must be managed via risk assessment and mitigation.

- Process Controls which include

  − Procedural controls

    A comprehensive set of facility, equipment and quality system controls which result in robust and reproducible operations supporting the production of product of the appropriate quality. These controls are supported by a quality risk management system.

  − Process parameter controls

    Critical process parameters that are linked to Critical Quality Attributes (CQAs) that when controlled within the limits of the design space ensure product quality. Key process parameters that are linked to Key performance Attributes (KPAs) that when controlled within the limits of the design space ensure product consistency. The control strategy during A-VAX manufacture will include the identification of CPPs and KPPs. The parameters will require process controls to ensure they remain in the limits identified to ensure the overall process meets its CQA and KPAs. The identification of the process controls is an evolutionary process developed using risk assessment and DOE.

  − Process development: During process development a preliminary list of CQAs has been developed to meet the requirements of the TPP. From these CQAs a process would be developed to produce a product that meets requirements. This process will be developed with little process variability in mind. Process parameters will be identified through the use of prior knowledge, literature searches and pilot lots. These same methods will be used to identify set points that each of the parameters will be run at during the development process. At this point, we are looking to develop a process that will produce a product that meets the TPP and the preliminary CQAs but not concerned with understanding the inherent variability of the process.

  − Process Characterization:Once a process has been identified and proven to meet the product CQAs a second risk assessment will be performed to identify those parameters that truly have an effect on the CQAs. Here the first attempt to define the ranges for the CPPs will be performed. If this step is performed with prior knowledge techniques only, the CPPs and their ranges will be identified using prior experience with similar products, previously published experimentation and scientific knowledge. The use of Design of Experiment techniques will identify CPPs that influence the CQAs as main affects and if the proper techniques are used interactions can be identified. If no interactions are identified the ranges used during the DOE exercises will be used as the ranges for the process. If interactions are identified then Response Surface Modeling DOE techniques should be used to identify the extent of the interactions and also set the ranges for the

1497   CPPs. Those parameters that are not identified as CPPs might not be included in the
1498   control strategy.

1499

1500 • Test Controls

1501   As part of a comprehensive approach to the control and verification that the process can
1502   produce product that meets the assigned CQAs a testing strategy is employed to verify that
1503   the process and procedural controls performed as expected. The control strategy includes:

1504   − In-process testing

1505   o Measurements typically conducted using analytical test methods or functionality
1506   tests to ensure that selected manufacturing operations are performing satisfactorily
1507   to achieve the intended product quality.

1508   − Specifications (release testing)

1509   o Tests with associated acceptance criteria conducted at final lot release on a set of
1510   quality attributes to confirm quality of drug substance for forward processing and
1511   drug product for distribution.

1512   − Characterization or comparability testing

1513   o Testing of certain attributes outside of lot release testing for the purposes of
1514   demonstration of comparability. A specific testing plan would be developed based
1515   on risk to product quality.

1516   − Process monitoring

1517   o Testing or evaluation of selected attributes and/or parameters to trend product
1518   quality or process performance within the design space and/or to enhance
1519   confidence in an attribute's normal distribution. The frequency of monitoring is
1520   periodically reviewed and adjusted based on trends. The process monitoring
1521   program may include limits for evaluating data trends.

1522

1523 • Continuous Process Verification (Process Monitoring)

1524   − The control strategy approach to this point has been focused on developing a process
1525   that will produce product that meets the predetermined CQAs and KPAs utilizing
1526   parameters identified as critical. This identification is based on risk assessments,
1527   univariate and multivariate experimentation and validation performed in process
1528   development. Using multivariate and univariate statistical process control, data
1529   generated during the manufacturing process will be evaluated to verify that the most
1530   influential parameters were chosen to control the process and to also identify
1531   manufacturing trends. The set of parameters that constitutes the quality product profile
1532   is routinely monitored to ensure consistency of the manufacturing process.

1533

1534 3.3.1.   A-VAX Process Controls

1535 Process control and control of material inputs are both elements of a robust control strategy for
1536 the manufacture of A-VAX. In characterizing the process through a combination of risk
1537 assessments and the resulting multivariate and univariate experimental designs, the CPPs that
1538 control the CQAs and KPAs are identified. For the limited set of CQAs discussed in this case
1539 study, the correlation between the CQAs and KPAS and the CPPs and KPPs is given below.

1540   Control of material inputs (either directly through knowledge of first principles or deduced from
1541   observed correlations) can be assessed in a similar manner to process parameters.
1542
1543   Three attributes were studied in the fermentation of the polysaccharide: number of unitrepeats,
1544   polysaccharide length and percent lysis, which influences the polysaccharide length. A risk
1545   assessment identified four variables that have potentially significant effects on these CQAs: the
1546   concentrations of raw material 1 (RM #1) and characteristics of raw material 2 (RM#2) as a
1547   material input, as well as time to inactivation and incubation temperature. The number of unit
1548   repeats was influenced by RM #2. The percent lysis was influenced by time to inactivation and
1549   incubation temperature.
1550
1551   Five attributes, purity (as measured by DNA, protein and lipids), SDS-PAGE profile and percent
1552   monomer were studied for the manufacture of VLP. A preliminary risk assessment determined
1553   that the quality attributes chosen for study were predominantly affected in the primary
1554   recovery of the VLP. A second risk assessment identified nine parameters in primary recovery as
1555   potentially critical. After an initial screening DOE, four parameters were identified for further
1556   study: homogenization pressure, pass number, temperature and time of solubilization. The data
1557   for the upstream operations is given in 9.
1558
1559   **Table 3-8: CQA/CPP Correlation for Upstream Operations**

	PS Fermentation	VLP Primary Recovery
CQAs to Control	1. Number of unit repeats 2. Percent Lysis	1. Purity (DNA, protein, lipid) 2. SDS-PAGE profile 3. % Monomer
CPPs Identified	1. RM #2[1] 2. RM #2, Time to inactivation and incubation temperature	Data for CPP vs CQAs to be collected post licensure and control strategy updated

1560
1561   Downstream operations were also studied using risk assessments in conjunction with
1562   multivariate and univariate experimental designs. Three downstream steps of the manufacture
1563   were studied: PS extraction, PS activation and PS/VLP conjugation.
1564
1565   The risk assessment process identified temperature, pH and horrificase concentration as
1566   potential CPPs for the extractions step. The QAs and CQAs measured were PS size, O-acetyl
1567   content and residual peptidoglycan content. Both residual peptidoglycan content and PS size
1568   were significantly affected by the temperature and pH, but none of the three operating
1569   parameters affected the O-acetyl content.
1570

---

[1] RM 2 as a material input is treated in a manner equivalent to a CPP, though strictly speaking it is not a process parameter, though individual attributes of the material act to influence the process much as a process parameter does.

1571   Time, pH and PS concentration were similarly identified as potential CPPs for the activation step.
1572   Quality attributes measured were reducing activity, PS size and O-acetyl content. These
1573   attributes are not necessarily CQAs but are required to ensure successful conjugation to the VLP.
1574   A screening DOE revealed that temperature, over the range studied, had no effect on the quality
1575   attributes. However, time, pH and PS concentration were observed to have effects on the three
1576   quality attributes. The PS size was also measured at line by HPSEC HPLC to ensure the size was
1577   less than 15,000 kD. This is a true in-process test.
1578
1579   DAPS and VLP concentrations, temperature, agitation during VLP addition, NaCNBH$_4$
1580   concentration and time were identified as potential CPPs for conjugation. The CQAs measured
1581   were free PS, VS/VLP ratio and PS/VLP size. Only the DAPS and VLP concentrations had a
1582   significant impact, over the ranges studied, on the measured CQAs. The results for each of these
1583   three downstream operations are summarized in Table 3-10.
1584
1585   **Table 3-9: CQA/CPP Correlation for Downstream Operations**

	PS Extraction	PS Activation	PS/VLP Conjugation
CQAs to Control	1. Residual peptidoglycan content 2. PS size 3. O-Acetyl content	1. Reducing activity 2. PS size 3. O-Acetyl content	1. Free PS 2. PS/VLP ratio 3. PS/VLP size
CPPs Identified	1. Temperature, pH 2. Temperature, pH 3. No effect of Temperature, pH or enzyme concentration	1. pH, Time, PS concentration 2. pH 3. Time, PS concentration	1. DAPS concentration 2. No effect of parameters studied 3. DAPS concentration, VLP concentration

1586
1587   Two process steps, drug product formulation and lyophilization, were addressed in this
1588   case study. Again, extensive use was made of risk assessments to aid in the design of
1589   multivariate experiments.
1590
1591   In the first set of experiments, the excipients, sucrose and NaCl, along with pH and AlPO$_4$ were
1592   varied to determine the effects in binding of the PS/VLP to the aluminum adjuvant. Sucrose, pH
1593   and NaCl concentrations had significant impact on the binding of the five PS/VLP serotypes to
1594   the adjuvant. In the second set of experiments, the concentrations of excipients sucrose,
1595   histidine and polysorbate 80 were varied and the formulated PS/VLP containing all five
1596   serotypes was lyophilized under standard conditions. No significant effects of the excipients
1597   were observed on the VS/VLP binding, moisture content or reconstitution time.
1598
1599   Next, the lyophilization conditions were studied with the standard formulation. The parameters
1600   varied were sucrose concentration, chamber pressure, primary drying shelf temperature, shelf
1601   temperature ramp rate, secondary drying shelf temperature and secondary drying duration.

1602   Moisture of the cake, reconstitution time and potency were measured. The moisture level was
1603   impacted by the sucrose concentration, shelf temperature ramp rate and the secondary drying
1604   temperature and time, and the reconstitution time was impacted by the secondary drying
1605   temperature and time. None of the parameters had impact on potency or cake appearance. The
1606   results for each of these three downstream operations are summarized in Table 3-11.
1607
1608   **Table 3-10: CQA/CPP for Drug Product Operations**

	Formulation	**Lyophilization**
CQAs to Control	1. PS-VLP Binding 2. Moisture 3. Reconstitution time	1. Moisture 2. Reconstitution time 3. Potency 4. Cake appearance
CPPs Identified	1. pH, sucrose and NaCl 2. No effect of excipients 3. No effect of excipients	1. Sucrose, Shelf temperature ramp rate, SD temperature, SD time 2. SD temperature and SD time 3. No significant effects of the parameters studied 4. No significant effects of the parameters studied

1609
1610   **Test Control**
1611
1612   1. The control strategy during A-VAX manufacture includes raw material testing, in-process
1613   testing, intermediate polysaccharides (Ps) and virus-like particle (VLP) acceptance testing as
1614   well as drug substance and drug product release testing. Raw material testing is discussed in
1615   Section X.X.X. In-process tests have been developed for fermentation operations as well as
1616   for the downstream and conjugation processes.
1617   2. The testing component of the integrated approach to the control strategy is given in Table
1618   3-12 through Table 3-14. Table 3-12 lists the release and stability CQAs and associated
1619   assays registered for the initial filing for both release and in-process testing. It is
1620   comprehensive and includes the CQAs assayed at not only the drug product stage, but also
1621   for the process intermediates. In addition, Table 3-2 lists several CQAs that are assayed but
1622   not registered at the initial filing and are used for additional process monitoring. Finally,
1623   Table 3-3 lists those CQAs for which additional clearance studies will become available or
1624   are assayed earlier in the process and may be redundant. If, after suitable validation and
1625   continuous process monitoring, these CQAs are under control they would be eliminated
1626   from the control strategy.

1627   **Testing Controls**
1628
1629   **Table 3-11: Initial DRAFT of Control Strategy: Registered Release Tests** [a, b]

1630   **Specification Tests**
1631

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Physical Properties							
pH		5.5-6.5	✓	5.5-6.5	5.5-6.5	5.5-6.5	Compendial
Appearance	White to off white powder	Clear, colorless & essentially free from visible particles	✓	White to off-white cake	Homogeneous white suspension	Homogeneous white suspension	Compendial
Residual Moisture*	≤ 5%			3-9%			Compendial
Quantity	95% monosaccharides	0.9-1.1 mg/mL				✓	PS: High-pH HPAEX-PAD VLP: BCA
Size*	Type 1: 6.6-9.2kD Type 2: 8.8-12.3kD Type 3: 6.6-9.2kD Type 4: 11.0-15.3kD Type 3: 13.2-18.4kD	20-50 nm diameter ≤ 0.07 polysispersity index	50 nm				PS: HPSEC-MALS-RI VLP: DLS

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Conjugation Sites*	> 0.5 site/repeating unit						1H-NMR
Ps/VLP Ratio*			✓	0.2-0.4Ps/ VLP monomer			Calculated from Extent-of-Conjugation Data
Quantity (as PS Content)			5 mcg each of Ps 1-4 50 mcg Ps 5	5 mcg each of Ps 1-4 50 mcg Ps 5		5 mcg each of Ps 1-4 50 mcg Ps 5	DS:HPLC
Quantity (as Protein Content)			TBD g/mL				BCA
Reconstitution Time						≤ 180 sec	Visual
Particle Size*					5-40 μm		Particle sizer
Zeta Potential*					-10 mV		Zeta potentiometer
Fill Volume in Container					≥ 0.5 mL	≥ 0.5 mL	Compendial
Aluminum Content					0.3±0.05 mg/mL as AlPO$_4$	0.3±0.05 mg/mL as AlPO$_4$	Compendial

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Identity							
Identity	Western blot – positive for each subtype	ELISA – positive	Western blot – positive for each subtype	Western blot – positive for each subtype	Homogeneous white suspension. Positive for aluminum	Western blot – positive for each subtype	Specific to drug intermediate, substance, adjuvant or drug product.

1632   a.   CQAs marked with an "*" are stability indicating.

1633   b.   CQA in grayed cells are marked for potential removal

1634

1635   **Table 3-12: Registered Release Tests (continued)**[a,b]

1636   **Specification Tests**

1637

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Potency							
Serotypes 1-4 (correlation) *			70-130%	70-130%		70-130%	mAb-based Competitive ELISA
Serotype 5 (no correlation)*			70-130%	70-130%		70-130%	Rate Nephelometry
Purity							
%Purity*	≥ 95%						1H-NMR
Integrity and Degradation Products*	≤ 5%						1H-NMR
Monomer*	≤ 5%	80-90%	≥ 95%	≥ 95%		≥ 95%	VLP: Asymmetrical Flow FFF DS and DPLyo: Reducing CGE
Complexes* (dimer + trimer)		≤ 10%	≤ 10%	≤ 10%		≤ 10%	VLP: Asymmetrical Flow FFF DS and DPLyo: Non-reducing CGE
Aggregates* (>trimer)	≤ 5%	≤ 1%	✓				PS: HPSEC-MALS-RI VLP: Asymmetrical Flow FFF DS: DLS
Fragments*		≤ 1%	≤ 7%	≤ 7%		≤ 7%	VLP: Asymmetrical Flow FFF

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
							DS and DPLyo: Reducing CGE
Post-Translational Modifications		Comparable to reference standard					Peptide map
Free Amino Groups*		Comparable to reference standard					Peptide map
Host Cell Proteins	< 10 ng/mg	< 10 ng/mg					Anti-HCP ELISA
Host Cell DNA	≤ 10 ng/100 mcg	≤ 10 ng/100 mcg					qPCR
Free Ps*			≤ 10%	≤ 10%			High-pH HPAEX-PAD
Free VLP			✓				Reducing CGE
Conjugation Reactants			✓				RP-HPLC
Free Phosphate					✓		Compendial

1638   a.   CQAs marked with an "*" are stability indicating.
1639   b.   CQA in grayed cells are marked for potential removal for final control strategy
1640

1641   **Table 3-13: Control Strategy: Registered Release Tests** [a,b]

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Safety							
Endotoxin	< 5EU/ kg of body mass	< 5EU/k g of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	< 5EU/kg of body mass	Compendial
Sterility					Meets compendial requirement s	r	Compendial
General Safety				Meets compendi al requireme nts	Meets compendial requirement s	Meets compendia l requireme nts	Compendial

1642

1643   **In-process tests**

Attribute	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Bioburden	< 10 cfu/mL	Meets compend ial requirem ents	Meets compendial requirement s	Meets compendial requiremen ts			Compend ial
Reducing Activity (PAT)			Activation:				HPSEC
Polysacchar ide size			Activation				PS: HPSEC

1644   a.   CQAs marked with an "*" are stability indicating.
1645   b.   CQA in grayed cells are marked for potential removal for final control strategy
1646

1647 **Table 3-14: Additional Release Tests for characterization, Not Registered [a]**

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Assay
Critical Epitope(s)*			Report results	Report results			mAb-based Competitive ELISA (1-4) or Rate Nephelometry (5)
Linear & Conformational Epitopes		Report results					mAb-based ELISA (desorbed) or Peptide Map
Mass-to-charge ratio		Report results	Report results				CZE
Quantity (as protein content)				Report results			BCA

1648 a. CQAs marked with an "*" are stability indicating.
1649
1650 **Table 3-15: Tests Targeted for Removal**

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
Host Cell Proteins	✓	✓					Process validation demonstrates easily removed.
Host Cell DNA	✓	✓					Process validation demonstrates easily removed.
Ps/VLP Ratio*				✓			Measured on drug substance
Fill Volume in Container				✓			More relevant with adjuvanted diluents.
Quantity (as PS content)						✓	Applies to DPLyo, no change upon

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
							dilution
Aluminum Content						✓	Applies to adjuvant only, no change upon dilution of DPLyo
Ps/VLP/Adjuvant Ratio*						✓	Validated to use stability
Serotypes 1-4 (correlation)*						✓	Applies to DPLyo , no change upon dilution
Serotype 5 (no correlation)*						✓	Applies to DPLyo , no change upon dilution
Monomer*						✓	Applies to DPLyo no change upon dilution
Complexes*						✓	Applies to DPLyo , no change upon dilution
Sterility						✓	Applies to DPLyo and Adjuvent only. Reconstitution not performed under aseptic conditions.
Endotoxin*						✓	Applies to DPLyo and Adjuvent only. Reconstitution not performed under aseptic conditions.

CQA	PS	VLP	DS	DPLyo	Diluent	DP/Diluent	Comments
Rabbit Pyrogenicity				Meets compendial requirements	Meets compendial requirements	Meets compendial requirements	Compendial; Test replaced by endotoxin test

Input Materials Control

1651

1652 • Input materials required for the manufacture of A-VAX are determined by process
1653   development and are controlled by procedures within the quality control and quality
1654   assurance organizations. Quality control is responsible for executing the appropriate tests to
1655   ensure that the materials meet pre-determined specifications. Quality assurance is
1656   responsible for procedures to ensure the operations fall within cGMP guidelines including
1657   receipt, testing, and storage, order of use and disposal of out-dated materials. Compendial
1658   and well-characterized input materials are tested by analytical methods appropriate for
1659   each chemical.

1660 • Input materials that are not well characterized are assayed for ability to promote the
1661   expected response in an appropriate biological system. The lack of ability to assay these
1662   materials by more precise methods requires additional procedures to ensure that they meet
1663   use specifications on a regular and continuing basis. Such additional procedures include
1664   regular audits of the supplier(s) ensure that the input material manufacturing processes
1665   remain consistent and that any changes are communicated to the A-VAX manufacturer to
1666   ensure that such changes do not affect A-VAX production in an adverse manner.

1667 • A robust development program is in place to identify the critical and active components of
1668   the not well-characterized input material mixture. As information is developed it will be
1669   communicated to the input material manufacturer to determine if there are opportunities
1670   to upgrade the manufacturing process to gain a more consistent and robust control of the
1671   incoming raw material. Also in place is a procedure of process monitoring (refer to
1672   Continuous Process Verification section) to identify shifts and changes in the process. This
1673   process can identify important aspects of an input material. For example, process
1674   monitoring for complex raw material #2 for the polysaccharide fermentation indicated a
1675   reduction in variability occurred after a vendor change (refer to Upstream section) The
1676   subsequent investigation revealed that the new vendor had better control of nitrogen levels
1677   which ultimately affected OD levels in the fermentation. With this information the
1678   specification for the material was changed to include a requirement for nitrogen levels. In
1679   the event of any potential change to the raw material manufacturing process, multiple lots
1680   will be evaluated for performance in the A-VAX manufacturing process. Such evaluations
1681   would include, but are not limited to, process performance and consistency as well as
1682   process validation including characterization of the intermediate materials, drug substance
1683   and drug product, in a comparability study.

1684

Continuous Process Verification (or Process Monitoring)

1685

1686 • At the completion of developing a control strategy for the processes involved in the
1687   manufacture of A-VAX, continuous process verification should be implemented to ensure
1688   that the control strategy is appropriate. Multivariate Statistical Process Control (MSPC) will
1689   be used for the process parameters implemented in the upstream and downstream
1690   processes. Univariate SPC will be used for attributes. Routine monitoring of data will further
1691   increase the understanding of the sources of variation in the process and ensure the most
1692   influential parameters were selected to control the process.

1693 • The data for MSPC will be collected from the various processes via online and at-line
1694   collection points. The advantage of MSPC vs. Univariate SPC is that it can detect shifts in the
1695   mean or the relationship (covariance) between several related parameters. After the

1696   collection of a minimum of 30 lots of data, control limits should be put in place. Control
1697   limits will be reevaluated after process changes are implemented.

1698 • The data for Univariate SPC on the attributes will be collected from release testing. After the
1699   collection of data from a minimum of 30 lots control limits should be put in place. Run rules,
1700   eg. Western Electric Run rules can also be utilized to further enhance the process and can
1701   detect more subtle shifts in processes. Control limits should be reevaluated after process
1702   changes are implemented.

1703 • The level of monitoring should be statistically sound and appropriate based on the criticality
1704   and impact of the parameters and should be reevaluated on a routine basis.

1705 • Learnings from the verification process should be evaluated on a regular basis to determine
1706   if changes are required for the control strategy.

1707
1708   **Annex 1**
1709   The following formula was used in the analysis:
1710
1711   **Equation 3-1: Process Capability Analysis Formula**

$$\mathrm{Cp} = \frac{(\mathrm{Maximum} - \mathrm{Minimum}) - \mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}},$$

1712

$$\mathrm{Maximum} - \mathrm{Minimum} = 6 \cdot \mathrm{Cp} \cdot s_{\mathrm{Process}} + \mathrm{Release\ Ranges},$$

where s_{Process} is the variability estimated from manufacturing data
or obtained from manufacturing modeling.

1713
1714   This is related to a capability index, Cpm, which is commonly used to assess the impacts of
1715   process variability on process capability.

$$\mathrm{Cp} = \frac{(\mathrm{Maximum} - \mathrm{Minimum}) - \mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}},$$

1716

$$= \frac{\mathrm{Maximum} - \mathrm{Minimum}}{6 \cdot s_{\mathrm{Process}}} - \frac{\mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}}$$

$$= \mathrm{Cpm} - \frac{\mathrm{Release\ Ranges}}{6 \cdot s_{\mathrm{Process}}}.$$

1717
1718   Cpm is related to the proportion of lots which are predicted to fall outside of release limits. Thus
1719   for example Cpm=1.0, which corresponds to 3 standard deviations on either side of the process
1720   mean, is associated with a failure rate equal to 0.0027, or 3 in 1000 failures. Cpm=0.67 is
1721   associated with a rate of 1 in 20 failures.

1722
1723   Release ranges are calculated for the upper release limit and the lower release limit as follows:
1724

1725 **Equation 3-2: Release Range Formula**

$$\text{Upper Release Range} = t_{\alpha,df} \cdot s_{Assay},$$

$$\text{Lower Release Range} = t \cdot b + t_{\alpha,df} \cdot \sqrt{(t \cdot s_b)^2 + s_{Assay}^2},$$

1726   where $t_{\alpha,df}$ = value from t-distribution with error degress of freedom (df),

            s_{Assay} = release assay variability estimated from stability evaluation,

            b = estimated loss rate at labelled storage temperature,

            s_b = standard error of the estimated loss, and

            t = product shelf-life a t lablelled storage temperature (24 months).

1727
1728 Summary measures from analyses of manufacturing and stability data for a licensed product
1729 similar in process and in the potency assay to A-VAX, along with the calculated upper and lower
1730 release ranges are presented in **Error! Reference source not found.**. The results are expressed in
1731 log (natural log) units due to the distributional characteristics of the potency measurements of
1732 the licensed product.
1733
1734 **Table 3-16: Summary measures from analyses of manufacturing and stability data for a similar**
1735 **licensed vaccine**

Component	Process Variability (sProcess)	Loss Rate (b)	Standard Error (sb)	Assay Variability (sAssay)	Upper Release Range	Lower Release Range
A-VAX$_1$ - A-VAX$_4$	0.0608	0.0100	0.0062	0.0461	0.0800	0.5101
A-VAX$_5$	0.1596	0.0100	0.0062	0.1210	0.2098	0.5726

1736
1737 The loss rate, standard error of the loss rate, and assay variability (for A-VAX$_1$ - A-VAX$_4$) were
1738 obtained from an analysis of stability data for 3 lots of the similar vaccine. The t-value associated
1739 with the estimate of assay variability is equal to $t_{0.10,18}$ = 1.734. This gives upper and lower
1740 release ranges as follows:
1741
1742 **Equation 3-3: Release Range Calculation**

$$\text{Upper Release Range} = t_{\alpha,df} \cdot s_{Assay} = 1.734 \cdot 0.0461 = 0.0800,$$

1743
$$\text{Lower Release Range} = t \cdot b + t_{\alpha,df} \cdot \sqrt{(t \cdot s_b)^2 + s_{Assay}^2}$$
$$= 24 \cdot 0.0100 + 1.734 \cdot \sqrt{(24 \cdot 0.0062)^2 + 0.0461^2} = 0.5101.$$

1744

1745   The release ranges for A-VAX$_5$ were calculated from the stability results obtained from the in
1746   vitro assay, but using the assay variability for the *in vivo* assay (s = 0.1210 from long term control
1747   data for the *in vivo* assay). Process variability was likewise scaled up in proportion to the
1748   difference in variability of the *in vivo* and *in vitro* assays.
1749
1750   The minimum to maximum potency ranges and values supporting several levels of process
1751   capability are given in Table 3-5.
1752
1753   **Table 3-17: Potency ranges and minimum and maximum potencies for values two levels of**
1754   **process capability (probability of OOS)**

Component	Cpk	Prob(OOS)	Range (loge)	Minimum at Expiry	Minimum at Release	Maximum at Release
A-VAX$_1$ - A-VAX$_4$	0.67	0.05	0.8340	0.53	0.80	1.22
	1	0.003	0.9555	0.50	0.77	1.30
A-VAX$_5$	0.67	0.05	1.4208	0.40	0.60	1.70
	1	0.003	1.7400	0.35	0.50	2.00

1755
1756   The minimum and maximum potencies are derived from the target potencies for the 5
1757   components of A-VAX (1.00). Potencies were determined to support good process capability
1758   (Cpm=1.0). It should be noted that the probability of OOS for one or more of the serotypes is
1759   equal to $1 - (1-0.003)^5 = 0.015$ (i.e., 1.5%). Target potencies, together with minimum and
1760   maximum potencies are given in **Error! Reference source not found.**.
1761
1762   **Table 3-18: Target potencies, and minimum and maximum potencies**

Component	Target	Minimum	Maximum
A-VAX$_1$ - A-VAX$_4$	1.00	0.50	1.30
A-VAX$_5$	1.00	0.35	2.00

1763
1764   It should be noted that minimum and maximum potencies are not (geometrically) symmetric
1765   about the target (1.00). This is caused by including stability in the determination of minimum
1766   expiry potency.
1767   The forecast minimum and maximum potencies were utilized to guide manufacture of clinical
1768   lots to be performed in Phase III clinical studies. The clinical lots were manufactured from
1769   common conjugated bulks in order to preserve the planned differences (minimum to maximum)
1770   in potencies. The source conjugate bulks were tested in an enhanced potency assay format in
1771   order to better target clinical lot potencies.
1772
1773   **Annex 2:**
1774   This begins with determining an appropriate level of risk of batch failure due to one or more
1775   false positive (false OOS) results. The overall failure rate is a function of the number of tests and
1776   the risk of failure in each individual test. The overall risk associated with either 95% or 99%
1777   limits for various numbers of tests is given in Table 3-8.

1778   **Table 3-19: Overall risk for various numbers of tests**

No. Tests	95% Limits	99% Limits
1	5%	1%
2	10%	2%
3	14%	3%
6	26%	6%

1779
1780   Significant overall risk results from using 95% limits. The overall risk using 99% limits results in a
1781   more realistic false failure rate for a moderate number of tests. The number of tests can be tests
1782   on multiple components of a vaccine (e.g., multiple polysaccharides) or multiple quality
1783   attributes.
1784   Excess risk also results from redundant or correlated tests. Tests which measure the same or
1785   related properties of a vaccine will be correlated. Thus for example, potency measured by both
1786   an *in vivo* assay and an *in vitro* assay will likely be highly correlated, resulting in higher than
1787   expected product failure. Effort should be made to select a single measure of a quality attribute,
1788   or to utilize an alternative strategy for controlling the vaccine such as multivariate quality
1789   control.
1790
1791   Acceptance criteria which have been established from process data are estimates of the true
1792   limits and subject to uncertainty. Like all statistical estimates, their reliability may be a function
1793   of the number of data points (batches) used to calculate the limits. The risks associated with
1794   estimating acceptance criteria using simple 2- or 3-sigma limits are high for small numbers of
1795   batches. Tolerance limits are utilized to control risk of false failure for small and large numbers
1796   of batches alike. This comes at a cost, however, of excessively wide limits with small numbers of
1797   batches. A lifecycle approach to establishing acceptance criteria using tolerance limits should be
1798   utilized. Early limits should be updated when a sufficient number of batches (and adequate long
1799   term experience with the process) has been acquired.
1800
1801

1802 # 4. Upstream (Polysaccharide) Section

1803 ## 4.1. Executive Summary

1804 In the manufacturing process for polysaccharide,, a well-defined upstream process is required to
1805 provide sufficient material (bulk volume) with well-defined quality attributes for the
1806 downstream processing.
1807
1808 This document describes the polysaccharide fermentation process and the effects of the
1809 complex raw materials, fermentor operating parameters, and inactivation parameters. Prior
1810 knowledge from published literature and process risk assessments are used to ascertain the
1811 factors that will be evaluated further. Ishikawa diagrams and cause-and-effect matrices facilitate
1812 the identification of process steps for further exploration via design of experiments (DOEs) or
1813 one factor at a time (OFAT) evaluations. Failure modes and effects analysis is used to assess the
1814 process risks and to develop appropriate strategies for managing critical process attributes.
1815
1816 ## 4.2. Brief Description of Each Process Step

1817 The following is a step-wise description of each process step at Phase 2 starting with the *H.*
1818 *horrificus* background. Post-Phase 2 changes are discussed at the appropriate section of the
1819 document.
1820
1821 *H. horrificus* is a lactic acid-producing, gram-negative anaerobic bacteria. It is aero-tolerant;
1822 however, it is sensitive to vigorous mixing and prolonged exposure to elevated levels of oxygen.
1823 It typically grows as single cells. There are 11 serotypes, of which eight are pathogenic in
1824 otherwise healthy individuals. Five serotypes are responsible for >95% of clinically reported
1825 cases in both the developed and developing worlds, although the distribution among the five
1826 varies by region. The serotype-specific capsular polysaccharide (Ps) is constitutively expressed
1827 through the growth cycle. Therefore, Ps yield correlates with biomass. Under stressed
1828 conditions, such as nutrient limitation, *H*. *horrificus* expresses the enzyme polysaccharidase,
1829 which will digest the capsular Ps to monomer units.
1830
1831 ### 4.2.1. Cell Banks

1832 Master and stock cell bank vials are prepared in the logarithmic growth phase according to
1833 standard procedures to generate a sufficient inoculum per vial to initiate a viable culture of the
1834 organism. The choice of a glycerol-based cryo-preservative was made based on characteristics of
1835 the organism. Maximum viability of freshly thawed vials will ensure a robust process.
1836

1837   ### 4.2.2. Media

1838   There is significant prior knowledge for the media. It is a proprietary media with two complex
1839   non-animal-derived components (raw materials designated RM 1 and RM 2). Glycerol is the
1840   carbon source (5 g/L for shake flasks and 10 g/L for seed and production fermentors) and is the
1841   limiting nutrient. Experimental results indicate that the media can support fourfold biomass
1842   achieved in fermentor, given a concomitant increased in glycerol. Remaining media components
1843   are amino acids, salts, and one growth factor/vitamin. The only other difference in shake flask
1844   media contains 1M PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)). Fermentor pH is
1845   controlled with the automated addition of a 1N sodium hydroxide solution.
1846
1847   ### 4.2.3. Shake Flask: Stage 1

1848   The stage 1 shake flask purpose is to robustly culture the organism after cryo-preservation and
1849   increase the biomass for the shake flask stage 2 inoculation. Two (1.5ml each) WCB vials are
1850   thawed for 20 minutes at room temperature. The vials inoculate 72ml shake flask media (4%
1851   v/v) in 250ml disposable shake flasks. The flasks are incubated at 25 ±5 RPM and 37 ±2 °C.
1852   Transfer to stage 2 is triggered at an optical density (OD) target of 2 Absorbance Unit (AU)
1853   (range 1.5 to 3).
1854
1855   ### 4.2.4. Shake Flask: Stage 2

1856   The stage 2 shake flask purpose is to robustly culture and increase the biomass for the seed
1857   fermentation inoculation. Inoculate 2 x 768ml media in 2L disposable shake flasks with 32ml (4%
1858   v/v) each from the stage 1 culture. The flasks are incubated at 30 ± 5 RPM and 37 ± 2 °C.
1859   Transfer to stage 2 at an OD target of 2 AU (range 1.5 to 3). In Table 4-1, the shake flask data is
1860   summarized from prior knowledge.
1861
1862   **Table 4-1: Shake Flask Data from Prior Knowledge**

Process Step	Doubling Time (h)	Lag (h)	Total Time to Transfer (h)
Shake Flask Stage 1	0.83 ± 0.06	2.3 ± 0.23	7.8 ± 0.6
Shake Flask Stage 2	0.80 ± 0.05	N.D	4.7 ± 0.5
Seed Fermentor	0.74 ± 0.05	1.08 ± 0.15	5.8 ± 0.6

1863
1864
1865   ### 4.2.5. 50L Seed Fermentation

1866   The seed fermentor purpose is to increase biomass for the production fermentor inoculation,
1867   and it is performed as a batch fermentation. Inoculate 38.4L fermentor media with 1.6L (4% v/v)
1868   stage 2 culture. Transfer to the production fermentor is triggered at an OD target of 3 AU (range
1869   2.5–5). The fermentor operation parameters are summarized in Table 4-2.
1870

1871 **Table 4-2: Seed Fermentor Parameters**

Parameter	Set-point and Range
Back Pressure	2 ± 1 psig
Air Overlay	4 ± 2 LPM
Temperature	37 ± 2 °C
pH	7 ± 0.5 pH units
Agitation	40 ± 10 RPM

1872

1873 **Figure 4-1: Seed Fermentation Transfer Criterion Data from Prior Knowledge**

Plot of Glycerol (g/L) versus OD at Transfer. $y = -1.0916x + 9.4579$, $R^2 = 0.9332$
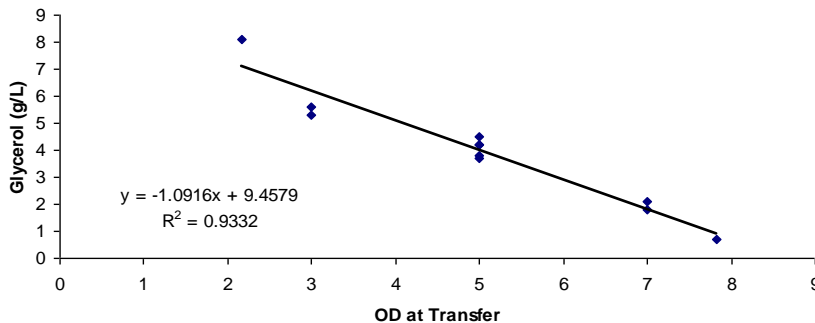
1874

1875 ## 4.2.6. 1,000L Production Fermentation

1876 The purpose of the production fermentation is to provide sufficient biomass for a consistent
1877 culture substrate for the down stream inactivation step. It is a batch fermentation in which 760L
1878 of fermentor media is inoculated with 40L (2% v/v) of seed fermentor culture. The fermentor
1879 operation parameters are summarized in Table 4-3.
1880

1881 Because the process involves cultivation of an aero-tolerant anaerobe, mixing and aeration
1882 conditions were not deemed critical to quality and conditions from a previous production
1883 platform were implemented. Temperature and pH ranges were established at typical ranges for
1884 this production platform based on a series of early stage experiments, which are not included
1885 herein. Phenol is added 60 minutes post glycerol exhaustion.
1886

1887 **Table 4-3: Production Fermentor Parameters**

Parameter	Set-point and Range
Back Pressure	2 ± 1 Psig
Air Overlay	10 ± 2 LPM
Temperature	37 ± 2 °C
pH	7 ± 0.5 pH units

Agitation	30 ± 10 RPM

1888 **Figure 4-2: Effect of Seed Fermentor Transfer on Lag from Prior Knowledge**

$$y = -4.8149Ln(x) + 10.646$$
$$R^2 = 0.906$$
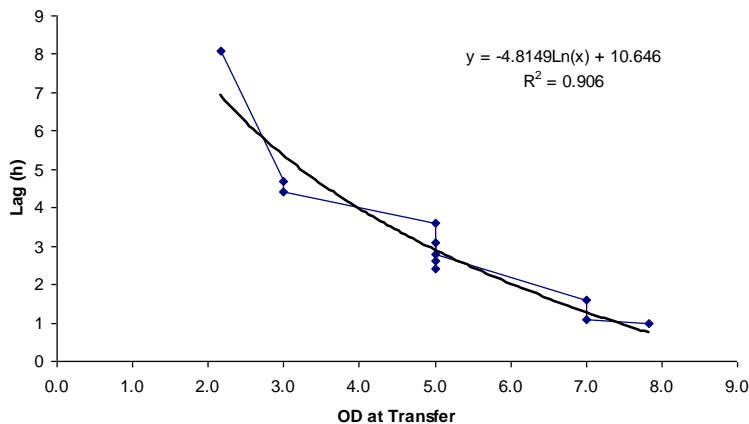
1889

4.2.7. Inactivation

1891 Phenol is added to a final concentration of 1% (w/w). Studies to determine inactivation
1892 kinetics were performed prior to initiating development work. The results were mostly
1893 independent of serotype. A 7-log reduction in viable cells is achieved in 27 ± 3 minutes at
1894 the stated inactivation conditions. A threefold safety factor was used to determine the 90-
1895 minute time for inactivation. After 90 minutes, a sample is submitted to confirm culture
1896 inactivation. After inactivation, *X. Horrificus* culture OD (600nm) is adjusted at 5 with Water
1897 for Injection (WFI) to normalize the biomass. Assuming a constant peptidoglycan content in
1898 the cell wall, this dilution is expected to normalize the enzyme substrate concentration. The
1899 diluted inactivated broth is then sent to purification.
1900

1901   4.2.8.   Process Diagram

1902   **Figure 4-3: Process Diagram**
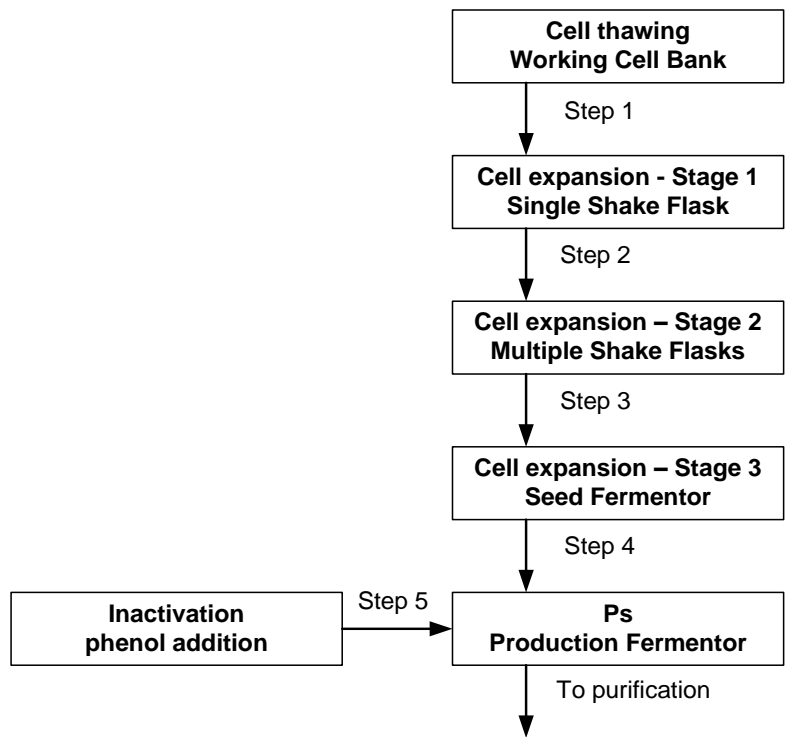
1903
1904
1905   4.2.9.   Source of Prior Knowledge

1906   Numerous articles exist giving general cultivation parameters such as pH and temperature.
1907   Literature also exists for media and nutritional requirements but is less numerous. The process
1908   risk assessment was executed by subject matter experts. Similar data is available from other Ps
1909   processes (one licensed, one in development) derived from other species of lactic acid-
1910   producing bacteria. Also, the final manufacturing facility is planned to be the same facility as the
1911   licensed Ps product.
1912
1913   **4.3.**   Process Risk Assessment

1914   The following section summarizes the process of defining and executing the risk assessment.
1915
1916   4.3.1.   Process Analysis (Ishikawa Diagram)

1917   This Ishikawa diagram illustrates a comprehensive analysis of how all aspects of the
1918   development and manufacturing process potentially impact drug substance quality. The
1919   process-specific parameters are only a subset of the parameters to control the overall process.
1920   Nonetheless, these parameters are the most direct routes to ensure consistent product quality.
1921

1922   **Figure 4-4: Ishikawa diagram built around parameters that include process, materials, people,**
1923   **and facilities.**
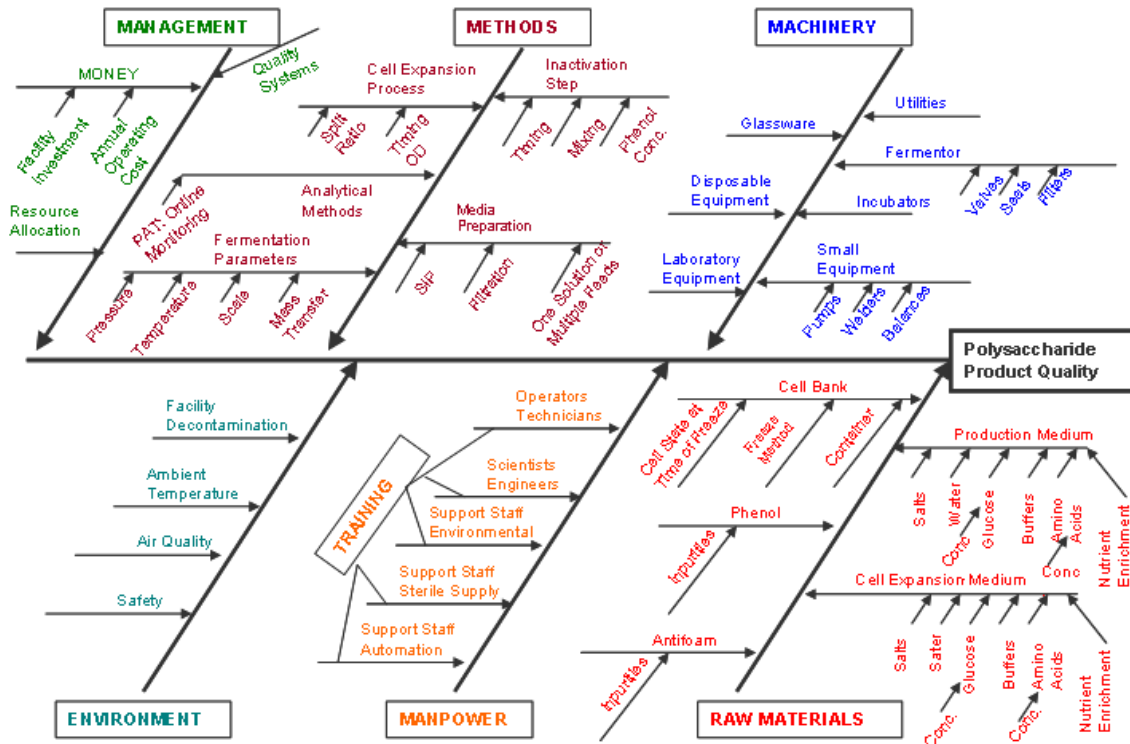
1924
1925
1926   Figure 4-5 is an expanded Ishikawa diagram built around the process-related factors. The
1927   expansion was performed to identify the key parameters at each process step. This information
1928   will be used for analysis once the process step for Quality by Design (QbD) analysis is identified.
1929

1930 **Figure 4-5: Process Ishikawa Diagram**
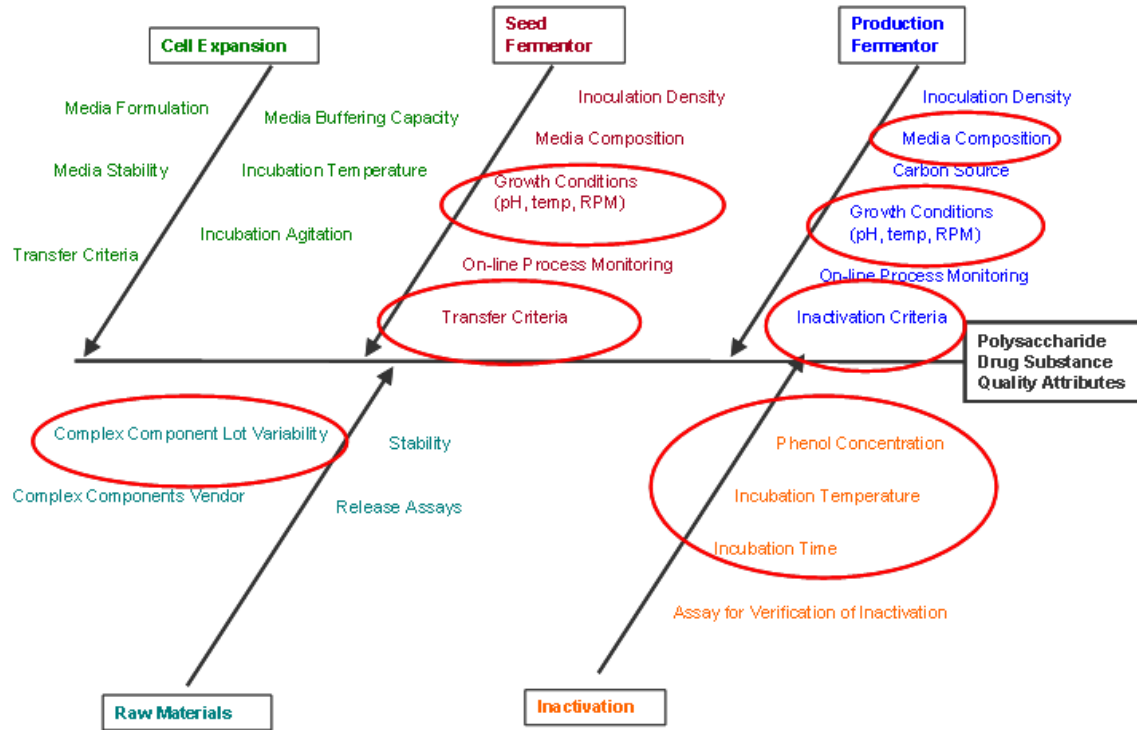
1931
1932
1933 This Ishikawa diagram isolates process-specific parameters for their potential impact on drug
1934 substance quality attributes. The outcome of this analysis is a list of parameters that can be
1935 taken forward for further analysis or experimentation to begin identifying key and critical
1936 process parameters. Font colors have been assigned to each process step to better visualize the
1937 specific parameters involved in it.
1938
1939 Elements of prior knowledge were used to identify process steps (cell Expansion, seed
1940 fermentor) that were NOT taken forward with additional QBD approaches. Various parameters
1941 of raw materials, production fermentor, and inactivation steps (see circles) were analyzed with
1942 further QbD approaches.
1943

1944 4.3.2. Rationale for Selecting the Production Fermentation/Inactivation as a Unit of
1945   Operation for QbD Analysis

1946 The results of the Ishikawa analyses and cause-and-effect matrix identified process steps and
1947 parameters that required further experimentation to define critical and key parameters. Most of
1948 the "no relationship" scores were based on prior knowledge. The "relationship known" or
1949 "relationship expected" scores were determined based on scientific first principles. The
1950 quantitative ranking structure was based on a typical scoring matrix.
1951
1952 Table 4-4 defines the weight given to each ranking value. A total score of 66 was estimated to
1953 represent "greater than moderate impact" (i.e., score of 5.5) across all 12 quality attributes.
1954 Process steps with scores or 66 or higher were taken forward for further exploration via DOEs
1955 and OFAT experiments to determine critical parameters and ranges. The scores are shown in

1956   Table 4-5 and illustrated in the Pareto chart in

	PS Yield	Host-cell Protein	Host-cell DNA	Size	Integrity & Degradation Products	Aggregation	Conjugation Sites	Polysaccharide Length	Quantity (as Monosaccharide Content)	Endotoxin	Bioburden	Appearance	TOTAL
Cell Bank Vial Thaw	4	1	1	4	1	1	4	1	1	1	1	4	24
Cell Expansion	4	1	1	4	4	1	4	1	4	1	1	4	30
Seed Fermenter	7	4	4	7	4	4	4	4	4	4	7	4	57
Production Fermenter	10	7	7	4	4	7	4	7	7	4	7	7	75
Inactivation	7	10	10	7	7	7	4	7	4	10	7	7	87
Harvest Process	4	4	4	4	7	7	4	4	4	10	4	7	63
Raw Materials	10	4	4	7	4	4	4	7	7	4	7	4	66

1957
1958
1959   Figure
1960   Figure 4-6. Those steps with borderline scores (seed fermentor and harvest process) were not
1961   considered for further experimentation in this case study, although prior knowledge was used to
1962   mitigate risk around these steps.
1963
1964   **Table 4-4: Cause-and-Effect Ranking Definition**

Rank	Input Process Steps to Critical Quality Attributes (CQA) and Key Process Attributes (KPA)
10	Relationship Known
7	Relationship Suspected or Unknown
4	Slight Relationship
1	No Relationship

1965

1966 **Table 4-5: Cause-and-Effect Process Step Ranking**

	PS Yield	Host-cell Protein	Host-cell DNA	Size	Integrity & Degradation Products	Aggregation	Conjugation Sites	Polysaccharide Length	Quantity (as Monosaccharide Content)	Endotoxin	Bioburden	Appearance	TOTAL
Cell Bank Vial Thaw	4	1	1	4	1	1	4	1	1	1	1	4	24
Cell Expansion	4	1	1	4	4	1	4	1	4	1	1	4	30
Seed Fermenter	7	4	4	7	4	4	4	4	4	4	7	4	57
Production Fermenter	10	7	7	4	4	7	4	7	7	4	7	7	75
Inactivation	7	10	10	7	7	7	4	7	4	10	7	7	87
Harvest Process	4	4	4	4	7	7	4	4	4	10	4	7	63
Raw Materials	10	4	4	7	4	4	4	7	7	4	7	4	66

1967
1968

1969 **Figure 4-6: Pareto Chart**
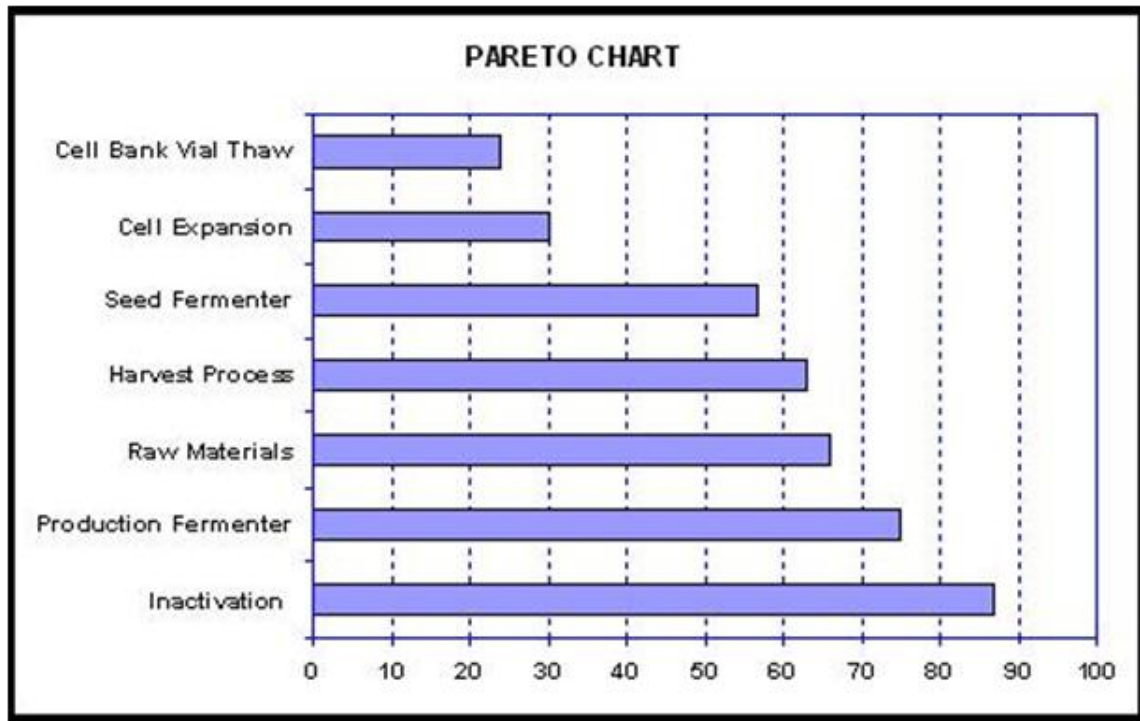
1970
1971

1972 ## 4.4. Design of Experiment

1973 Based on a combination of historical knowledge and process risk assessment (cause-and-effect
1974 analysis (Table 4-5), the raw materials, fermentor operating parameters, and inactivation
1975 parameters (see Pareto Chart, Figure 4-6) were analyzed through a multivariable central
1976 composite design of experiments.
1977
1978 Note that at this stage the central composite design was selected in place of a more routine
1979 screening design for a number of reasons. First, it was known from early process development
1980 (and prior knowledge from similar programs) that polysaccharide production yield and quality
1981 are directly tied to biomass production. Therefore, conditions that promoted optimal biomass
1982 productivity would generate optimal Ps yields. As the production process involves cultivation of
1983 an aero-tolerant anaerobe, screening of mixing and aeration parameters was not prioritized.
1984 Instead, greater emphasis was applied to identify potential interacting parameters using an
1985 experimental design that was best suited for this. The following factors were explored:
1986 •   Concentration of complex RM #1 (18–22 g/L)
1987 •   Concentration of complex RM #2 (8–12 g/L))
1988 •   Time to inactivation (time post glycerol depletion) (-30 – 150 minutes)
1989 •   Incubation temperature (35–39˚ C)
1990 Appropriate analytical tools were developed through the early stages of process development to
1991 determine the cell lysis during the fermentation process. In addition, analytical methods were
1992 developed to determine the polysaccharide repeat units and quantify the yields at the
1993 laboratory scale.

1994 ## 4.4.1. Response Surface and Analysis of Variance for Repeat Units

1995 Response variables were identified primarily by leveraging prior knowledge from early and late
1996 stage upstream process development of a polysaccharide production platform. Early
1997 development indicated that polysaccharide length and the number of polysaccharide repeat
1998 units were variable with incorporation of upstream process changes. Polysaccharide yield is a
1999 major process economics consideration. Most critically, the link between polysaccharide length
2000 and percent lysis was well established early on in the upstream process development. Extended
2001 time post lysis resulted in degradation of mean polysaccharide lengths and therefore negatively
2002 impacted product quality. The following response variables were explored:

2003 • Number of polysaccharide repeat units, identity, and integrity are measured by 1H-NMR.
2004 This parameter impacts potency CQA, measured in Ps-VLP through ELISA.

2005 • Polysaccharide size is measured by HPSEC-MALLS-RI on the purified Ps, following
2006 fermentation. Furthermore, each type is sized to a particular molecular weight in the
2007 downstream purification process (summarized in Table 7-12 in the Control Strategy section).
2008 The final size of the Ps impacts potency CQA and is measured in Ps-VLP through ELISA.

2009 • Ps yield (key process attributes, referred to as quantity in the cause-effect matrix) is
2010 measured through the hydrolysis of the purified polysaccharide using high-pH HPAEX-PAD

2011 • Percentage lysis, which is tied to Ps length and subsequently the potency critical quality
2012 attribute (CQA)

2013 The outcome of the DOE is to understand interactions and identify potential Critical Process
2014 Parameters (CPPs), without defining clear parameter limits or ranges. The CPP candidates
2015 identified from the DOE underwent further analysis via FMEA and OFAT experiments to
2016 conclusively define their overall criticality and establish ranges.

2017 **Figure 4-7: Response Surface for Impact of RM 1 and RM 2 on the Polysaccharide Repeat Units**

2018
2019

CMC-VWG
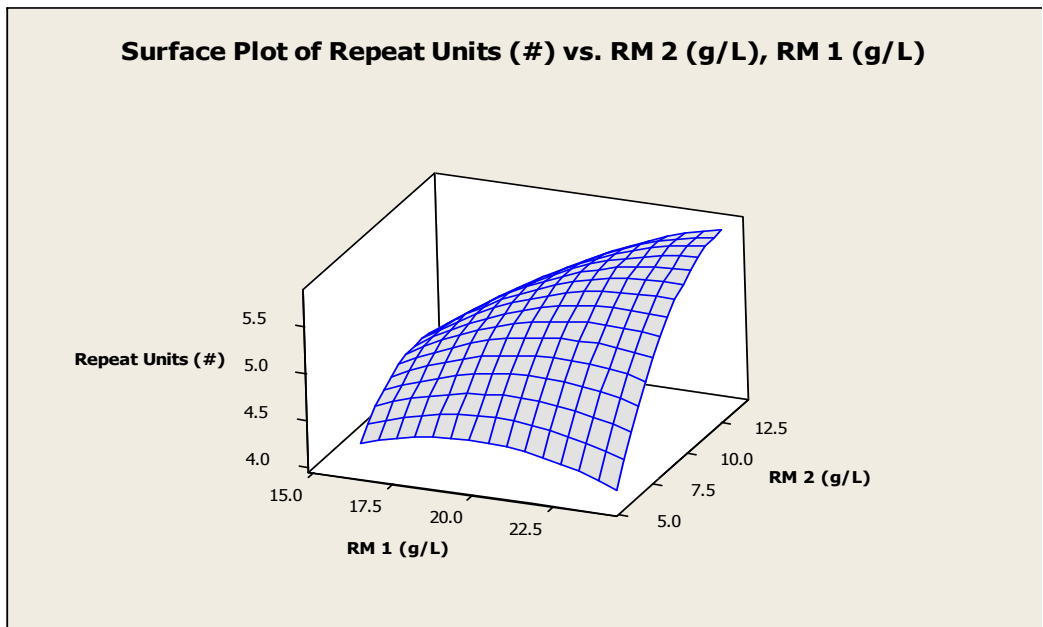
2020 The results of the DOE (see surface response plot Figure 4-8) indicate that the concentration of
2021 RM 2 has a direct impact on the number of polysaccharide repeat units. Considering the direct
2022 impact of RM 2 concentration on this critical quality attribute, this parameter was defined as a
2023 CPP.
2024
2025 The following table lists the analysis of variance (ANOVA) regression for the number of repeat
2026 units versus block, RM 1, and RM2. The analysis was performed using coded units.
2027
2028 Table: 4-6 Response Surface Regression: Number of Glucose Repeats versus Block, RM 1 (g/L),
2029 RM 2 (g/L). Estimated Regression Coefficients for number of glucose repeat Term.
2030

	Coef	SE Coe	T	P
Constant	5.30000	0.18982	27.921	0.000
Block 1	-0.01000	0.12005	0.083	0.935
Block 2	0.04500	0.12005	0.375	0.714
RM 1 (g/L	0.17083	0.09491	1.800	0.095
RM 2 (g/L)	0.23333	0.09491	2.458	0.029
Inactivation Time (min)	0.02083	0.09491	-0.220	0.830
Temperature	0.14583	0.09491	1.537	0.148
RM 1 (g/L)*RM 1 (g/L	-0.06563	0.08878	-0.739	0.473
RM 2 (g/L)*RM 2 (g/L	0.11563	0.08878	-1.302	0.215
Inactivation Time (min)*Inactivation Time (min)	0.00312	0.08878	-0.035	0.972
Temperature*Temperature	0.01562	0.08878	-0.176	0.863
RM 1 (g/L)*RM 2 (g/L)	0.10000	0.11624	0.860	0.405
RM 1 (g/L)*Inactivation Time (min)	0.01875	0.11624	0.161	0.874
RM 1 (g/L)*Temperature	0.09375	0.11624	0.807	0.434
RM 2 (g/L)*Inactivation Time (min)	0.08750	0.11624	0.753	0.465
RM 2 (g/L)*Temperature	0.15000	0.11624	1.290	0.219
Inactivation Time (min)*Temperature	-0.03125	0.11624	-0.269	0.792
S = 0.464961 PRESS = 17.2224				
R-Sq = 57.56% R-Sq(pred) = 0.00% R-Sq(adj) = 5.32%				

2031
2032 RM 2 was the only significant (p<0.05) term for this response, while RM 1 had borderline-
2033 significant response (p<0.10). Because inactivation time and temperature were shown not to
2034 impact the number of repeat units in this experiment, these variables were excluded from the
2035 DOE analysis to repeat the statistical analysis with increased degrees of freedom. When the DOE
2036 was re-analyzed with number of repeats as the response variable and only RM 1 and RM 2 as

2037 the model effects (Table: 4-7), RM 2 again was the only significant factor (p<0.05), with RM 1
2038 showing borderline significance (p< 0.10).
2039
2040 The following table lists the ANOVA regression for the number of repeat units versus RM 1 and
2041 RM 2. The analysis was performed using coded units.
2042
2043 Table: 4-7, Response Surface Regression: Number of Repeat Units versus Block, RM 1 (g/L), RM 2
2044 (g/L) Estimated Regression Coefficients for number of repeat units:
2045

Term	Coef	SE Coef	T	P
Constant	5.28125	0.12282	43.000	0.000
Block 1	-0.01000	0.10985	-0.091	0.928
Block 2	0.04500	0.10985	0.410	0.686
RM 1 (g/L)	0.17083	0.08685	1.967	0.062
RM 2 (g/L)	0.23333	0.08685	2.687	0.013
RM 1 (g/L)*RM 1 (g/L)	-0.06328	0.07977	-0.793	0.436
RM 2 (g/L)*RM 2 (g/L)	-0.11328	0.07977	-1.420	0.170
RM 1 (g/L)*RM 2 (g/L)	0.10000	0.10637	0.940	0.357
S = 0.425462 PRESS = 8.10926				
R-Sq = 39.86% R-Sq(pred) = 0.00% R-Sq(adj) = 20.73%				

2046
2047 The range for RM 2 was subsequently determined by OFAT experiments. Since no interaction
2048 effects were shown in the DOE, an OFAT experiment was chosen to better define the response
2049 to a range of RM 2 values.
2050
2051 4.4.2. Response Plots for Polysaccharide Yield

2052 **Polysaccharide yield is sensitive to inactivation time (not RM 2)**
2053 Concentration of phenol required for inactivation of the bacterial strain was obtained from prior
2054 knowledge. Considering the historical data, it was deemed not to be a critical parameter, as long
2055 as it was well controlled above a threshold. Inactivation time was critical to maintaining high
2056 polysaccharide yield. Ps yield was insensitive to changes in concentration of RM 2. Maximum
2057 polysaccharide yield was obtained when inactivation was initiated 50–100 minutes following
2058 glycerol depletion.
2059

2060 **Figure 4-8: Impact of Polysaccharide yield on Inactivation Time**
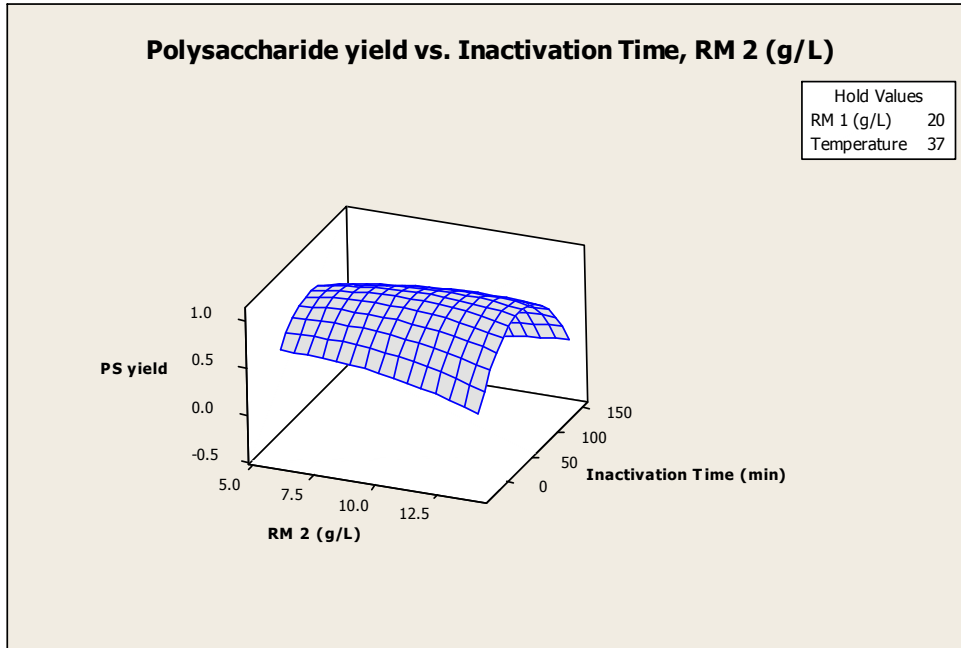
2061
2062
2063 **Polysaccharide yield (potency) is sensitive to temperature (not raw material)**
2064 Ps yield was sensitive to fermentation temperature but not to the concentration of RM 1.
2065 Incubation temperatures of 36–38˚C delivered the highest polysaccharide yield relative to the
2066 lowest and highest temperatures explored.
2067

2068 **Figure 4-9: Impact of Polysaccharide yield on Temperature**
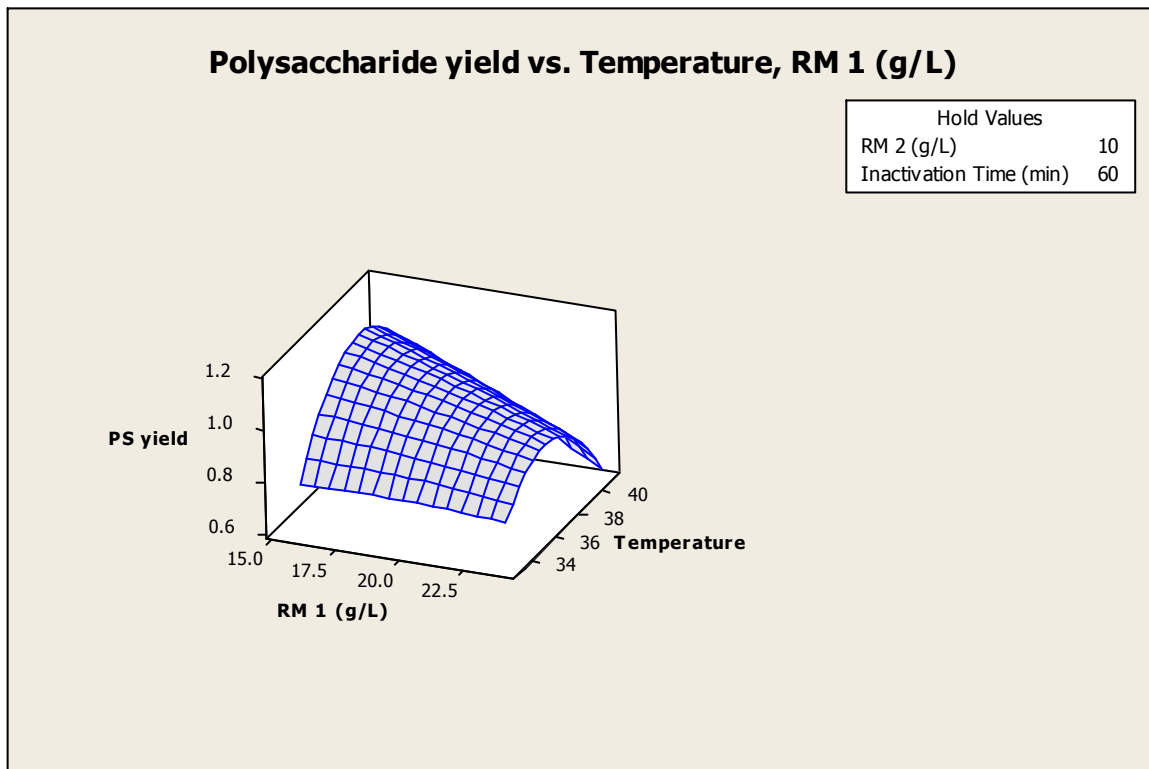
2069
2070
2071 **Polysaccharide yield is sensitive to Inactivation Time (not RM 1)**
2072 Inactivation time was critical to maintaining high polysaccharide yield. While it is known that the
2073 enzyme polysaccharidase is expressed under these conditions, therefore reducing the Ps overall
2074 MW, it is balanced with the rate of Ps release yield. Ps yield was less sensitive to changes in
2075 concentration of RM 1. Maximum polysaccharide yield was obtained when inactivation was
2076 initiated 50–100 minutes following glycerol depletion.
2077

2078   **Figure 4-10: Impact of Polysaccharide yield on Inactivation time**
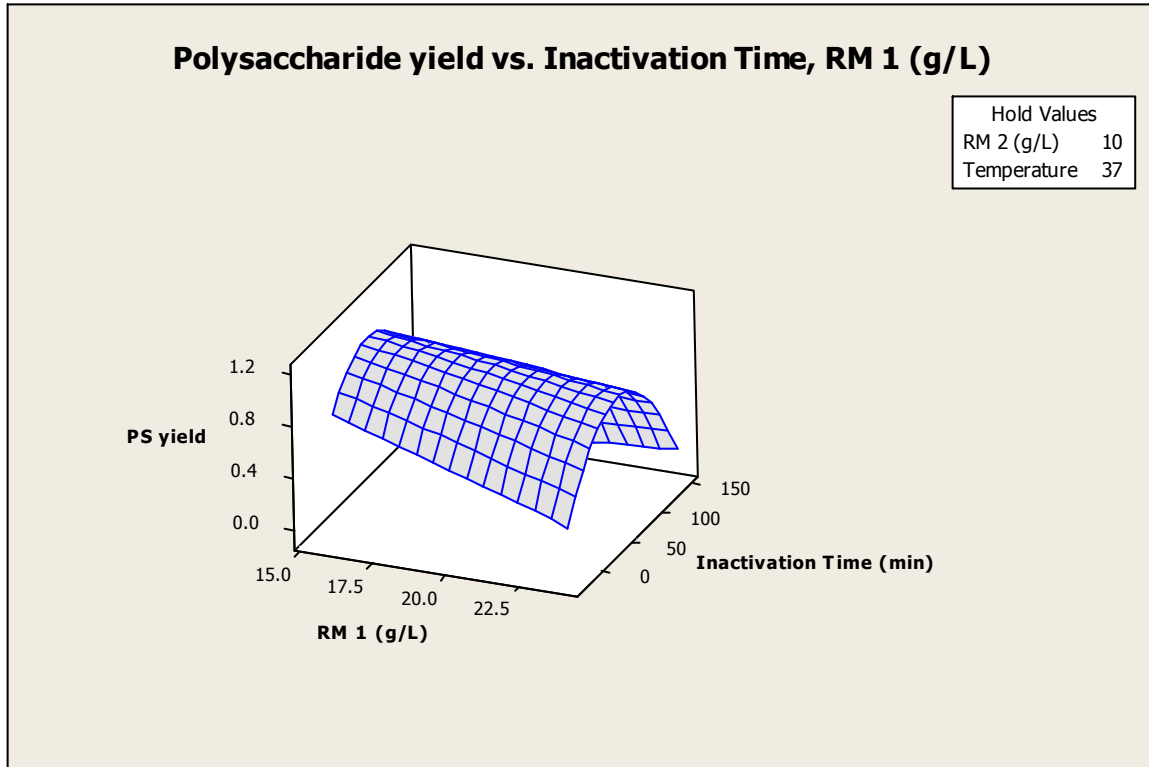
2079
2080

2081   **Polysaccharide yield is sensitive to both inactivation time and temperature**

2082   Polysaccharide yield was most sensitive to changes in inactivation time and temperature, as
2083   described in previous slides. Considering the direct impact of these process parameters to
2084   polysaccharide critical quality attributes, these two parameters were defined as CPPs.

2085

2086 **Figure 4-11: Impact of Polysaccharide yield dependence on Inactivation time and Temperature**
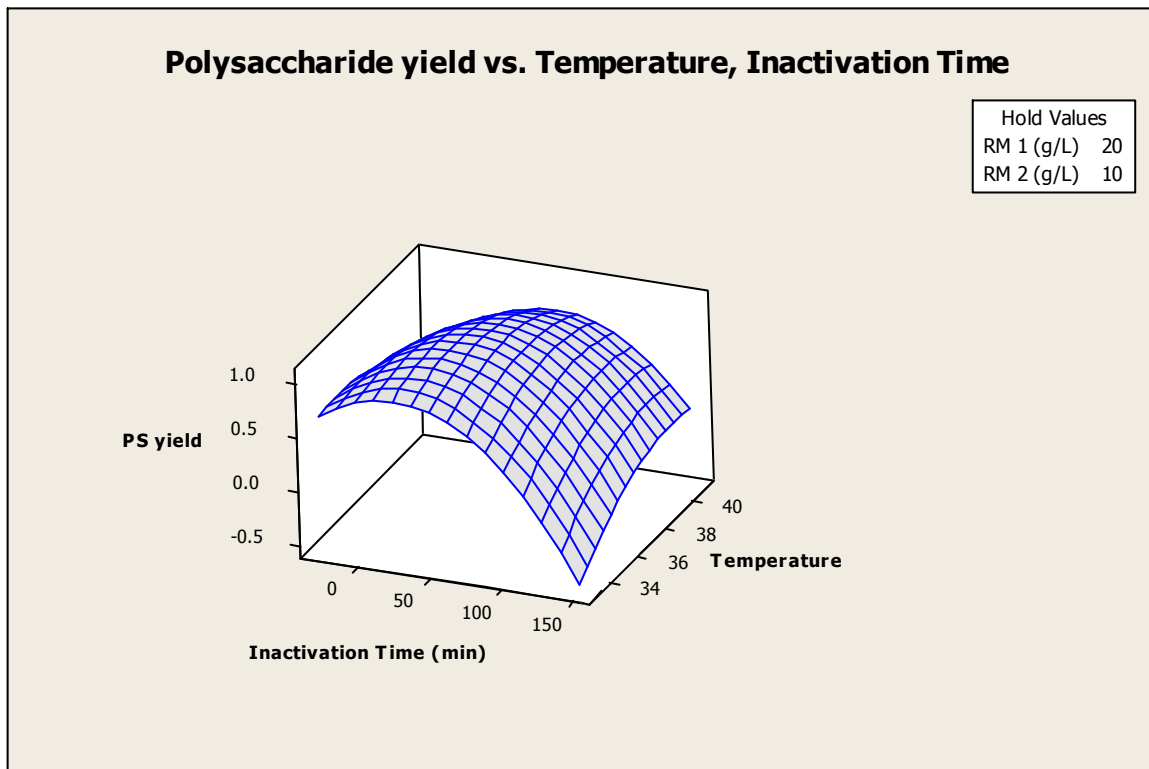
2087
2088

4.4.3. Response Surface Plots for Cell Lysis

2090 Figures below illustrate the impact of DOE parameters on % lysis (a key process attribute and
2091 measure of overall process performance). Cell lysis is a negative attribute that is coupled with
2092 cellular degeneration and endotoxin release. Factors explored included temperature,
2093 inactivation time, and raw materials 1 and 2 concentration.
2094

2095 **Cell lysis is sensitive to inactivation time and temperature**
2096 Minimal cell lysis was observed when inactivation was initiated by 50 minutes post glycerol
2097 depletion. This is also within the window of maximum polysaccharide yield as described in
2098 previous figures. Longer time prior to inactivation is coupled with increased cell lysis and higher
2099 risk of exceeding endotoxin limits, which is a CQA.
2100

2101 **Figure 4-12: Impact of Inactivation time and Temperature on cell lysis**

2102

Surface Plot of % lysis vs. Temperature, Inactivation Time (min)
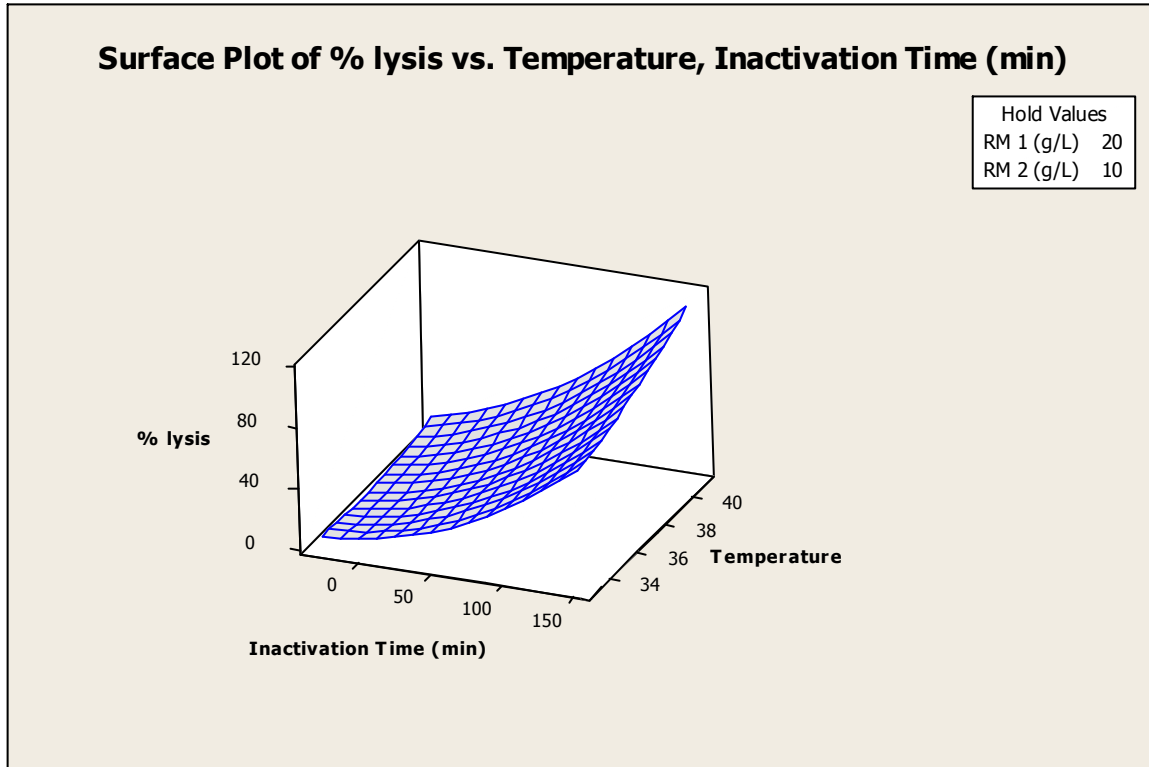
2103

2104 Cell Lysis is sensitive to temperature (not RM 2)

2105 Higher levels of cell lysis occurred when fermentation was incubated above 37°C. This correlates
2106 with higher endotoxin levels and therefore is undesirable.

2107

2108   **Figure 4-13 Impact of Temperature and RM2 on cell lysis**
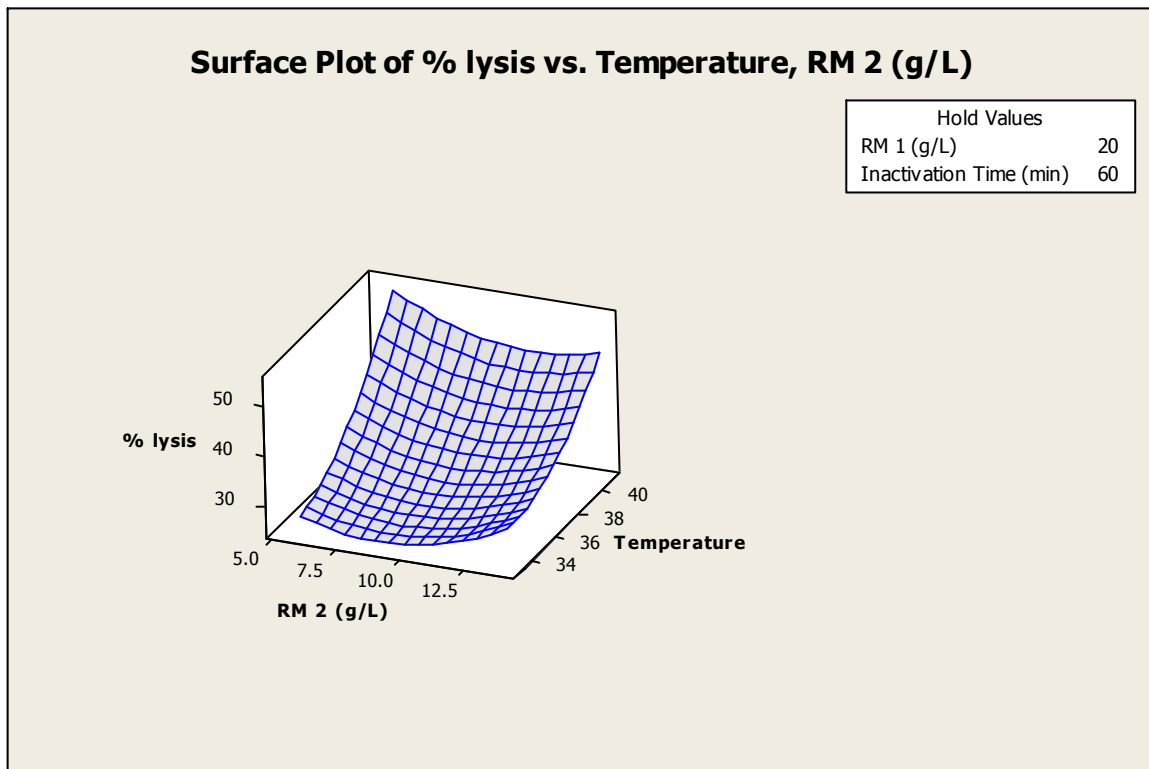
2109
2110

2111   **% lysis is sensitive to inactivation time (not RM 1)**
2112   Minimal cell lysis was observed when inactivation was initiated by 50 minutes post glycerol
2113   depletion. This is also within the window of maximum polysaccharide yield as described in
2114   previous figures. Longer time prior to inactivation is coupled with increased cell lysis and higher
2115   risk of exceeding endotoxin limits, which is a CQA. Concentration of RM 1 and/or 2 did not
2116   impact the degree of cell lysis.
2117

2118 **Figure 4-14: Impact of Temperature and RM2 on cell lysis**
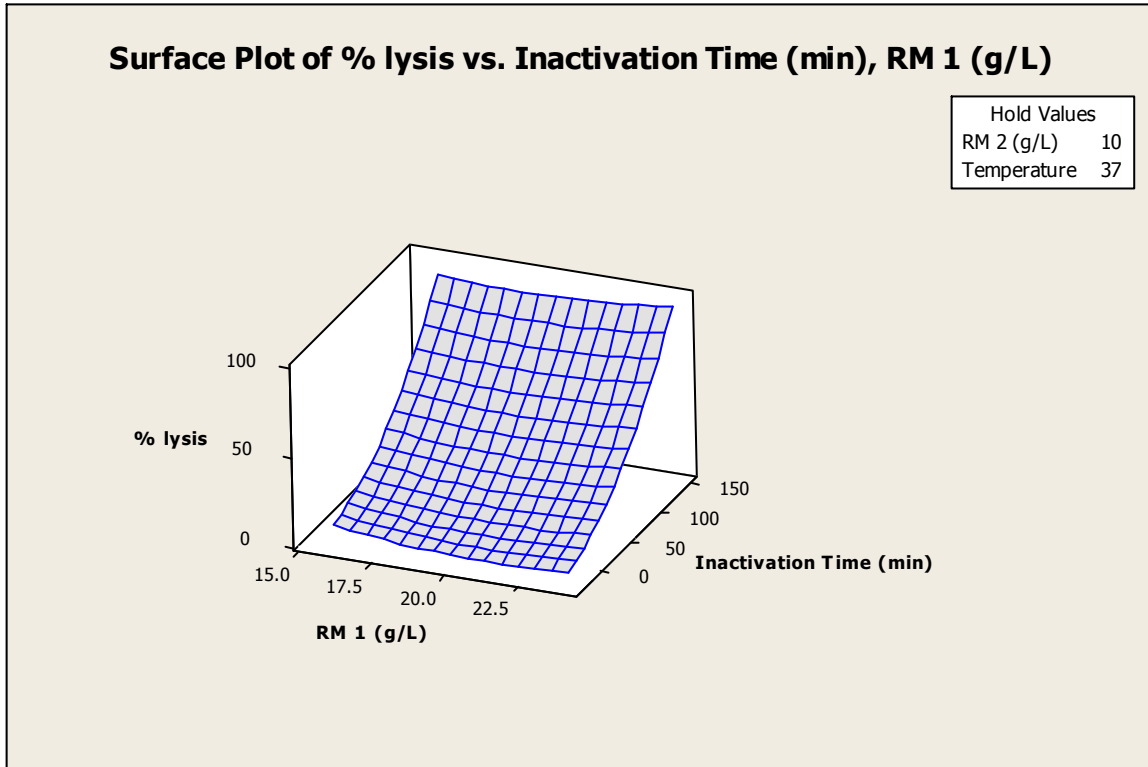
2119
2120
2121 **Figure 4-15: Impact of Inactivation Time and RM2 on cell lysis**
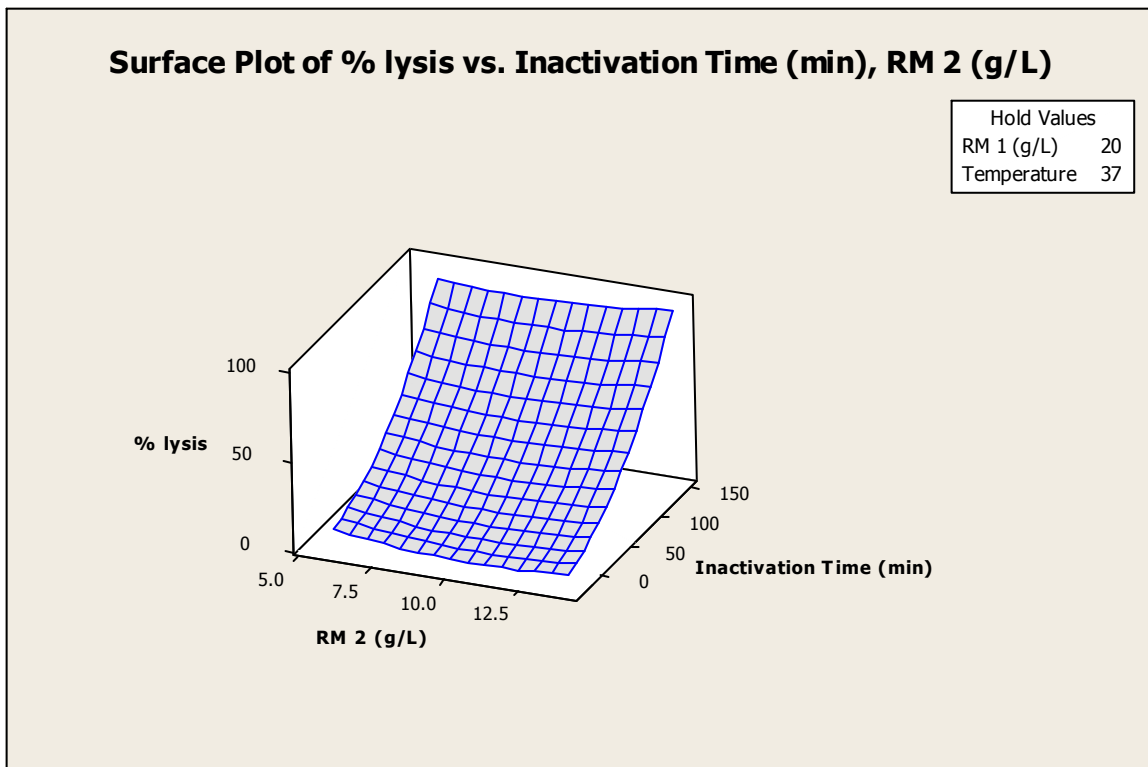
2122

2123 **% lysis is sensitive to temperature (not RM 1)**

2124 High incubation temperature promoted a higher degree of cell lysis. Target temperature (35–38
2125 C) supported lower levels of cell lysis.

2126

2127 **Figure 4-16: Impact of Temperature and RM1 on cell lysis**

2128

Surface Plot of % lysis vs. Temperature, RM 1 (g/L)

Hold Values
RM 2 (g/L) 10
Inactivation Time (min) 60
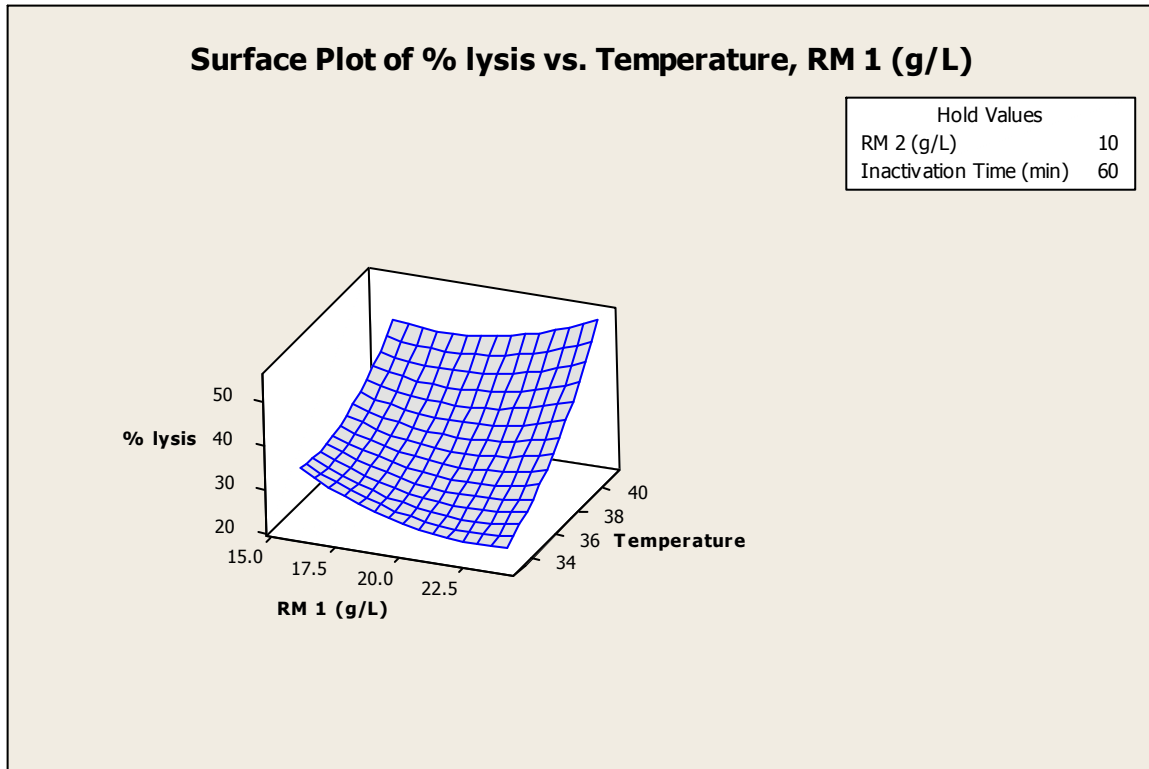
2129

4.5.   Selection of Critical Process Parameters (CPPs)

2131 Parameters, that influence the number of polysaccharide repeat units, polysaccharide yields and
2132 lysis of the cells, were identified using the design of experiments (DOE) and one factor at a time
2133 (OFAT). The factors are summarized in Table: 4-8.

2134

2135 Table: 4-8, Summary of Production Bioreactor Parameters' Impact on Polysaccharide CQAs
2136 Parameter ranges were defined based on DOE and OFAT experiments (provided in next section).

Process Parameter	IMPACT: Number of PS Repeat Units	IMPACT: Polysaccharide Yield (potency)	IMPACT: % Lysis	OVERALL Parameter Assessment
Concentration of Complex RM #1 (18 - 22 g/L)	NO	NO	NO	NOT a CPP
Concentration of Complex RM #2 (8 – 12 g/L)	YES	NO	NO	Key Operating Parameter
Time to Inactivation (time post glycerol Depletion): 30 – 150 min)	NO	YES	YES	CPP
Incubation Temperature (35 – 39?C)	NO	YES	YES	Well Controlled CPP

2137
2138

2139 Note that while the Ps is sized to a particular molecular weight (MW) in downstream steps, it is
2140 possible that the fermentation could produce a Ps of a MW less than the minimum size needed.
2141 This may also happen if the number of repeat units differs significantly. A well-controlled CPP
2142 has been defined in this case when redundant automation system in the overall manufacturing
2143 process is able to control the operating parameter in a very narrow range, as compared with the
2144 design space.
2145

2146 ## 4.6. One Factor at a Time Experiments to Establish Critical Process
2147 Parameters (CPPs) Range

2148 After the DOE and CPP selection, the critical ranges were determined for each parameter by
2149 OFAT. Again OFAT was chosen to define the range since there were no significant interactions
2150 among the parameters as determined by the DOE. Both the RM 2 concentration and time to
2151 inactivation) were further defined around their respective set points using experimentation.
2152 Incubation temperature was not further explored by experimentation despite being a CPP since
2153 it was determined to be a well-controlled parameter and a sufficient range was tested in the
2154 initial DOE.
2155

2156 For RM 2, the concentration was explored in the range of 7 to 11 g/L. The experimental range
2157 was skewed to the lower concentration since the effect on the response in the DOE was much
2158 more pronounced. The experimental results are shown in Figure 4-17.
2159

2160 **Figure 4-17: Polysaccharide Repeat Response to RM 2**
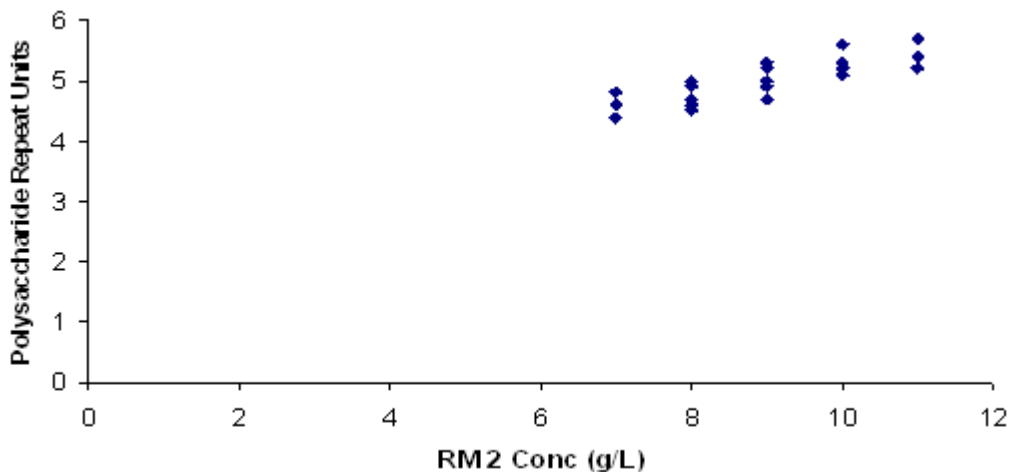
2161
2162

2163 This figure shows a threshold concentration of RM 2 is needed to yield a consistent number of
2164 polysaccharide repeat units. This RM 2 value is 10 ± 2 g/L.
2165

2166   4.7.   Exploration of RM 2 Lot-to-Lot Variability

2167   Because of the fact that RM 2 concentration is a CPP and the material is derived from an
2168   undefined plant, an initial screen was performed to assess the lot-to-lot variability. This was
2169   accomplished via an OFAT experiment with three independent lots of RM 2. The results are
2170   illustrated in Figure 4-18. Note that results of post-implementation early manufacturing data
2171   with more than 100 lots in consideration (section 1.9) subsequently revealed that lot-to-lot
2172   variability in RM 2 led to variability in product yield, which was not evident through this initial
2173   series of OFAT experiments.
2174
2175   **Figure 4-18: Polysaccharide Repeat Response to RM 2 Lots**
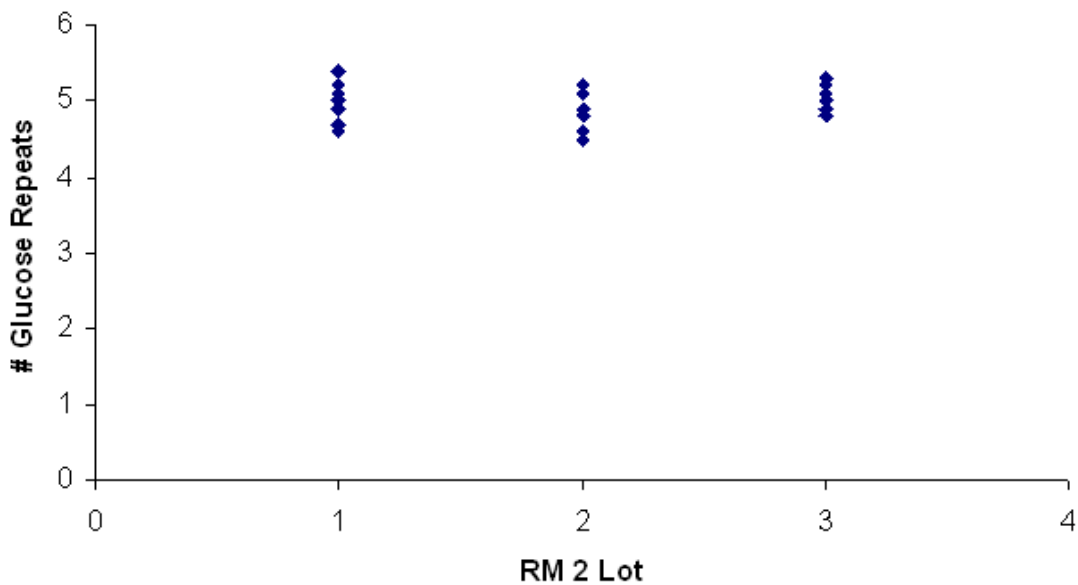
2176
2177   The results were compared by a T-test analysis, and there is not a significant difference among
2178   the lots (p < 0.05).
2179

2180   4.8.   Failure Modes and Effects Analysis

2181   4.8.1.   FMEA Methodology

2182   The failure modes and effects analysis is a risk assessment tool used to proactively identify and
2183   mitigate potential failure scenarios. The initial step in the analysis is to generate a list of process
2184   parameters to assess in the FMEA. Next, a risk prioritization number (RPN) is generated for each
2185   parameter based on assessment of the severity (S), occurrence (O), and the ability to detect (D)
2186   failures (see FMEA for full list). The product of these scores is used to determine the RPN
2187   (Equation 4-1), which enables a semi-quantitative ranking of process parameters.
2188

2189 **Equation 4-1: FMEA Risk Prioritization Number (RPN)**

2190 S x O x D = RPN
2191 Severity was defined based on the potential impact to the process and/or product as evaluated
2192 by the effect on in-process CQAs and final release tests (which include final product CQAs).
2193 Occurrence was defined as the likelihood that the failure mode would take place. The detection
2194 score was defined as the ability to recognize the potential failure (i.e. excursion of measured
2195 parameter from a pre-defined range) of a process parameter before the consequences are
2196 observed either in additional processing or after product release. A summary of the parameters
2197 is given in Table 4-9: FMEA Scoring System. The levels were chosen with weighting of 1, 3, or 9
2198 to clearly delineate the results.
2199
2200 **Table 4-9: FMEA Scoring System**

			Frequency
SEVERITY (S)	1 =	No impact to customer	
	3 =	Probable generation of impact on CQA	
	9 =	Known product quality impact or likely to fail release testing	
OCCURRENCE (O)	1 =	Likelihood of occurrence is remote	1 in 100
	3 =	Moderate failure rate without supporting documentation	1 in 20
	9 =	Assured of failure based on warranty data or significant testing	1 in 2
DETECTION (D)	1 =	Certain that failure will be found (including calibration errors)	
	3 =	Moderate chance that failure will be undetected (or detected after additional processing, but before release)	
	9 =	Certain that failure will be undetected (or detected after release)	

2201
2202
2203 In addition to the RPN, the FMEA was also used to evaluate operating ranges and process
2204 control. All parameters and potential failure modes were discussed and agreed upon jointly by a
2205 cross-functional team. Table 4-10: RPN Results Classification summarizes the classification of
2206 RPN results and the classification of the parameters as a CPP, non CPP, or potential CPP. The
2207 Failure Modes Effects Analysis is summarized in Table 4-11
2208
2209 **Table 4-10: RPN Results Classification**

RPN RESULT	CLASSIFICATION
1–8	Not a CPP
9–26	Potential CPP
27–729	CPP test experimentally for process range

2210 **Table 4-11: Failure Modes Effects Analysis**

Failure Modes Effects Analysis

Process Step or Variable or Key Input	Operating Parameter	Typical Operating Range	Sensitive to Scale of Operation	Potential Failure Effects	SEV	Potential Causes or Route of Failure	OCC	Current Process Controls	DET	RPN	Actions Recommended
What is the process step?	What is the operating parameter?	What is the targeted operating range?	Is the processing step sensitive to scale? (Y/N)	What is the impact on the Key Output Variables?	How Severe is affect to the customer?	What causes the Key Input to go wrong? (How could the failure mode occur?)	How frequent is cause likely to Occur?	What are controls that prevent the failure mode from occurring or detect it should it occur?	How probable is Detection of cause?	Risk Priority # to rank order concerns	What are the actions for reducing the Occurrence of the cause, or improving Detection? Should have actions on high RPN's or Severity of 9 or 10.
1000L Fermentation	Innoculate from Seed Fermentor	40 +/- 8L	N	Growth Failure	3	Failure in due to extreme varaition in growth paramter (pH, temperature)	1	On-line monitoring with automated alarms	1	3	N/A
	Media Addition	800 +/- 40L	N	Slight variability in volume of inoculum	3	load cell miscalibration	1	depends on load cell or not? Scale check?	1	3	N/A
	Complex RM 1 addition	16000 +/- 1600 g	N	possibility growth inhibition at high concentration; at low slight inpact to growth	3	Incorrect wieghment	1	Docuemenation	1	3	N.A
	Complex RM 2 addition	8000 +/- 800 g	N	High: impact to PS structure variability (glucose repeats); low = minimal impact to biomass; lot to lot variability may lead to non-robust productivity or PS structure variability	9	Incorrect wieghment	1	Docuemenation	3	27	Procedural Controls, scale calibration
	Glycerol Addition	8000 +/- 800 g	N	Change in final biomasl	3	Incorrect weighment	1	Documentation, scale calibration	1	3	NA
	Agitation	100 +/- 20 RPM	Y	Loss of agaition	3	Mechanical Failure	1	Preventative Maintenance, on-line monitoring with alarms	1	3	NA
	Pressure	Target 2 PSIG	N	Contamination	3	Loss of back pressure valve control	1	Preventative Maintenance, redundant control, on-line montoring with alarms	1	3	NA
	Air Overlay	10 +/- 1 LPM	N	Contamination	1	Loss of clean air, used for pressure control	1	Preventative Maintenance, on-line monitoring with alarms	1	1	NA
	pH	7 +/- 0.5 units	N	transient: minimal impact to biomass; sustained excursion: growth inhibition	3	Value failure, probe failure	1	On-line monitoring with automated alarms	1	3	NA
	Temperature	37 C +/- 2 C	N	transient: minimal impact to biomass; sustained excursion: growth inhibition	9	Value failure, probe failure, steam and or glycol loss	1	On-line monitoring with automated alarms	1	9	N/A
	Inactivation Criterion (glycerol concentration)	inactivation 30 minutes (+/- 10 minutes) post glycerol depletion (<0.1 g/L)	N	Cell lysis	9	Incorrect glycerol measurement, instrumentation failre	3	on-line monitoring manual hourly recorded off-line final sample	3	81	Maintian back up insturment
Phenol Inactivation	Phenol Concentration	0.5 +/- 0.1%	N	Incomplete inactivation / safety	9	Incorrect weighment	1	Documentation, scale calibration	3	27	In process phenol assay
	Incubation Time	1h +/- 15min	N	Incomplete Ps release, yeild loss	3	Human error	1	Documentation	1	3	N/A
	Incubation Temp	37 +/- 2 C	N	Incomplete inactivation / safety	9	Probe failure, temp control loss	1	On-line monitoring with automated alarms	1	9	N/A
	Agitation Rate	60 +/- 20 RPM	N	Incomplete inactivation / safety	3	Mechanical Failure	1	Preventative Maintenance, on-line monitoring with alarms	1	3	N/A

2211
2212
2213 The results from the FMEA are as follows. The inactivation criterion had the highest RPN score of
2214 81 and is a CPP. RM 2 had a score of 27 and is a CPP as a result of the significance of the
2215 concentration on the PS.

2216 Note that two parameters resulted in borderline RPN scores of 9, although upon further analysis
2217 only one of these parameters was carried forward as a CPP because of its differential in
2218 potential impact on product quality. The phenol concentration had a score of 9 due to the safety
2219 aspect for completing inactivation, but because it does not have direct quality impact on the
2220 product it was not determined to be a CPP. Incubation temperature also had a score of 9,
2221 although this was determined to be a CPP because of its impact on the quality attribute Ps size.
2222 However, as the redundant automation systems in the process are able to control the
2223 processing parameter in a very narrow range, as compared with the design space, incubation
2224 temperature is classified as a well-controlled CPP. The previously mentioned parameters would
2225 all require special attention during the scale-up to final manufacturing.
2226
2227 In addition, as part of the scale-up to final manufacturing, the ability of the downstream process
2228 to consistently clear residual host cell impurities, including proteins and host cellular DNA, is
2229 verified through process validation.
2230

2231 4.9. Continuous Improvement Based on Process Understanding

2232 Proactive monitoring of the fermentation process was implemented to leverage new technology
2233 to build scientific understanding. During the manufacturing, multivariate tools (random forest
2234 analysis) were used as a proactive process monitoring initiative to identify correlations between
2235 variability among input parameters to variability in process attributes such as OD at harvest. The
2236 random forest analysis has the ability to evaluate hundreds of process input parameters with
2237 respect to their impact on a given process attribute.
2238
2239 The multivariate analysis identified that variable nitrogen content contained in various lots of
2240 complex RM 2 was related to variability in cell mass at harvest. By controlling nitrogen content
2241 through setting acceptability criteria and implementing a release test and/or by procuring large
2242 volumes of a single lot of raw materials within these specifications, the variability in cell yield at
2243 the production stage was reduced.
2244

2245 **Figure 4-19. Control Chart of Fermentation Output (Optical Density)**
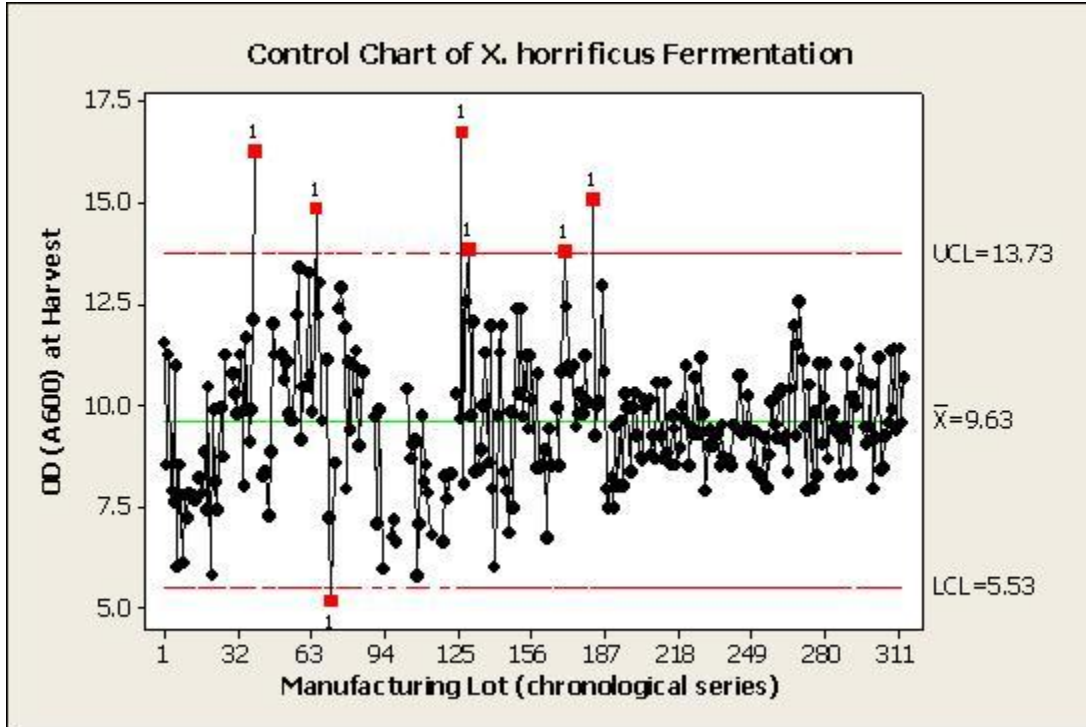
2246

2247 # 5.   Upstream (VLP) Section

2248 ## 5.1.   Executive Summary

2249 In the manufacturing process for recombinant VLP in gram negative organisms, the criticality of
2250 the final attributes is largely determined by the efficiency of the downstream processing.
2251 However, there should be a well-defined upstream process to provide a sufficient yield of
2252 upstream material with well-defined quality attributes for the downstream processing.
2253
2254 This document assesses the contribution of the upstream process in E. coli VLP production. Also,
2255 it looks at the potential impact of the quality attributes of the upstream material on the critical
2256 attributes of the bulk VLP. The harvest step of the upstream VLP production step was selected
2257 as an example of the applications of tools that would provide operational confidence in selecting
2258 input parameters that potentially can affect the quality attributes of the VLP.
2259
2260 Several commonly used tools have been explored throughout the document to illustrate the
2261 approach for selection of critical parameters and the design space, which support the
2262 operational ranges for continuous production post validation. Examples of post-validation
2263 changes that may or may not affect the quality attribute have also been shown. A rational
2264 approach to evaluate the risk of process changes associated with vaccine production has been
2265 taken. Common tools such as cause-and-effect (C&E) matrices and failure modes and effects
2266 analysis (FMEA) have been used to assess the risk of individual process parameter changes. Also,
2267 a DOE-based approach has analyzed the effects of these process parameters on the product
2268 quality attributes.
2269
2270 For the case study, the responses measured upstream do not directly impact the critical
2271 attributes of the bulk VLP after downstream processing. However, the downstream process
2272 involves a series of purification steps to achieve the final vaccine's desired critical attributes,
2273 such as size distribution, tertiary structure, purity etc.. So the overall efficiency of sizing depends
2274 on modeling a downstream process based on expected specific protein activity of the inclusion
2275 bodies upstream while assessing the initial purity of the material to ensure consistency of
2276 material delivered for downstream purification. The critical quality attributes of the bulk VLP will
2277 be defined downstream of the VLP harvest step.
2278
2279 For the E. coli VLP primary recovery steps, the following response parameters were assessed:
2280 protein content, pellet mass for each wash, purity (DNA, protein, lipid), SDS-PAGE profile, and
2281 percentage of monomer measurement. The scale-down models were used to reduce the
2282 number of parameters in series of fractional and full factorial designs. For the screening
2283 experiments (DOE #1), all these tests were performed for 16 runs in a fractional factorial design
2284 with all eight parameters.
2285

2286   In DOE #2, the design space was also defined using scale-down models from four factors that
2287   were selected from DOE #1. For the optimization studies to define the design space, a central
2288   composite rotatable design with 29 runs was used, and the design space was defined from
2289   analysis simulations using MATLAB software to generate response surface models. The control
2290   space was verified at scale with 16 repeat runs at the same conditions. This provided enough
2291   confidence to establish the protein content expected downstream for the VLP process. In all, the
2292   eight parameters were eventually reduced to four by relative importance for the harvest step.
2293
2294   For the purposes of illustration, only responses for protein content are used throughout the
2295   document. Primarily, the reason is that the quality and quantity of the protein upstream impact
2296   the downstream processing, during which the critical quality attributes of the bulk VLP are
2297   assessed for the vaccine. These responses will then be monitored on a continuous basis.
2298   Downstream processing tests will include tests for purity and percentage of monomers.
2299
2300   Combining with the downstream purification and drug product analysis, this document can
2301   contribute to development of a more systematic way to validate the manufacturing processes at
2302   late stages of vaccine development and production.
2303

2304 5.2. Process Descriptions

2305 Figure 5-1: General Process Flow Diagram (Upstream)

INPUTS		OUTPUTS

Seed

INPUTS:
- Inoculum Volume
- Thaw Method
- Preservative (ex. glycerol)
- Freezing Storage Temperature
- Enclosure

OUTPUTS:
- Viability at Thawing
- Viable Cell ~~Total~~ Concentration ~~of Live~~
- Lag Phase Duration

Starter Flask

INPUTS:
- Media
    - Base
    - Trace Elements
    - Supplements
- Inoculum
    - Volume
    - Concentration
- RPM
- Temperature
- pH
- Antibiotic Selection'

OUTPUTS:
- Sterility
- % Viability/live
- Final OD
- Plasmid Retention

Pre-Induction: 20L Fermentor

INPUTS:
- Media
    - Base
    - Trace Elements
    - Supplements
- Inoculum (conc., %viability)
- Temperature
- pH
- Air/O_2
- Growth Phase Duration
- Glucose Feed Rate Duration

OUTPUTS:
- Initial OD
- Pre-Induction OD
- % Viability
- Plasmid Vector and Insert Retention (copy no., qPCR, RNA expression levels)

Induction: 20L Fermentor

INPUTS:
- Temperature
- pH
- Air/O_2
- IPTG Concentration
- Induction Duration

OUTPUTS:
- Final OD
- Yield (specific yield, biomass)

Primary Recovery

INPUTS:
- Homogenization
    - Method
    - Pass No.
    - Cooling
- Washes
    - Number
    - Urea Concentration
- Centrifugation
    - Speed
    - Duration
    - Temperature
- Solubilization
    - Urea Concentration
    - pH
    - Reducing Agent
        - Type (ex. L-cys, DTT)
        - Concentration
    - Duration

OUTPUTS:
- Specific Yield
- Pellet Mass for Each Wash
- Total Protein
- Purity
    - DNA
    - Protein
    - Lipid
- SDS-PAGE Profile
- % Monomer Measurement (GF, HPLC, native SDS)

2306

2307 ## 5.2.1. Brief Description of Each Process Step

2308 The following is a brief description of the process steps outlined in the proposed VLP primary
2309 recovery process. Variables and key considerations are presented where applicable.

2310 **5.2.1.1. Seed**

2311 Seed vials are prepared in a logarithmic growth phase according to standard procedures to
2312 generate sufficient inoculum per vial to initiate a viable culture of the desired recombinant
2313 organism. Antibiotic selection on the culture prior to cryopreservation is optional but likely in
2314 order to ensure a high percentage of recombinant organisms at the time of thawing. If present,
2315 nonrecombinants may overwhelm a culture, resulting in reduced protein content per biomass.
2316
2317 The choice of preservative is made based on characteristics of the host organism and for
2318 bacterial hosts is likely to be a glycerol-based cryopreservative. Maximum viability of freshly
2319 thawed vials will ensure a prompt initiation of the culture in the starter flask, reducing process
2320 time and maximizing expression levels. Plasmid copy number is to be assessed at the end of the
2321 starter culture.
2322

2323 **5.2.1.2. Starter Flask**

2324 Generally richer than cultures in subsequent steps, the starter culture ensures maximal recovery
2325 of an organism post cryopreservation. Organisms are usually in logarithmic growth at the end of
2326 culturing, creating a consistently high concentration of cells prior to inoculation into the pre-
2327 induction fermentor. Vial-to-vial variations in total number of organisms, concentration, volume,
2328 viability, etc., are usually minimized during starter flask culturing such that the inoculum for the
2329 20L fermentor is consistent from batch to batch.

2330 **5.2.1.3. Pre-induction Culture: 20L Fermentor**

2331 The pre-induction culture is inoculated with sufficient starter culture to initiate a logarithmic
2332 growth of the organism in the absence of an inducer. Log phase cells are maximally viable such
2333 that once they are induced, a maximum amount of VLP monomer is expressed. Final pre-
2334 induction optical density should be maximized while ensuring that the culture remains at log
2335 phase prior to induction. Protein contents depend on culture condition at the time of induction.

2336 **5.2.1.4. Induction Culture: 20L Fermentor**

2337 Induction is performed by addition of an appropriate inducer and as defined by the host vector
2338 expression system. Duration, temperature, and concentration at induction all affect the final
2339 protein content. The desired conditions at this stage are those that maintain the metabolism of
2340 the cell for the longest time to maximize continued expression of the desired VLP monomer. The
2341 expressed VLP monomers are accumulated as an inclusion body (IB) in the recombinant
2342 organisms.

2343 **5.2.1.5. Primary Recovery**

2344 Recovery of the product from inclusion bodies requires disruption of the cell wall/membrane
2345 such that IBs are released. Passage through a homogenizer or microfluidizer can result in heat
2346 transfer and cause enzymatic and/or thermal degradation of the product. To minimize this
2347 potential negative effect, cooling is often employed during IB release. In addition, ineffective

2348 homogenization may cause incomplete release of IBs from the cell and thus their loss in
2349 subsequent centrifugation steps. Passage number, channel width, and other factors including
2350 pressure determine the efficiency of cell disruption.
2351
2352 Furthermore, denaturation/solubilization is a critical step in primary harvest. It separates the
2353 aggregated IB mass and generates individual proteins, which can then be recovered by standard
2354 chromatographic techniques. Inefficient denaturation/solubilization results in aggregated
2355 material and poor recovery of VLP monomer, especially during subsequent centrifugation steps.
2356 Duration of denaturation and denaturant concentration both affect the degree of solubilization
2357 and overall protein content.
2358

5.2.2. Prior Knowledge

2360 The primary objective of the upstream process is to have a maximal amount of product for
2361 downstream processing while taking into consideration any conditions that will impact the
2362 purity percentage of the IBs going downstream. Impurity at the IB stage is generally less than 5%
2363 and dependent on inclusion body washing efficiency. Based on this prior knowledge, purity
2364 assessment as a potential CQA for the upstream process has been excluded. The overall
2365 efficiency of sizing the downstream process to achieve the desired CQAs is dependent on
2366 modeling a process based on expected protein contents of the IBs upstream. The CQAs of the
2367 VLP will be defined downstream of the VLP harvest step.
2368
2369 The purification of IBs from over-expressing host cells generally involves the process of cell lysis
2370 and subsequent centrifugation. The IBs are a high-density, intracellular body resistant to the
2371 effects of cell lysis. Once lysis is complete, the IBs are released and easily separated from all
2372 other solubilized cell debris by low-speed differential centrifugation. The pellet resulting from
2373 such centrifugation is highly enriched in over-expressed protein. However, resolubilization of
2374 the pellet without further washing fails to remove contaminating proteins, which are readily
2375 identified by SDS-PAGE. IB washes result in a much cleaner product, but the washes are often
2376 accompanied by some product loss.
2377
2378 From prior knowledge, the presence of the contaminating material results mainly from
2379 nonspecific adsorption on the surface of the inclusion bodies following cell lysis and
2380 contaminating proteins/nucleic acids, etc., that are not likely integrated into the IB.
2381 Furthermore, the IB can be considered a highly pure aggregate of the over-expressed protein of
2382 interest, which if purified appropriately should yield protein purity levels >95%.

2383 **5.2.2.1. Quality at Upstream/Primary Recovery**
2384 Unlike most other cell-derived recombinant products, proteins over-expressed in hosts such as
2385 E. coli are segregated into inclusion bodies that do not preserve the secondary and tertiary
2386 structure of the protein of interest. As such, the product is recovered during primary recovery as
2387 a nonfunctional protein, which is refolded during intermediate processing steps into a functional
2388 product with the desired structure. Subsequent purification steps are employed to remove
2389 residual impurities as well as product that lacks the desired functional structure.
2390
2391 Since the quality of the product is determined only during the intermediate refolding steps, the
2392 harvest and primary recovery steps that precede this refolding play no role in the final product

2393 quality beyond the yield of the intact (full-length) protein within the structure of the inclusion
2394 body. Inclusion bodies effectively remove the product from the general metabolism of the cell,
2395 notably from the action of proteases that would otherwise degrade the product. As such, the
2396 recovered product from inclusion bodies tends to be full-length intact protein, abrogating the
2397 need for additional design requirements to ensure product quality. This leaves the overall
2398 product yields as a priority in a well-designed upstream process.

2399 **5.2.2.2. Optimizing Yields Vs. Optimizing Purity at Primary Recovery**

2400 Although it is pointed out that yields are potentially higher if modified conditions are applied
2401 during primary recovery, this increase in yields comes at the cost of decreased purity of product.
2402 Although the downstream process can be modified to accommodate a larger impurity capacity,
2403 this generally becomes cost prohibitive relative to the gains achieved in product yield.
2404
2405 The proposed criteria for the primary recovery are expected to generate estimated impurity
2406 levels that are well within the capacity of the downstream process to remove them. The loss of
2407 product is therefore offset by the reduced costs downstream. It is a common occurrence that a
2408 compromise between product yield and purity is made throughout a mature purification
2409 process. It is also possible that the desired compromise can be adjusted depending on protein
2410 expression levels, product value, downstream processing costs, etc. These can be finalized once
2411 the process is better defined.
2412
2413 5.2.3. Rationale for Selecting Primary Recovery as a Unit of Operation for Quality by
2414   Design Analysis

2415 Primary recovery is the last step in VLP production prior to purification. It is complex and is
2416 known to be affected by more than a dozen process parameters. This is twice as many as some
2417 other single steps during the upstream manufacturing process, considering the number of
2418 factors that affect product quality and quantity.
2419
2420 The primary recovery step is also impacted by other changes accumulated through the upstream
2421 process optimization and manufacturing. Thus, it can be a direct measurement of the effect of
2422 these process modifications. In addition, what is generated through this step is used in the next
2423 stage of the VLP production. The step has a significant impact on all subsequent manufacturing
2424 processes, especially purification, which takes place following completion of the primary
2425 recovery step. Finally, risk assessment using cause-and-effect (C&E) matrices suggests the
2426 primary recovery impacts the quality of VLP to a considerable extent during VLP production.
2427
2428 The complexity of the primary recovery step and its bridging function in determining the protein
2429 content and initial quality characteristics of the VLP for downstream processing demonstrate its
2430 importance to be chosen as a unit of operation for the VLP Quality by Design case study.
2431

2432 **Figure 5-2: Pareto Graph (by Process Step)**
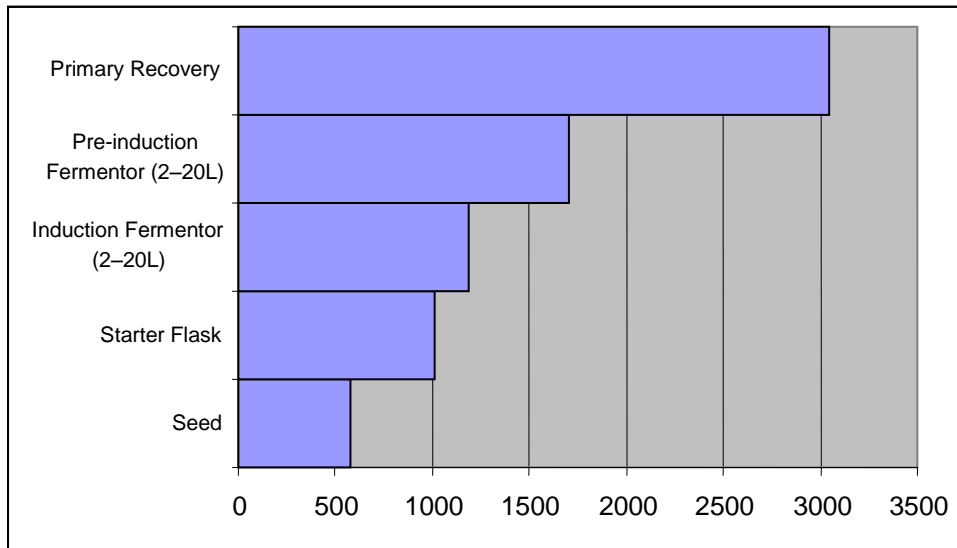
2433
2434

2435 5.2.4.   Summary Process Flow Diagram of VLP Primary Recovery Step

2436 **Figure 5-3: Summary Process Flow Diagram of VLP Primary Recovery Step**
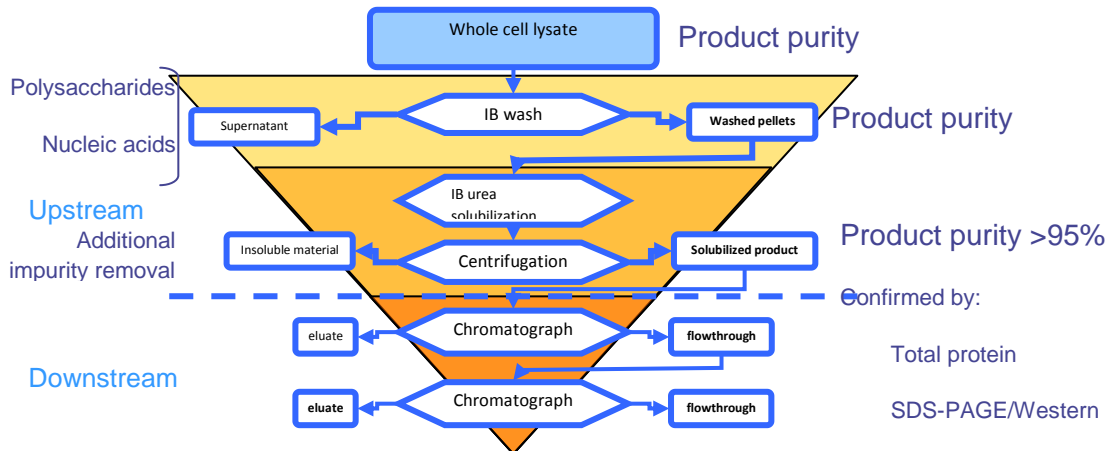
2437

2438 ## 5.3.   Process Risk Assessment

2439 ### 5.3.1.   Risk Assessment Using Cause-and-Effect Matrices

2440 **Table 5-1: Scoring of Process Parameters and Quality Attributes**

Process Parameters		Attributes[1]	
Impact Score	Ranking Criteria	Weight Score	Ranking Criteria
10	Strong relationship is known based on available data and experience.	10	Established or expected direct impact on safety and/or efficacy of product.[2]
7	Strong relationship is expected.	7	Moderate or indirect impact on safety and/or efficacy. Direct impact on efficiency.
5	Not-so-strong relationship is expected or unknown.	5	Low or unlikely impact to product safety and/or efficacy. Moderate or indirect impact efficiency.
1	Known to not have a relationship.	1	No impact to product safety and/or efficacy. Low or unlikely to impact efficiency.

2441 [1] Process performance attributes may have no direct impact on product quality, safety, or efficacy but are assessed where they are important indicators of focus area
2442 function or performance consistency. Examples include step recoveries and overall protein content.
2443 [2] May include efficiency attributes, but most efficiency attributes are not a 10 unless they significantly impact product viability.
2444
2445 Total Score = ∑ (impact score * weight score)
2446

2447 **Table 5-2: Cause-and-Effect Matrix**

		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
Quality Attributes Score		5	5	5	10	10	7	
Process Step	Parameter							
Seed	Inoculum Volume	5	5	7	1	1	1	112
	Thaw Method	5	5	7	1	1	1	112
	Preservative (ex. glycerol, DMSO)	5	5	7	1	1	1	112
	Freezing Storage Temp.	5	5	7	1	1	1	112
	Enclosure	5	5	10	1	1	1	127
Starter Flask	Base Media + Trace Elements/Supplements	5	10	10	1	1	1	152
	Inoculum Volume	5	7	10	1	1	1	137
	Inoculum Conc.	5	7	10	1	1	1	137
	RPM	5	7	10	1	1	1	137
	Temp.	5	7	10	1	1	1	137
	pH	5	7	10	1	1	1	137
	Antibiotic Selection	10	10	10	1	1	1	177
Pre-induction Fermentor (2–20L)	Base Media + Trace Elements/Supplements	10	10	10	1	5	1	217

		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
	Inoculum (conc., % viability)	10	10	10	1	5	1	217
	Temp.	10	10	10	1	5	1	217
	pH	7	10	10	1	5	1	202
	Air/O2	7	10	10	1	5	1	202
	Growth Phase Duration	10	10	10	1	5	1	217
	Glucose Feed Rate	10	10	10	1	5	1	217
	Glucose Feed Rate Duration	10	10	10	1	5	1	217
Induction Fermentor (2–20L)	Temp.	10	10	10	1	10	1	267
	pH	7	10	10	1	5	1	202
	Air/O2	10	10	10	1	10	1	267
	IPTG Conc.	10	5	5	1	10	1	217
	Induction Duration	10	7	7	1	10	1	237
Primary Recovery	Pressure - Homogenization	1	10	10	10	10	1	312
	Pass No. - Homogenization	1	10	10	10	10	1	312
	Cooling - Homogenization	1	5	5	7	10	1	232
	Number of Washes	1	10	10	7	10	1	282
	Urea Conc. - Washes	1	10	10	10	10	1	312
	Speed - Centrifugation	1	7	7	7	1	1	162

		Protein Content (Specific Activity by ELISA)	Pellet Mass for Each Wash	Total Protein	Purity (DNA, Protein, Lipid)	SDS-PAGE Profile	% Monomer Measurement (GF, HPLC, Native SDS)	Total Score
	Duration - Centrifugation	1	5	5	10	1	1	172
	Temp. - Centrifugation	1	5	5	5	7	1	182
	Urea Conc. - Solubilization	1	10	10	1	10	10	285
	pH - Solubilization	1	5	5	1	1	7	124
	Reducing Agent Type (ex. L-cys, DTT) - Solubilization	1	1	1	1	7	10	165
	Reducing Agent Conc. - Solubilization	1	7	7	1	7	10	225
	Duration - Solubilization	1	7	7	1	7	10	225

5.3.1.1. Parameters with the Highest Potential Impact on Quality Attributes

From the Pareto, the parameters with the highest potential to impact any of the response attributes have been highlighted. These attributes include protein content measured as specific activity by ELISA as the critical quality attribute of the primary recovery step.

2451   **Figure 5-4: Pareto Graph (Primary Recovery Step)**
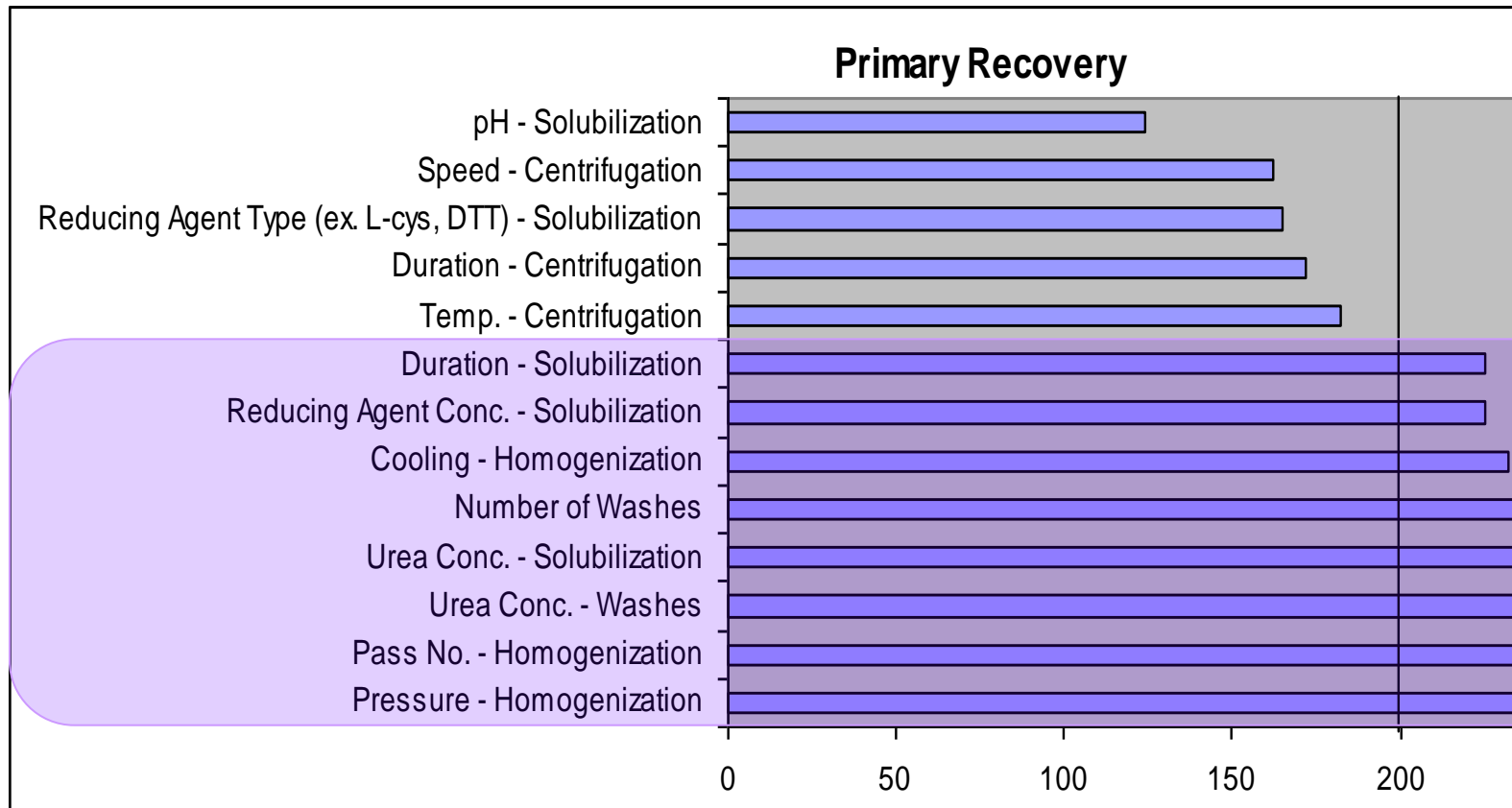
2452

2453   ## 5.4.   Addressing High-Risk Process Parameters/Material Attributes

2454   ### 5.4.1.   Selection of Parameters (from Primary Recovery Step) for DOE

2455   **5.4.1.1. Parameters' Selection Scoring Guidelines**

2456   • **Technical impact:** Using technical literature and/or theory as a guide, how important is this process variable?

2457   – 1 = Not important

2458   – 3 = Relatively important

2459   – 9 = Extremely important

2460   • **Ability to adjust:** When working with the manufacturing process, how easy is it to make changes to this process variable?

2461   – 1 = Difficult

2462   – 3 = Moderate difficulty

2463   – 9 = Very easy to change

2464   • **Support by process data:** When assessing the process control and performance, how much does the process data support the

2465   relative importance of this variable?

2466   – 1 = No importance observed

2467   – 3 = Moderate importance observed

2468   – 9 = High level of importance

2469   **5.4.1.2. Parameters' Selection Scores**

2470 **Figure 5-5: Parameters' Selection Scores**

Process Input or Factor	Purpose	Investigational Range		Units	Type	Technical Impact	Ability to Adjust	Supported by Process Data	Importance Index
		Low	*High*			*3*	*1*	*9*	
Pressure – Homogenization	release of the product from intracellular compartment	10000	20000	psi	Continuous	9	1	9	109
Pass No. – Homogenization	no. of repeats with which to achieve maximum product release	1	3	N/Ap	Continuous	9	9	9	117
Cooling – Homogenization	prevention of product degradation due to excessive heat buildup	5	15	min	Continuous	3	9	9	99
Number of Washes	removal of impurities	1	4	N/Ap	Continuous	3	9	9	99
Urea Conc. – Washes	efficiency of impurity removal	1	5	M	Continuous	3	9	9	99
Speed – Centrifugation	pelleting of product inclusion bodies	10000	20000	g	Continuous	1	9	3	39
Duration – Centrifugation	pelleting of product inclusion bodies	10	60	mins	Continuous	1	9	3	39
Temp. – Centrifugation	minimizing of product enzyme degradation	4	24	°C	Continuous	3	9	3	45
Urea Conc. – Solubilization	solubilization of product	5	10	M	Continuous	9	9	9	117
pH – Solubilization	solubilization of product	5	10	N/Ap	Continuous	3	3	3	39

CMC-VWG

Process Input or Factor	Purpose	Investigational Range		Units	Type	Technical Impact	Ability to Adjust	Supported by Process Data	Importance Index
		Low	High			3	1	9	
Reducing Agent Type (ex. L-cys, DTT) – Solubilization	solubilization of product – reduction of disulfide cross-linking	DTT	L-cys	N/Ap	Discrete	3	3	3	39
Reducing Agent Conc. – Solubilization	solubilization of product – reduction of disulfide cross-linking	0.5	50	mM	Continuous	9	9	9	117
Duration – Solubilization	solubilization of product – reduction of disulfide cross-linking	3	15	hrs	Continuous	9	9	9	117

2471

2472   **Top 80% of parameters ranked by importance index chosen as candidate factors for DOE**

2473   • Minimum: 39

2474   • Maximum: 117

2475   • Selection Boundary: 94 (= 0.8*117)

CMC-VWG

2476 ## 5.5. DOE #1: Fractional Factorial Design (Scale-Down Model – 2L Fermentor)

2477 DOE#1 consisted of 16 runs using eight factors - Resolution 4, designed to assess some two-factor interactions.
2478 From the C&E and selection of parameter analysis, eight factors are potentially critical to all the performance attributes at the VLP
2479 harvest step. Since there are eight factors, a fractional factorial design at a small scale is used as the first screening step to assess
2480 interaction and confounding effects and to select the parameters that have the highest impact for the next series of experiments.
2481 ### 5.5.1. Analysis of the Fractional Factorial Design

2482 **Table 5-3: Analysis of the Fractional Factorial Design (DOE #1)**

Run #	Pattern	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Number of Washes	Urea Conc. – Washes	Urea Conc. – Solubilization(M)	Reducing Agent Conc. – Solubilization (mM)	Duration – Solubilization (hrs)	Specific Activity Protein Content
1	––––––––	10000	1	5	1	1	5	0.5	3	0.92
2	–––++++–	10000	1	5	4	5	10	50	3	1.03
3	––+–++–+	10000	1	15	1	5	10	0.5	15	1.34
4	––++––++	10000	1	15	4	1	5	50	15	1.43
5	–+––+–++	10000	3	5	1	5	5	50	15	1.19
6	–+–+–+–+	10000	3	5	4	1	10	0.5	15	1.24
7	–++––++–	10000	3	15	1	1	10	50	3	1.23
8	–++++–––	10000	3	15	4	5	5	0.5	3	0.97
9	+––––+++	20000	1	5	1	1	10	50	15	1.40
10	+––++––+	20000	1	5	4	5	5	0.5	15	1.29
11	+–+–+–+–	20000	1	15	1	5	5	50	3	1.34

Run #	Pattern	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Number of Washes	Urea Conc. – Washes	Urea Conc. – Solubilization(M)	Reducing Agent Conc. – Solubilization (mM)	Duration – Solubilization (hrs)	Specific Activity Protein Content
12	+–++–+––	20000	1	15	4	1	10	0.5	3	1.50
13	++––++––	20000	3	5	1	5	10	0.5	3	0.51
14	++–+––+–	20000	3	5	4	1	5	50	3	0.50
15	+++––––+	20000	3	15	1	1	5	0.5	15	1.17
16	++++++++	20000	3	15	4	5	10	50	15	1.18

2483   **5.5.1.1. Pareto Plot of Estimates**

2484   **Figure 5-6: Pareto Plot of Estimates (DOE #1)**

Term	Estimate
Pass No. - Homogenization(1,3)	-0.1412500
Duration - Solubilization(3,15)	0.1400000
Cooling - Homogenization(5,15)	0.1300000
Pressure - Homogenization*Pass No. - Homogenization	-0.1300000
Pressure - Homogenization*Cooling - Homogenization	0.0562500
Urea Conc. - Solubilization(5,10)	0.0387500
Urea Conc. - Washes(1,5)	-0.0337500
Pressure - Homogenization(10000,20000)	-0.0287500
Pressure - Homogenization*Reducing Agent Conc. - Solubilization	-0.0287500
Reducing Agent Conc. - Solubilization(0.5,50)	0.0225000
Pressure - Homogenization*Duration - Solubilization	0.0087500
Pressure - Homogenization*Number of Washes	0.0037500
Pressure - Homogenization*Urea Conc. - Solubilization	-0.0025000
Number of Washes(1,4)	0.0025000
Pressure - Homogenization*Urea Conc. - Washes	0.0025000

2485

2486   **5.5.1.2. Conclusions from DOE #1**

2487   Main factors Pass No. – Homogenization, Duration – Solubilization, Cooling – Homogenization, and
2488   interaction Pressure – Homogenization*Pass No. – Homogenization show relatively higher estimates
2489   compared with the other factors based on the Pareto Plot (Figure 5-6). Thus, these four factors will
2490   be used for the next experimental design runs.
2491

2492   ## 5.6.   DOE #2: Central Composite Design for Control/Manufacturing Space
2493   ## (Scale-Down Model – 2L Fermentor)

2494   Based on the knowledge learned from the first run, four factors (Pressure – Homogenization, Pass
2495   No. – Homogenization, Cooling – Homogenization, and Duration – Solubilization) were used for a
2496   central composite design run in DOE #2. DOE #2 consisted of 29 runs using the four factors, designed
2497   to assess the design space and optimum responses.

2498   ### 5.6.1.   Analysis of the Central Composite Design

2499   **Table 5-4: Analysis of the Central Composite Design (DOE #2)**

Run #	Pattern	Pressure-H (x1)	Pass #-H (x2)	Cooling-H (x3)	Duration of Solubilization (x4)	Specific Activity by ELISA - Protein Content (y)
1	–+––	1000	3	5	3	*0.68*
2	+–++	2000	1	15	15	*0.91*
3	––++	1000	1	15	15	*0.65*
4	00a0	1500	2	0	9	*0.45*
5	0	1500	2	10	9	*0.95*
6	+–+–	2000	1	15	3	*0.89*
7	++––	2000	3	5	3	*0.44*
8	+++–	2000	3	15	3	*0.7*

Run #	Pattern	Pressure-H (x1)	Pass #-H (x2)	Cooling-H (x3)	Duration of Solubilization (x4)	Specific Activity by ELISA - Protein Content (y)
9	−−−+	1000	1	5	15	0.49
10	+−−+	2000	1	5	15	0.52
11	++++	2000	3	15	15	0.69
12	−−−−	1000	1	5	3	0.59
13	000A	1500	2	10	21	0.58
14	00A0	1500	2	20	9	0.73
15	0	1500	2	10	9	0.77
16	0a00	1500	0	10	9	0.5
17	0	1500	2	10	9	1.05
18	−+++	1000	3	15	15	0.65
19	−++−	1000	3	15	3	0.53
20	0	1500	2	10	9	1.23
21	++−+	2000	3	5	15	0.78
22	−−+−	1000	1	15	3	0.71
23	000a	1500	2	10	-3	0.49
24	+−−−	2000	1	5	3	0.53
25	A000	2500	2	10	9	0.41
26	−+−+	1000	3	5	15	0.64
27	a000	500	2	10	9	0.43
28	0A00	1500	4	10	9	0.98
29	0	1500	2	10	9	1

5.6.1.1. Conclusions from DOE #2

The central composite design data in Table 5-4 is used to develop a quadratic Response Surface Model RSM model (second-degree polynomial) that can capture the curvature in the data.

The RSM model :

$y = 0.65+4.18E\text{-}06*x1+0.03*x2+0.01*x3+0.004*x4-4.71E\text{-}09*(x1-15000).^2-3.75E\text{-}06*(x1-15000).*(x2-2)-0.038*(x2-2).^2+1.95E\text{-}5*(x1-15000).*(x3-10)-0.013*(x2-2).*(x3-10)-0.003*(x3-10).^2+8.75E\text{-}07*(x1-15000).*(x4-9.2069)+0.006*(x2-2).*(x4-9.2069)-0.0003*(x3-10).*(x4-9.2069)-0.003*(x4-9.2069).^2;$

where y = Specific activity by ELISA protein content , x1 = Pressure – H, x2 = Pass # - H, x3 = Cooling – H, and x4 = Duration of solubilization

5.7.   Constraints for Maximum Protein Content

2513

2514   Monte Carlo simulation was performed to obtain the optimal constraints for maximum protein
2515   content. In the simulation, 100,000 realizations were sampled from normally distributed populations
2516   to evaluate the RSM model for protein content. The mean values used were the optimum point
2517   based on the model, and the standard deviations were tuned to reduce the chances for the protein
2518   content to fall below a value of 0.77. The optimum constraints based on ± 3σ are given below (the
2519   values are rounded):

	X1 Pressure(psi)	X2 Pass #	X3 Cooling(min)	X4 Duration(min)
min	10,000	1	7	5
max	19,000	3	16	14

2520   • The statistics of the resulting protein content distribution are given as follows:

2521   Mean = 0.94, Std = 0.003
2522   The histograms of the inputs as well as the protein content are shown below.
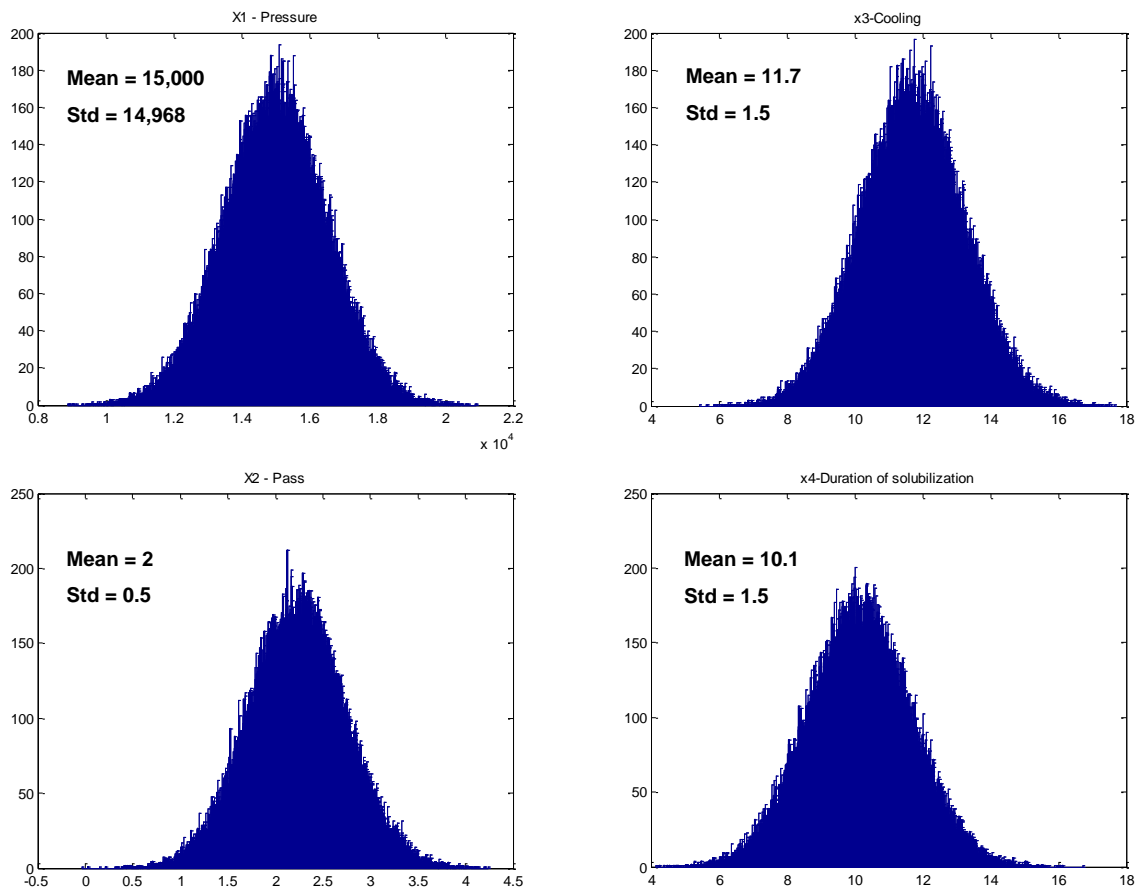
2523
2524
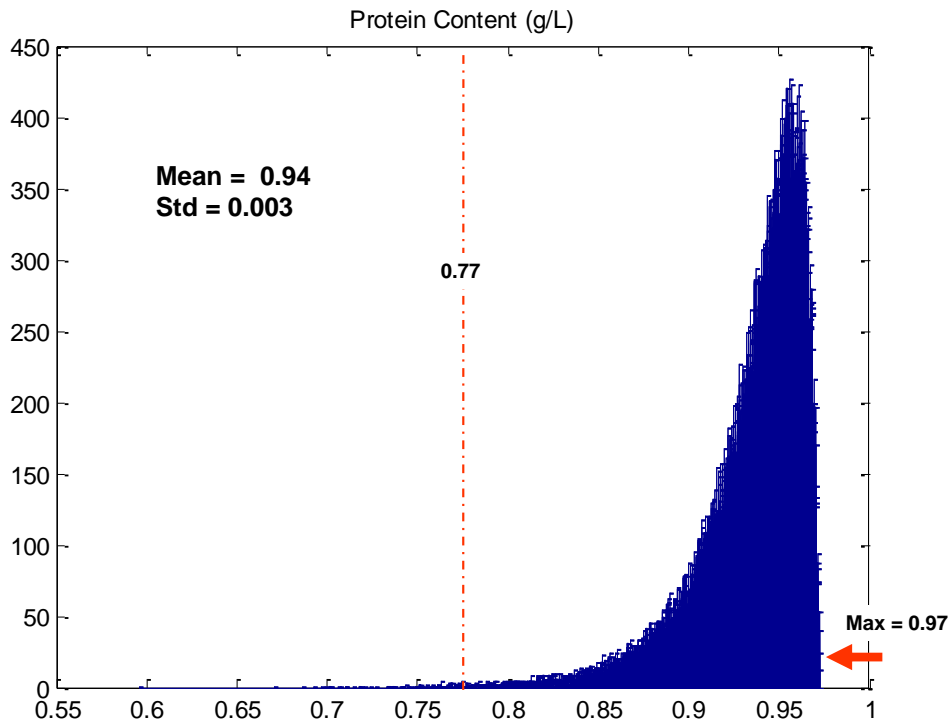
2525
2526
2527   The shape of the resulting distribution is skewed toward the maximum value of 0.97, as can be seen
2528   in the protein content histogram.
2529

5.8.   Design Space for the VLP Primary Recovery Step

2531   •   A Central Composite Rotatable Design (CCRD) (Schmidt and Launsby, 1992) is chosen to optimize
2532   the VLP recovery step. This design is more useful in practice than other designs; it requires fewer
2533   experimental points to determine polynomial coefficients and also measures the lack of fit of the
2534   resulting equation.
2535   •   A CCRD was used to study how variations in Pressure – Homogenization, Pass No. –
2536   Homogenization, Cooling – Homogenization, and Duration – Solubilization affect the purity and
2537   quantity of protein content responses of VLP from the primary recovery step.
2538   •   Responses, namely protein content, pellet mass for each wash, total protein, purity (DNA,
2539   protein, lipid), SDS-PAGE profile, and percentage of monomer measurement (GF, HPLC, native
2540   SDS-PAGE), were studied.
2541   •   Optimization of the protein content is provided as surface plots to illustrate the process
2542   capability within the design space.

5.9.   Design Space Identification

2544   Simulations were performed in MATLAB using the RSM model from the factors in DOE #2. The worst-
2545   case protein content was set to 0.77, and the sweet spot plot was then used to visualize the
2546   resulting design space based on the model. The area is encapsulated with the relaxed boxlike space,
2547   which is given by the following vertices:
2548

	X1 Pressure (Psi)	X2 Pass #	X3 Cooling (min)	X4 Duration (min)
min	9,000	0	1	3

max	22,000	4	20	18

2549
2550   It should be pointed out that with the relaxed space, some combinations when selected will result in
2551   protein content lower than the worst-case value of 0.77. Also, to be close to the optimum
2552   operations, the control space should be strictly inside the space represented by the sweet spot plot
2553   because the boundary itself is associated with uncertainty resulting from model errors (i.e., close to
2554   the 0.77 boundary).
2555
2556   The surface response profiles and sweet spot plots are shown in the following figures for all binary
2557   combinations. In these plots, perturbations were made around the optimal point obtained from
2558   maximizing the protein content based on the RSM model. The maximum protein content obtained is
2559   0.97 (according to the model) with the following optimum conditions: X1 = 15000, X2 = 2, X3 = 12,
2560   and X4 = 10.
2561
2562   DOE #3 will be designed to confirm the model and assess the noise in the control space. Based on
2563   analysis of the contour plot, DOE #3 will be repeat runs with Pressure – Homogenization (15,000),
2564   Pass No. – Homogenization (2), Cooling – Homogenization (12), and Duration – Solubilization (10).
2565

2566   **Figure 5-7: Surface Response Profile and Sweet Spot Plot (Pressure and Pass Number)**
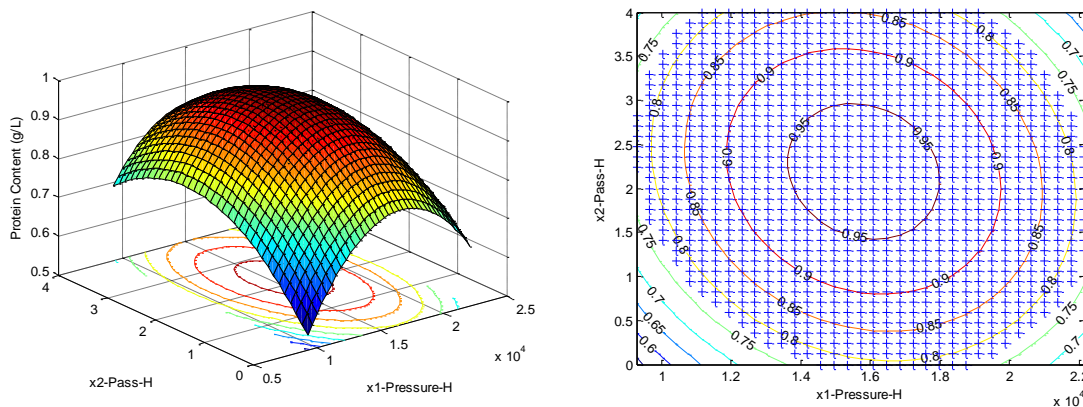
2567
2568
2569   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2570   between the **pressure and pass number.** The optimum is inside the operating range.
2571

2572   **Figure 5-8: Surface Response Profile and Sweet Spot Plot (Pressure and Cooling)**
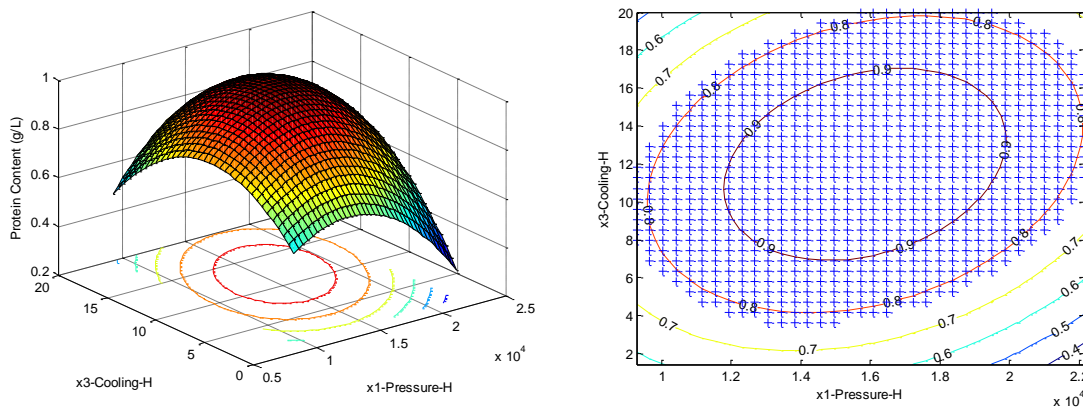
2573
2574
2575   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2576   between the **pressure and cooling**. The optimum is inside the operating range.
2577

2578   **Figure 5-9: Surface Response Profile and Sweet Spot Plot (Pressure and Solubilization Duration)**
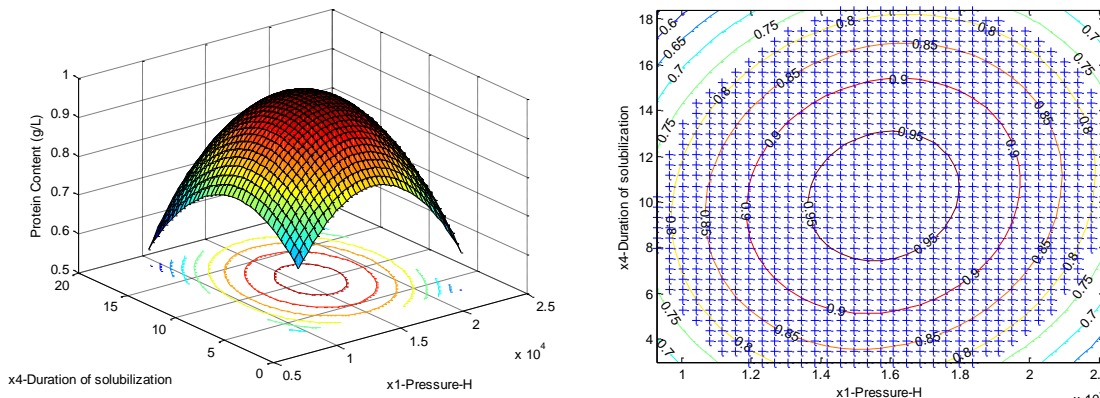
2579
2580   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2581   between the **pressure and solubilization duration**. The optimum is inside the operating range.
2582
2583   **Figure 5-10: Surface Response Profile and Sweet Spot Plot (Pass Number and Cooling Time)**
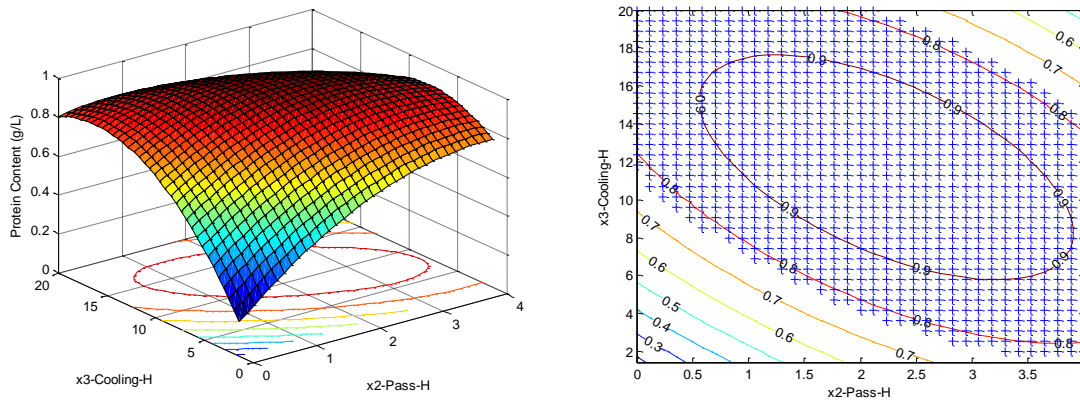
2584
2585   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2586   between the **pass number and cooling time**. The optimum is inside the operating range.
2587
2588   **Figure 5-11: Surface Response Profile and Sweet Spot Plot (Pass Number and Solubilization**
2589   **Duration)**
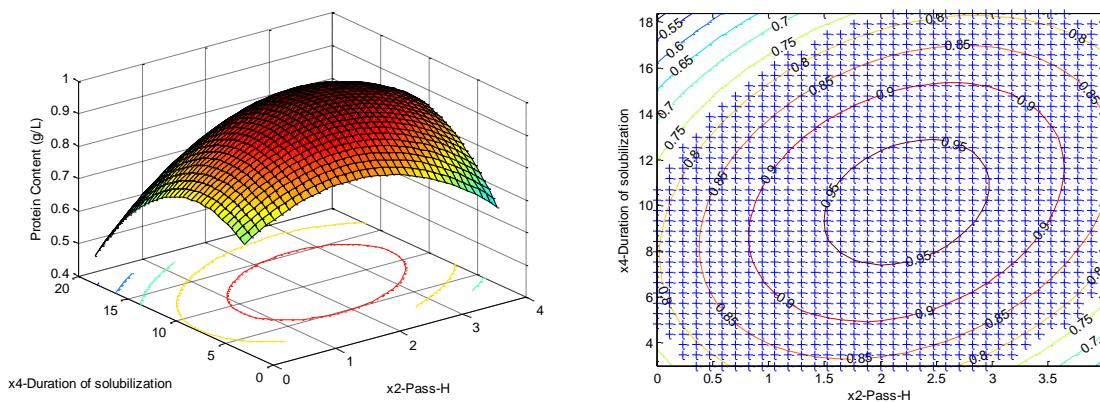
2590
2591   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2592   between the **pass number and solubilization** duration. The optimum is inside the operating range.
2593

Figure 5-12: Surface Response Profile and Sweet Spot Plot (Cooling Time and Solubilization
Duration)
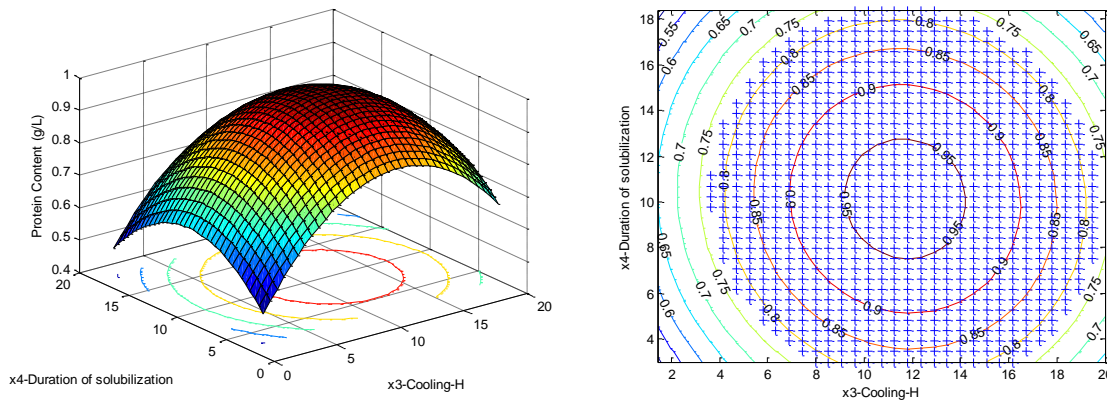
2596
2597
2598   Surface response profiles and the contour plot with sweet spot area (+) for the binary interaction
2599   between the cooling time and solubilization duration. The optimum is inside the operating range.
2600

5.9.1.1. Multivariate Interactions

2601
2602   To illustrate the multivariate interactions, 3D projections of all parameter combinations within the
2603   investigated space are shown below. Two space sets are shown. The wider range set represents the
2604   set corresponding to protein content better than or equal to 0.77, whereas the red square area
2605   represents a tighter space set that would result in a protein content better than or equal to 0.9.
2606
2607   The two sets are placed inside the investigated space. The RSM model was used to extract the two
2608   sets, and a design space based on the tighter space of 0.9 protein content is expected to have a
2609   more robust operation (one can extract a relaxed boxlike range around this tight space).
2610

2611   **Figure 5-13: Multivariate Interactions**
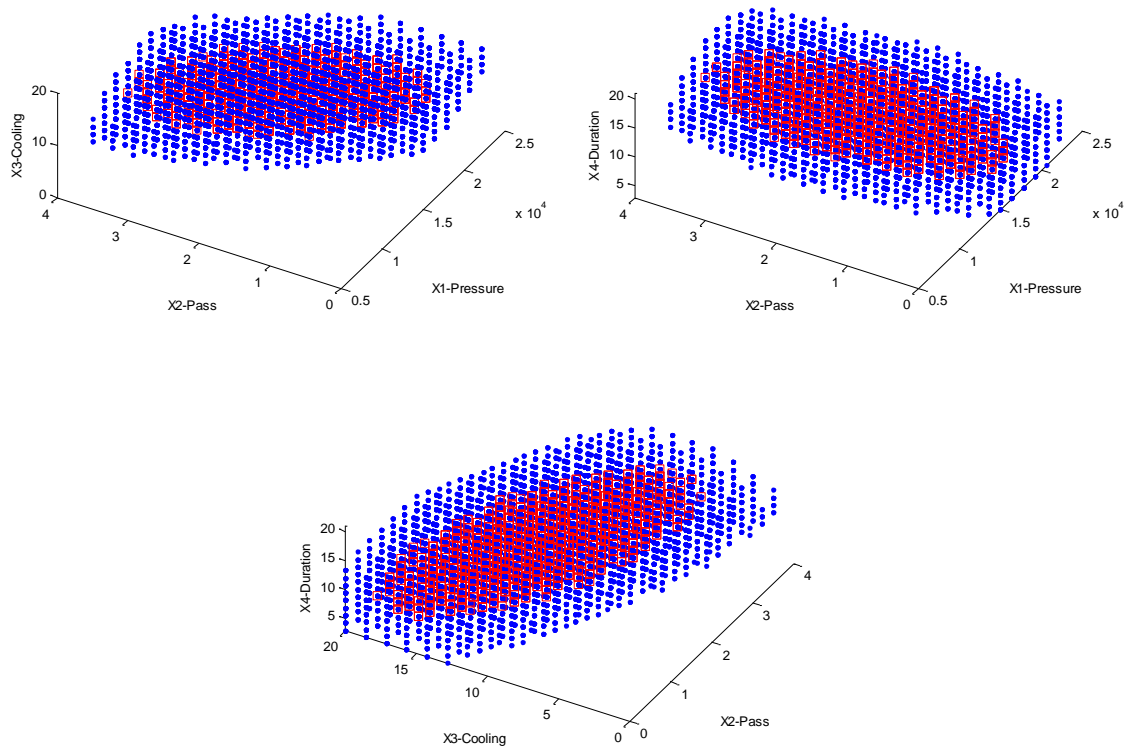
2612
2613
2614   These are 3D projections of the multidimensional interaction space. The investigated space is
2615   represented by the entire axes range (white area), the 0.77-bounded space (+), and the 0.9-bounded
2616   space (□)

5.10. Summary of Criticality of E. coli VLP – Primary Recovery Step

From DOE #2 and the prior knowledge assessment shown in section 5.4, criticality of each parameter has been assessed as shown in Table 5-5.

Table 5-5: Summary of Criticality of E. coli VLP – Primary Recovery Step

Parameter	Current Target	Control Range	Criticality
Pass No. – Homogenization	2 times	1-3 times	CPP
Cooling – Homogenization	12 mins	7-16 mins	CPP
Number of Washes	2x	1-4 times	KPP
Urea Conc. – Washes	3M	1-5 M	KPP
Pressure	15,000psi	10,000-19,000 psi	CPP
Duration – Centrifugation	30 min	10-60 mins	Non-KPP
Temp. – Centrifugation	8	4-24 °C	Non-KPP
Urea Conc. – Solubilization	8	5-10 M	KPP
pH Solubilization	6	5-10	Non-KPP
Reducing Agent (ex. L-cys, DTT) – Solubilization	L-cys	DTT, L-cys	Non-KPP
Reducing Agent Conc. – Solubilization	10	0.5-50 mM	KPP
Duration – Solubilization	10 hrs	5-14 hrs	CPP

5.11. DOE #3: Model Verification at Target Conditions of the Control Space (Full-Scale Model [e.g., 20L Fermentor])

2622
2623

2624 DOE #3 is a confirmation design from the analysis in DOE #2. The factors Pressure – Homogenization
2625 (15,000), Pass No. – Homogenization (2), Cooling – Homogenization (12), and Duration –
2626 Solubilization (10) were repeated for 16 runs.
2627
2628 ### 5.11.1. Analysis of the Full Factorial Design

Run #	Pressure – Homogenization (psi)	Pass No. – Homogenization	Cooling – Homogenization (min)	Duration – Solubilization (min)	Specific Activity by ELISA -Protein Content
1	15000	2	12	10	0.86
2	15000	2	12	10	0.79
3	15000	2	12	10	0.89
4	15000	2	12	10	1.25
5	15000	2	12	10	1.14
6	15000	2	12	10	0.94
7	15000	2	12	10	0.94
8	15000	2	12	10	0.98
9	15000	2	12	10	0.95
10	15000	2	12	10	0.75
11	15000	2	12	10	0.97
12	15000	2	12	10	1.39
13	15000	2	12	10	1.15
14	15000	2	12	10	1.47
15	15000	2	12	10	1.42
16	15000	2	12	10	1.33

2629 **5.11.1.1.   Error Estimation Attributable to Noise from the Control Space Analysis**
2630 The error estimate from the responses obtained from the repeated runs in DOE #3 is attributable to
2631 noise from the control space analysis. From DOE #3, the error estimate is calculated to be about
2632 0.23. This means that the expected protein content value of 1.0 could lie anywhere between 0.77
2633 and 1.23.

2634 **Distributions**
2635 **Protein content**
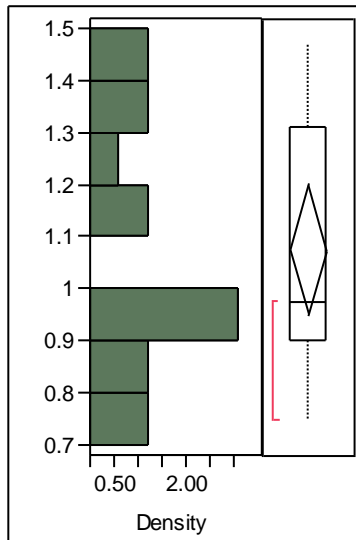
2636

Moments	
Mean	1.07625
Std Dev	0.2336914
Std Err Mean	0.0584228
upper 95% Mean	1.2007754
lower 95% Mean	0.9517246
N	16

2637
2638 Based on the error estimation of the validated runs at 20L, subsequent scale-up scenarios by any
2639 factor should factor in this noise in assessing protein content limits at the primary recovery step. This
2640 means that the robustness of the yield recoveries should be expected to fluctuate around the error
2641 estimate since the repeated runs have shown some fluctuations of the yield recoveries under the
2642 same conditions.
2643

2644 **5.11.1.2.   Control Charts of the Responses from the Validation/Verification at Target**
2645 **Conditions for Routine Manufacturing**

2646

2647 **Figure 5-14: Control Chart – Individual Measurement of Protein Content (DOE #3)**
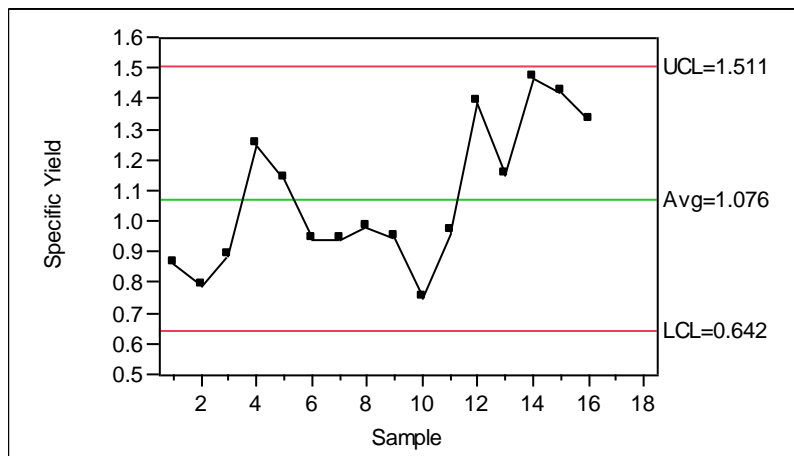
2648
2649

2650 **Figure 5-15: Moving Range of Protein Content (DOE #3)**
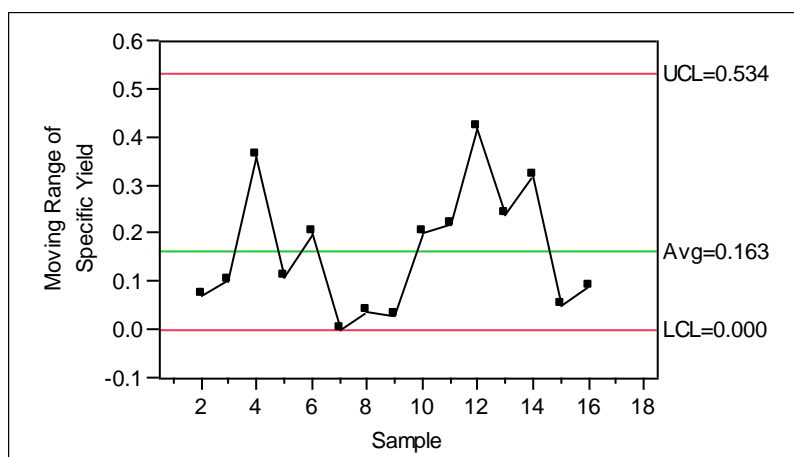
2651

2652 **5.11.1.3.   Conclusions from DOE #3**

2653 Analysis of DOE #3 showed the following:

2654 •   The level settings of the input parameters for Pressure – Homogenization at 15,000, Pass No. –
2655   Homogenization at 2, Cooling – Homogenization at 8, and Duration – Solubilization at 7 are
2656   capable of obtaining a protein content response of 1.0. The error estimate from the control
2657   space analysis should be factored in, however.

2658 •   DOE #3 was also capable of estimating the error in the control space because of the 16 repeated
2659   runs. The degrees of freedom df for center points alone was 15 (n-1).

2660 •   The control charts of the model validation runs show that responses from the model are stable
2661   (range chart) and the individual measurements are in control, with the common cause of
2662   variation attributable to noise in the control space.

2663 •   The upper and lower limits will be used as the protein content specs at the end of the primary
2664   recovery step.

2665 •   The limits of the protein content values were used to drive the design space of the in-process
2666   parameters.

5.12. Post Validation

After completion of manufacturing process validation, additional changes may still be introduced during commercial production. Thus, an ongoing program should be established to collect and analyze product and process data that relate to product quality and to ensure the process remains in the validated state.

When a change is observed, it will be evaluated to determine if it results in changes outside the validated range of critical process parameters and/or quality attributes. If the change is within the validated range, no additional action is deemed necessary, other than conducting continued monitoring and trending analysis both of critical process parameters and quality attributes according to the established procedures. If the change falls outside the validated range but within the design space, a risk assessment-based approach (FMEA) will be undertaken. In this section, we use potential changes during the urea wash step as an example to illustrate the risk assessment and post-validation plans.

Urea is obtained as a raw material and is used at two steps during harvest. It is prepared, used as 3M solution to wash the VLP protein-containing inclusion bodies, and then used as 8M solution to solubilize the VLP proteins. When the solution is prepared at an incorrect concentration, it can prolong the VLP protein solubilization time; this can impact the performance of the validated process and subsequently affect the quality of the harvest protein such as its protein content, pellet mass, purity, and proportion of monomers. These quality attributes have been determined to impact the final purified drug substance.

When a change of urea is noticed, we shall go through the above two-step analysis. If key performance attributes of the harvest step are within the validated range, no actions will be taken other than continuing monitoring and trending analysis according to the validated procedures. If the performance attributes are observed to be outside the validated range, a root-cause investigation will be conducted, which may lead to re-optimizing the individual process step. In such a case, a new DOE may be required to confirm the impact of the change.

2696 # 6.   Downstream Section

2697 ## 6.1.   Executive Summary

2698 The "Downstream" manufacturing process development section comprises three parts. The first two
2699 cover the purification of the polysaccharides and virus-like particles (VLPs) produced by the
2700 upstream processes, and the third part addresses the conjugation of the polysaccharides and VLPs.
2701
2702 These processes are "platform-like" in that a common set of unit operations (i.e. process steps) can
2703 typically be employed to purify polysaccharides and VLPs and conjugate them. Therefore, experience
2704 with similar processes and products supplies knowledge to guide downstream manufacturing
2705 development. However, the processes are not truly "platform" because of differences specific to the
2706 polysaccharides and VLPs involved, which may require unique bioprocess conditions.
2707
2708 As with the "Upstream" section, the "Downstream" section will use select unit operations for the
2709 three parts to illustrate how Quality by Design (QbD) principles can be applied to vaccine process
2710 development. For conciseness, not all data mentioned as part of the examples are shown, but these
2711 data would be available at the time of license application.
2712
2713 The three parts of the "Downstream" section, polysaccharide (Ps) purification, VLP purification, and
2714 Ps-VLP conjugation, encompass: (1) a description of the overall process with an explanation for the
2715 selection of the representative process step used as an example; (2) a summary of prior process
2716 knowledge, an initial process risk assessment, and early stage process development for each
2717 representative process step; (3) a late development stage process risk assessment followed by (4)
2718 the development of a design space; and (5) a description of a post-licensure process change.
2719
2720

6.1.1. Key Points from Downstream Section

1. Multiple approaches to conducting risk assessments are applicable for evaluating vaccine processes.
2. Defining a design space ensures robust process operation.
3. Enhanced process understanding of linkages between process parameters and the vaccine's quality attributes and process performance is possible.
4. Post-licensure changes benefit from a defined design space and enhanced process knowledge through use of QbD development.

6.1.2. QbD Elements for Vaccine Downstream Processes

This section of the case study summarizes how process development can be performed using different approaches to specific unit operations to define downstream manufacturing process steps based on principles of Quality by Design. The "Downstream" section includes exemplification of the following QbD principles:

1. Prior knowledge for process scale-up and mixing during process steps impacts the QbD approach used, from risk assessment to optimal use of scale-down models.
2. Risk assessments identify process parameters to evaluate impact on quality attributes and process performance through experimentation.
3. Prior process knowledge is used to determine process parameter ranges for process evaluations.
4. Prioritized and focused experimental efforts supply the data to define the design space based on (1) critical quality assurance (QA); (2) mandatory process performance attributes; and (3) high-risk process parameters (i.e., multivariate design of experiment setup for high-criticality QA/process attributes and high-risk process parameters and OFAT [one factor at a time] for less critical parameters).
5. Integrated models from multivariate and univariate experiments define a design space that optimizes process performance and ensures product quality.
6. Scale-down process models are confirmed to be applicable to full-scale performance.
7. Continuous improvement can provide further understanding and optimization of the process.

6.2.   Polysaccharide Process Description

6.2.1. Process Overview

The capsular polysaccharide is purified from inactivated fermentation broth after enzymatic extraction to release the Ps into the medium. Purification consists of a combination of precipitation, chromatographic, enzymatic, and ultrafiltration steps. The purified Ps is finally converted into a powder and stored at -70°C before conjugation to the VLP.

The downstream process flowsheet and the purpose of each step are summarized in Table 6-1.

6.2.2. Unit Operation Selected: Enzymatic Extraction

The enzymatic extraction step was selected as the Ps purification step to illustrate vaccine process development using QbD. For the sake of conciseness, other process steps were not addressed.

2762

2763   ***Step description***

2764

2765   *X. horrificus* capsular polysaccharide is released in the medium by enzymatic treatment using
2766   horrificase, a specific endopeptidase that cleaves the peptide cross bridges found in *X. horrificus*
2767   peptidoglycans.

2768

2769   • Horrificase is a commercial, nonrecombinant enzyme purified from the bacterium *X. lyticus,* a
2770   species closely related to *X. horrificus.*

2771   • After inactivation, X. horrificus culture is adjusted at pH 8.4 with 1M NaOH and treated with
2772   horrificase (100 U/ml) for 12 hours at 35°C under agitation in a stainless tank with marine
2773   impeller.

2774   • The resulting extract is filtered on a composite filter and the capsular polysaccharide is
2775   recovered in the filtrate, which is further processed by precipitation.

2776

2777   ***Rationale for selecting the extraction step as an example***

2778   • Extraction conditions may impact several critical quality attributes (CQAs) and key process
2779   attributes (KPAs) such as residual peptidoglycan content, Ps size, O-acetyl content, step yield,
2780   and filterability of the extract. On the basis of prior knowledge, the optimal operating range of
2781   the enzyme may impact Ps stability in terms of size and O-acetyl content. It can therefore be
2782   anticipated that optimizing all the attributes simultaneously will require a trade-off, which
2783   further reinforces the added value of using a DOE approach.

2784   • Uncontrolled sources of noise/**Error! Not a valid link.**variability arise at two levels:

2785   – Extraction is performed on a complex mixture subject to biological variability (fermentation
2786   broth).

2787   – The enzyme itself is a biological raw material. Background information on the stability and
2788   consistency of the enzyme is very limited since it is being used in an industrial process for
2789   the first time; there is no platform knowledge.

2790   • Assessing the impact of extraction parameters requires further processing all the way to the last
2791   Ps purification step for some CQAs (ex: Ps size cannot be measured accurately on the extract).
2792   This feature is typical of vaccines, especially when the process steps are far upstream of the
2793   purified active ingredient.

2794   • The quality of the extract can impact unit operations across several steps downstream in the Ps
2795   process. For example, extraction conditions leading to a small Ps size could impact the recovery
2796   at the ultrafiltration step (Ps leakage into the permeate). At the other extreme, suboptimal
2797   enzyme activity could result in large peptidoglycan fragments that will no longer be eliminated
2798   at the ultrafiltration step and will be poorly separated from the Ps in the subsequent size
2799   exclusion chromatography.

2800

2801   ***Subset of CQAs and KPAs used in example***

2802   Enzymatic extraction conditions most likely impact the following subset of CQAs and KPAs that will
2803   therefore be considered in the example (other CQAs and KPAs are not addressed for the sake of
2804   conciseness):

2805

2806   **CQAs**

2807   • Residual peptidoglycan content, because peptidoglycan is the substrate of horrificase.
2808   Peptidoglycans are assayed by H-NMR or HPAEC-PAD on purified Ps. Note that this Ps attribute
2809   was not considered as a CQA in the "TPP-CQA" section. It was assigned a borderline severity
2810   score of 24 and was classified as LCQA (see "TPP-CQA" section XX) after the design space was
2811   defined.

2812   • Ps size, because all five Ps serotypes contain a phosphodiester bond that is prone to hydrolysis in
2813   alkaline conditions (extraction performed at pH 8.4 at 35°C). Size distribution is determined by
2814   HPSEC-MALLS on purified Ps.

2815   • Ps structure (O-acetyl content), because de-O-acetylation could occur in the extraction
2816   conditions. O-acetyl content is assayed by H-NMR on the crude extract and on the purified Ps.
2817   The Ps structure is shown in Figure 6-1. The MW of the repetitive unit = 1530 g.mol-1 (without
2818   the counter-ion).

2819
2820   **KPAs**

2821   • Extraction yield, because it is directly related to peptidoglycan digestion. Ps is quantified by
2822   HPAEC-PAD or ELISA.

2823   • Filterability after extraction, because insufficient cell wall digestion leads to filter clogging.
2824   Filterability is assessed on small-scale filters in conditions that are qualified as representative of
2825   the large-scale process.

2826

2827   **Figure 6-1:** *X. horrificus* **serotype 2 capsular polysaccharide structure**

2828   →4)-α-D-Glc*p*(6OAc)-(1→3)-β-D-ManNAcA-(1→4)-α-L-Rha*p*(3OAc)-(1→2)-D-Ribitol(5−P-(O→
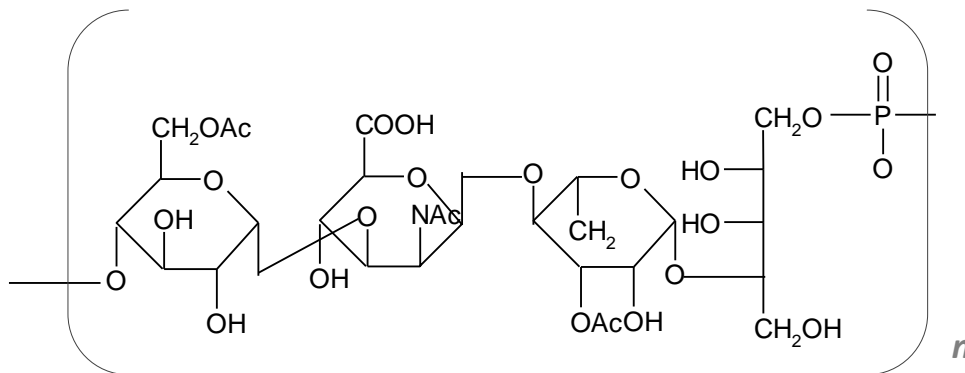
2829
2830

2831   **Table 6-1: *X. horrificus* serotype 2 Ps flowsheet and objectives of the different steps**

Fermentation harvest	
↓	
Inactivation – 30 min at 65°C 24 h at room temperature	
↓	
Transfer to downstream	
↓	

Process step	Objective
Enzymatic extraction with horrificase 100 U/ml – pH 8.4 – 12 h at 35°C	→ Releases Ps in the medium by cleaving peptidoglycan peptide cross-bridges
↓	
Clarification on composite filter	→ Removes cells and cell debris
↓	
Fractionated ethanol/CaCl₂ precipitation 25–75% – 10 mM CaCl₂ *(75% pellet resuspended in AEX buffer)*	→ Removes proteins, nucleic acids, peptidoglycans, and medium components
↓	
Anion exchange chromatography Elution with NaCl stepwise gradient	→ Removes proteins, nucleic acids, peptidoglycans, and horrificase
↓	
DNAse – RNAse treatment 100 U/ml – 3 h at 25°C	→ Digests residual nucleic acids
↓	
Ultrafiltration 100kD Concentration + Diafiltration	→ Removes digested nucleic acids, proteins, peptidoglycans, teichoic acids, and DNAse-RNAse
↓	→ Concentrates for subsequent SEC
Size exclusion chromatography	→ Removes teichoic acids
↓	
0.22μm filtration	→ Controls bioburden
↓	
Lyophilization	
↓	
Freezing at -70°C	
↓	
Bulk polysaccharide	

- Fractionated ethanol/$CaCl_2$ precipitation — 25–75% – 10 mM $CaCl_2$

6.3. Polysaccharide Extraction Early Process Development

6.3.1. Prior Knowledge

Most steps of the *X. horrificus* Ps purification process (ethanol precipitation, anion exchange chromatography, size exclusion chromatography, and nucleic acid digestion) have been used extensively in the manufacture of other bacterial polysaccharides and will not be further described here. Manufacture of *X. horrificus* capsular Ps requires enzymatic extraction or release, unlike other capsular polysaccharides that are spontaneously liberated into the medium upon bacterial inactivation. This enzymatic extraction is being used for the first time at an industrial scale. Early process development exploited prior knowledge gained from the following sources:

Literature: Six publications describe *X. horrificus* Ps extraction using horrificase. The operating ranges described in these articles are listed below:

- enzyme concentration   50 to 150 U/ml
- temperature   32 to 37°C
- pH   8.0 to 8.8
- duration   6 to 24 h

One of the papers also mentions that horrificase starts to denature at 38°C.

The horrificase enzyme manufacturer: The manufacturer specifies the optimal reaction conditions (based on a standardized assay using purified peptidoglycans). The manufacturer also stipulates that the enzyme should not be exposed to temperatures above 38°C.

Condition	Optimal (*)	Effective (**)
pH	8.4	8.0–8.8
Temperature	36°C	26°C–38°C

(*)   operating range in which horrificase retains $\geq$ 90% of its activity in a standardized assay
(**)   operating range in which horrificase retains $\geq$ 25% of its activity in a standardized assay

Polysaccharide structure: All five *X. horrificus* serotypes contain a labile phosphodiester bond that renders them prone to hydrolysis in mild alkaline conditions, especially at temperatures above 35°C–38°C (i.e., in conditions that are most suitable for horrificase activity). Therefore, stability data generated on purified polysaccharides in different pH and temperature conditions were used to define the testing ranges during early development and for the robustness DOE.

Other serotypes: Prior knowledge accumulated during development of the first serotype was leveraged to develop the others. For conciseness, only one serotype is discussed in this example.

2869 ### 6.3.2. Early Process Development

2870 Prior knowledge gained from the different sources described above was used to set up extraction
2871 conditions for Phase 1 and 2 batches. Development proceeded in two steps:

2872 • The time-course of extraction was studied at lab scale (0.5 L) at two pH levels and two temperatures
2873 at a fixed enzyme concentration of 100 U/ml. The reaction was followed using two readouts: the Ps
2874 extraction yield as determined by HPAEC-PAD and filterability of the extract. All conditions were
2875 tested on three different fermentation broths. The results were used to select four candidate
2876 conditions according to the following criteria: (1) maximum yield and (2) filterability of the extracts.

2877 • The four sets of extraction parameters were tested at Phase 1 and 2 scale (15 L), and the complete
2878 purification process was performed on the resulting extracts. Data obtained on the purified
2879 polysaccharides are presented in Table 6-2. Ps size and O-Ac content met the criteria and were fairly
2880 consistent in all four conditions. Residual peptidoglycan appears as the most impacted CQA;
2881 therefore, it was used as the criterion to identify the reference conditions of 12 h treatment at pH
2882 8.4 and 35°C because it lead to the lowest residual peptidoglycan content in the purified Ps.

2883 • The other CQAs and KPAs were met for all four conditions. Although values for the residual
2884 peptidoglycan CQA were also within target for two other conditions, they were close to the limit and
2885 these conditions were deemed borderline, especially owing to the limited process knowledge at this
2886 early development stage.

2887

2888 **Table 6-2: Results Obtained on Purified Ps Produced at 15 L Scale Using the Four Candidate Conditions**
2889 **Selected from the Extraction Time-Course Study**

pH	Temperature	Resid. PG (%)	Mean MW (kD)	O-Ac (mol/mol)
8.0	30°C	3.3	211 kD	1.85
	35°C	1.7	236 kD	1.72
8.4	30°C	1.8	208 kD	2.09
	35°C	0.7	187 kD	1.94
Target		*≤2%*	*150–300 kD*	*≥1.6*

2890

> **Reference conditions determined during early development and applied to
> Phase 1 and 2 batches**
>
> Enzyme concentration 100 U/ml
>
> Temperature   35°C
>
> pH   8.4

2891
2892

2893 ## 6.4.   Polysaccharide Extraction Early Process Risk Assessment

2894 A risk assessment approach is a useful way to categorize process variables and determine those that
2895 have an impact on product quality and process performance. This approach allows identification of
2896 parameters that require additional multivariate evaluation, those whose ranges can be supported by
2897 simpler univariate studies, and those that do not require additional experimental study but instead are
2898 supported by existing knowledge.
2899
2900 A variety of tools are suitable for risk assessment analysis. They can be broadly grouped into two
2901 categories: (1) basic tools including diagrammatic analysis, encompassing flowcharts, check sheets,
2902 process maps, and cause-and-effect diagrams; and (2) advanced tools including Fault Tree Analysis
2903 (FTA), hazard operability analysis (HAZOP), hazards analysis and critical control points (HACCP), and
2904 failure modes and effects analysis (FMEA). There is no single best choice among the available risk tools,
2905 but the methodology choice should be based on the complexity of the risk, depth of analysis required,
2906 and familiarity with the available tools. During early process development, basic tools such as risk rank
2907 and filtering and cause-and-effect analysis are generally adequate to differentiate parameters requiring
2908 multivariate or univariate evaluation. As the process matures and more process knowledge is available,
2909 a more sophisticated analysis is required to assess process risk (e.g., HACCP, FMEA).
2910
2911 A risk rank and filtering tool was used to screen the polysaccharide extraction parameters. The risk rank
2912 and filtering methodology classifies process variables based on their potential impact on quality and
2913 performance attributes. In addition to estimating the impact of individual process parameters, the
2914 method also assesses the potential interactive effects of multiple process parameters. This type of
2915 analysis is particularly useful in assessing situations where the risks and underlying consequences are
2916 diverse and difficult to characterize.
2917
2918 ***Risk Rank and Filtering***
2919 For the risk ranking and filtering analysis, a desired manufacturing range was identified for each process
2920 parameter and the impact on the presumptive CQAs (main effect) was measured over the parameter
2921 range. Any potential effect on other process parameters (interactive effect) was also assessed over the
2922 same parameter range.
2923
2924 The rankings for CQA impact (main effect and interaction effect) were weighted more severely than the
2925 impact to low-criticality quality attributes (LCQAs) or process attributes and Table 6-4). If no data or
2926 rationale were available to make an assessment, the parameter was ranked at the highest level.
2927
2928

2929   **Table 6-3: Impact Assessment of Attributes: Main Effect Ranking**

Impact Description	Impact Definition	Main Effect Ranking Based on Impact on Attributes	
		Critical Quality Attribute (CQA)	Low-Criticality Quality Attribute or Process Attribute
No Impact	Parameter is not expected to impact attribute – impact not detectable	1	1
Minor Impact	Expected parameter impact on attribute is within acceptable range	4	2
Major Impact	Expected parameter impact on attribute is outside acceptable range	8	4

2930

2931   **Table 6-4: Impact Assessment of Attributes: Interaction Effect Ranking**

Impact Description	Impact Definition	Interaction Effect Ranking Based on Impact on Attributes	
		Critical Quality Attribute (CQA)	Low-Criticality Quality Attribute or Process Attribute
No Impact	No parameter interaction; not expected to impact attribute – impact not detectable	1	1
Minor Impact	Expected parameter interaction; impact on attribute is within acceptable range	4	2
Major Impact	Expected parameter interaction; impact on attribute is outside acceptable range	8	4

2932

2933   Severity scores (Table 6-5)were determined by multiplying the potential for a parameter to impact a
2934   CQA or process attribute (main effect) by the potential of a parameter to impact a CQA or process
2935   attribute via interaction with another parameter (interaction effect). Only the largest main effect score

2936   (either CQA or process attribute) was multiplied with the largest interaction score (either CQA or
2937   process attribute).
2938
2939   *Severity score = Main effect x interaction effect*
2940
2941   The severity score provided the basis for determining whether process parameters required additional
2942   multivariate or univariate analysis or whether prior knowledge provided adequate characterization of
2943   the parameters. This assessment was used to rank parameters within individual unit operations. No
2944   attempt was made to estimate interactive effects of parameters across multiple unit operations.
2945
2946   **Table 6-5: Severity Score as a Function of Main and Interactive Rankings**

		Main Effect Ranking			
		1	2	3	4
Interaction Effect Ranking	8	8	16	32	64
	4	4	8	16	32
	2	2	4	8	16
	1	1	2	4	8

2947
2948   Severity scores were ranked from a minimum of 1 to a maximum of 64. Categorization of severity scores
2949   into those requiring multivariate analysis, univariate analysis, or no additional studies was based on the
2950   following principles (Table 6-6). Severity scores that exceeded 32 represent the cumulative combination
2951   of parameters where minimally one parameter (main or interactive) was ranked to have a major impact
2952   on CQAs or process performance attributes (i.e., parameter impact outside the acceptable range of the
2953   CQA). Because of this risk, additional multivariate studies to more accurately characterize the design
2954   space are recommended.
2955
2956   Severity scores between 8 and 16 generally involve a combination of parameters that are expected to
2957   have a minor impact on CQAs or process performance attributes (i.e., impact of the parameters on CQAs
2958   is within an acceptable range). These parameters could be further evaluated by either multivariate or
2959   univariate studies, depending on prior knowledge or experience with these parameters.
2960
2961   Severity scores that are less than 4 are the result of a combination of parameters that are not expected
2962   to have a measurable impact on CQAs or process performance attributes. Simple univariate studies or in
2963   some instances the use of prior knowledge is often adequate to characterize these parameters.

2964 **Table 6-6: Severity Classification**

Severity Score	Experimental Strategy	
≥ 32	Multivariate study	
8-16	Multivariate, or univariate with justification	
4	Univariate acceptable	
≤ 2	No additional study required	

2965

2966 The process parameters evaluated by the risk ranking and filtering tool for this example (Table 6-7) were
2967 identified from prior knowledge (see Section 6.3.1), including experience with similar enzyme
2968 extractions. Otherwise, approaches such as those shown in the "Upstream" chapter (Section 5) would
2969 be used to identify the process parameters for the risk assessment.

970 **Table 6-7: Severity Scores**

Parameter	Testing Range Low	Testing Range High	Rationale for Testing Range Low	Rationale for Testing Range High	Main Effect Rank[a] CQA	Main Effect Rank[a] KPA	Rationale for Main Effect Rank	Interaction Effect Rank[b] CQA	Interaction Effect Rank[b] KPA	Potential Interaction Parameters	Rationale for Interaction Rank	Severity Score (M x I)	Recommended Studies Based on Severity Score
pH (Reaction)	8.0	8.8	Insufficient pglycan clearance; Ps size distribution; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	8	4	Reaction characterized by narrow pH optimum; Ps is prone to hydrolysis and de-O-acetylation in alkaline conditions	4	4	Enzyme conc., polysaccharide concentration, pglycan conc., incubation time, incubation temperature	Moderate additive impact expected based on known relationship among pH, enzyme conc., and temperature	32	Multivariate
Enzyme Concentration	25 U/mL	200 U/mL	Insufficient pglycan clearance; low Ps yield and filterability	Insufficient pglycan clearance; low Ps yield and filterability	8	4	Conc. impacts kinetics; optimum conc. influenced by kinetics vs. cost	4	4	Pglycan conc., incubation time, incubation temperature	Moderate additive impact expected based on known relationship between pH, enzyme conc., and temp	32	Multivariate
Incubation Temperature	20°C	37°C	Insufficient pglycan clearance; Ps size distribution; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	8	4	Strong influence on reaction kinetics; Ps is prone to hydrolysis at higher temperatures	4	4	Pglycan conc., incubation time, incubation temperature, pH	Moderate additive impact expected based on known relationship between pH, enzyme conc., substrate conc., time, and temp	32	Multivariate
Incubation Time	10 hr	14 hr	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	Insufficient pglycan clearance; Ps size distribution and O-acetyl content; low Ps yield and filterability	4	4	Reaction most heavily influenced by pH, enzyme concentration, and incubation temperature	4	4	Pglycan conc., incubation time, incubation temperature, pH	Weak additive impact as pH, enzyme conc. and temperature drive Pglycan hydrolysis kinetics	16	Multivariate or univariate
Enzyme Batch	NA	NA	Variability among different batches of enzyme		4	1	Variability dependent on source	1	1	Pglycan conc., incubation time, incubation temperature	Weak additive impact as batch variability is expected to be small	4	Univariate

Parameter	Testing Range		Rationale for Testing Range		Main Effect Rank[a]		Rationale for Main Effect Rank	Interaction Effect Rank[b]		Potential Interaction Parameters	Rationale for Interaction Rank	Severity Score (M x I)	Recommended Studies Based on Severity Score
	Low	High	Low	High	CQA	KPA		CQA	KPA				
Fermentation Batch	?	?	Impact on kinetics	Impact on kinetics	1	1	Little impact on quality or recovery batch; variability is expected to be small	1	1	Pglycan conc., incubation time, incubation temperature	Weak additive impact as batch variability is expected to be small	1	Utilize prior knowledge
Filtration Rate	10 L/min	25 L/min	Recovery	Recovery	1	1	Little impact on quality or recovery	1	1	None expected	NA	1	Utilize prior knowledge
Mixing Rate	40 rpm	50 rpm	Reaction kinetics	Reaction kinetics	1	1	Reaction most heavily influenced by pH, enzyme concentration, and incubation temperature	1	1	None expected	NA	1	Utilize prior knowledge

971
972   [a, b] Rank based on impact to CQAs (peptidoglycan clearance, size distribution, O-Ac content) and process performance attributes (yield, filterability).

CMC-VWG

6.5. Polysaccharide Late Stage Risk Assessment

Process development following the early stage risk assessment seeks to understand the linkages between process parameters and both CQAs and KPAs so as to define an early design space and control strategy. A late development stage risk assessment is important to focus experimentation on characterizing the process and defining those parameters that will be most important for controlling process performance and product quality. A well-accepted tool to perform such a risk assessment is FMEA.

Failure Modes and Effects Analysis (FMEA)

FMEA is a tool for methodically evaluating, understanding, and documenting the potential for risks to the process operation/consistency and product quality — in other words, "what can go wrong" (Figure 6-2).

- What is impacted
- How frequently the event occurs
- Detection of the event

The FMEA provides a framework for a methodical approach to evaluating, understanding, and documenting the potential for failure in a process that might pose a risk to process consistency and product quality. The FMEA is conducted by a multidisciplinary team comprising process experts familiar with process development and characterization and manufacturing site representatives with expertise in manufacturing operations, manufacturing procedures, and equipment capabilities and controls.

The application of FMEA can be throughout the product commercialization stages in an iterative approach. This allows the initial FMEA template to be developed and refined with improved process knowledge and greater understanding of manufacturing capabilities.

3000   **Figure 6-2: The FMEA Work Process**
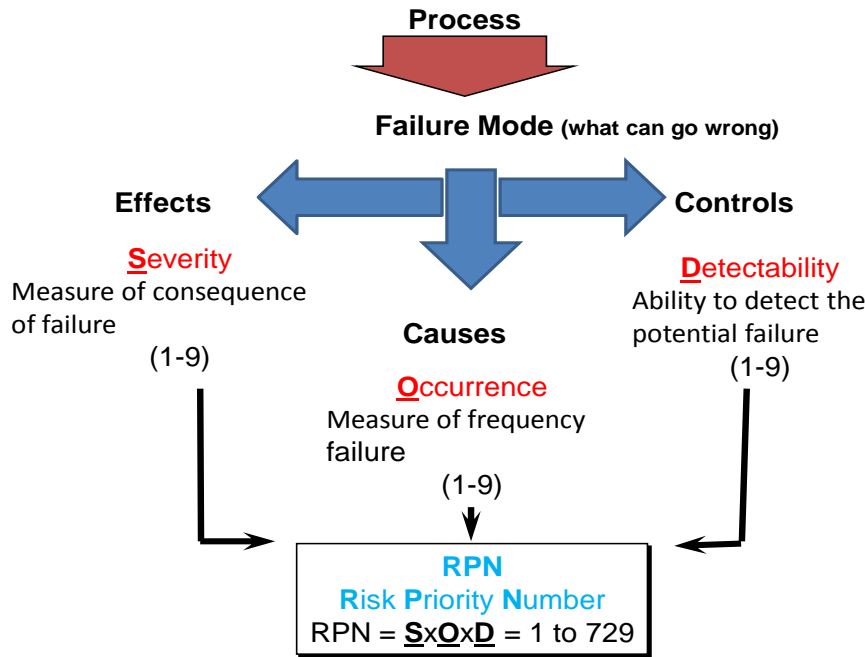
3001
3002
3003   The first stage of the FMEA is to assign process parameter severity (S) scoring (Figure 6-2) based on
3004   the parameter's potential impact on quality attributes and process performance. Quality attributes
3005   specific to process intermediates, final drug substance/drug product specification, or quality targets
3006   are considered. Process performance should be focused primarily on important performance
3007   indicators (e.g., conjugation step yield). The severity assessment is conducted with the primary input
3008   from process experts using prior knowledge gained from process characterization (DOE), pilot scale,
3009   and full-scale process batches.
3010
3011   Note that severity scores for FMEA should be consistent with risk ranking and filtering (RR&F) or
3012   cause-and-effect (C&E) outputs. Ideally severity scores may be directly translated from RR&F or C&E,
3013   providing consistent scoring definitions were applied. Alternatively, RR&F or C&E severity output
3014   may be calibrated to fit FMEA scoring definitions
3015
3016   The potential severity impact should be assessed over process parameter ranges extended well
3017   beyond the normal operating range (NOR), and the ranges proposed below are supplied for team
3018   guidance. Where NORs are established, the process parameter range for severity consideration
3019   should be extended by about three times the delta of the NOR from the parameter setpoint or
3020   target. For example, with a temperature setpoint of 20°C and NOR of 20 ±1°C, the range for the
3021   severity assessment was established at 3 x, equating to 20 ±3°C (17–23°C).
3022
3023   In other cases where NORs are not established, a range of ±10% from the parameter setpoint or
3024   target value may be used. Using the temperature example below (Table 6-8), a range of ±10%
3025   equates to ±2°C (18–22°C).
3026
3027   In all cases, good scientific judgment should apply when establishing ranges for severity, and
3028   rationale should be fully documented.

3029   **Table 6-8: Process Example for Defining FMEA Severity Ranges**

Temperature Setpoint	NOR	3 x NOR	±10% Setpoint
20°C	19–21°C (±1°C)	17–23°C (±3°C)	18–22°C (±2°C)

3030
3031   The highest severity score (9) is assigned to parameters with the greatest potential impact to
3032   product quality and process performance at the extended parameter ranges described. The scoring
3033   guidelines are listed in Table 6-9.
3034
3035   The second stage focuses on occurrence (O) (Figure 6-2). The scoring for occurrence (O) should focus
3036   on the likelihood of deviating beyond the specified NOR or setpoint/target for the process
3037   parameter assessed. The scoring scale is consistent with severity (1-9) with the highest occurrence
3038   assigned to parameters with the greatest likelihood of a deviation (Table 6-9). When considering
3039   occurrence, it's important to focus on common cause and not special cause events. Unexpected
3040   events (e.g., force majeure) are generally not considered. Other considerations may include prior
3041   knowledge, manufacturing history, equipment failure and human error and should be described in
3042   the FMEA worksheet.
3043
3044   The final stage of the FMEA is an assessment of detection (D) for detecting a potential deviation
3045   beyond the specified NOR or setpoint/target. The scoring range was consistent with scores assigned
3046   for severity and occurrence with the highest scores (7 and 9) assigned to process parameters with
3047   limited or no means of detection (Table 6-9). Considerations include equipment control capabilities,
3048   deviation alarms, and tracking procedures as described in the FMEA worksheet.
3049
3050   A final Risk Priority Number (RPN) number is assigned based on multiplying the scores for severity,
3051   occurrence, and detection (S x O x D) with appropriate rationales for each process parameter
3052   described. During the FMEA assessment, risk control or mitigation strategies are discussed and
3053   planned for implementation where appropriate. The RPN numbers for each unit operation are
3054   reviewed collectively and a cut off number (threshold) may be selected based on the data
3055   distribution to aid the selection of parameters for risk mitigation and/or criticality.

3056   **Table 6-9: FMEA Scoring Guidelines**

Score	Severity	Occurrence	Detection
9 "HIGH risk"	Process failure potentially impacting one or more critical product quality attributes leading to product rejection	> 20% (very frequent)	*No way to detect excursion. Not tracked or alarmed.*
7	Potential impact on product quality or consistency (e.g., product related substances). Investigation needed prior to product release.	*~ 5-20% (frequent)*	*Difficult to detect excursion, and not until after it has impacted the process.*
5	No impact on product quality, but deviation from manufacturing procedures requires justification. Likely deterioration in process performance (e.g., yield or operability).	*~ 1-5% (occasional)*	*Excursion can be detected, but not until after it has impacted the process.*
3	No impact on product quality. Potential for minor deterioration in process performance (e.g., yield or operability).	*< 1% (rare)*	*Excursion is usually detected and corrected prior to impacting the process.*
1 "LOW risk"	No impact to product quality or process performance.	*0% (never observed)*	*Excursion is obvious and always detected prior to impacting the process.*

3057
3058   The impact of severity on the process and product depends on the step and proximity to the final
3059   drug substance or drug product. For example, upstream processes have few if any quality attributes;
3060   as a result, an assessment against quality targets or final release specifications is challenging. In such
3061   cases, the impact on the process step is more meaningful.
3062
3063   Table 6-10 and Table 6-11 describe an FMEA analysis performed to identify critical process
3064   parameters as well as potential steps to mitigate their criticality. The evaluation has been arbitrarily
3065   divided between process parameters (intrinsically related to the process) and operational
3066   parameters that are associated with the design and operation of the process in a specific
3067   manufacturing environment. Critical parameters were judged as those that exceeded an RPN value
3068   of 175. An RPN of 175 was chosen because it represented a severity that minimally impacted
3069   product quality (≥7), occurred with a minimal frequency of ≥5 (≥ 1–5%), and had a detection
3070   capability of ≥5 (excursion can be detected but not until it has impacted the process). This results in
3071   a minimal RPN score of 175. Based on this analysis, enzyme concentration was the only parameter
3072   identified as a critical process parameter.

3073 **Table 6-10: FMEA Process – Process Parameters**

Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls and Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
Enzyme Concentration	25–200 U/mL	Operational and equipment	• Low enzyme conc. limits pglycan digestion and decreases recovery and filterability	9	• Operator error • Balance calibration • Poor enzyme dissolution	5	• Batch record check	5	• Double sign-off on critical reagents	225	Classify as CPP, include in DOE
pH (Rxn)	8.0–8.8	Operational and equipment	• High pH results in phosphodiester cleavage and altered Ps size distribution • Low pH results in poor peptidoglycan cleavage, low Ps recovery, and poor filterability	9	• Probe failure • Calibration error	5	• pH check prior to rxn initiation • Training	3	• Automated pH output and alarming condition	135	Study in DOE
Incubation Temperature	20°–37°C	Equipment	• Low temperatures result in poor pglycan digestion and low recovery and filterability • High temperatures result in increased phosphodiester cleavage and altered Ps size distribution	9	• Equipment failure • Mixing failure • Operator error	5	• Automated temperature readout	3	• Automated readout and alarming condition	135	Study in DOE
Enzyme Batch	>100 U/g	Significant variability in specific activity among enzyme lots	• Inadequate peptidoglycan digestion results in low step yield and poor filterability	5	• Enzyme quality	1	• Specific activity assay prior to enzyme use	5	• Prequalification of enzyme lots	25	Study in OFAT
Incubation Time	10–14 h	Insufficient reaction time	• Insufficient reaction time results in poor Pglycan digestion and low recovery and filterability	7	• Operator error	3	• Batch record check	1	• Double sign-off	21	Study in OFAT

3074   **Table 6-11: FMEA Process – Operational Parameters**

Substeps	Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls, Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
Transfer to Reaction Vessel	Transfer Time	≤ 1 h	Operational or equipment	• Product stability	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary
	Mass Transferred	22–26 kg	Operational or analytical	• Insufficient mass results in low step yield • Excessive mass results in high residual Pglycan, poor filterability and low yield	5	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	15	No Action Necessary
Raw Material Additions	Tank Tare Wt	200–210 kg	Equipment or calibration	• Incorrect reaction conditions	5	• Operator error • Equipment failure	1	• Batch record check	1	• Batch record recording	5	No Action Necessary
	Addition of Tris Base	1.5–1.7 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording and pH check	21	No Action Necessary
	Addition of Glycine	0.5–0.7 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording and pH check	21	No Action Necessary
	Addition of NaCl	0.1–0.2 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary
	Addition of Purified Water to Final Tare Wt	1,350–1,370 kg	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	7	• Operator error • Equipment failure	3	• Batch record check	1	• Batch record recording	21	No Action Necessary

CMC-VWG

Substeps	Process Parameter	Operating Range	Potential Failure Mode	Potential Effect(s) of Failure	Severity	Potential Cause(s) of Failure	Occurrence	Current Controls, Prevention	Detection	Current Controls and Detection	RPN	Recommended Action
	Agitation Rate	40–50 rpm	Operation or equipment	• Poor reaction kinetics and incomplete pglycan digestion	5	• Operator error • Equipment failure	3	• Batch record check	1	• Automated readout and alarming condition	15	No Action Necessary
Reaction Termination	Temperature Ramp	1 h	Operation or equipment	• Increased Ps hydrolysis	5	• Operator error • Equipment failure	3	• Batch record check	1	• Automated readout and alarming condition	15	No Action Necessary

6.6. Polysaccharide Extraction Design Space

6.6.1. Section Overview

This section describes the approach (outlined in Figure 6-3) used to define the design space for the Ps enzymatic extraction step. It comprises four subsections that can be summarized as follows:

- **Experimental design:** The outcome of risk assessment is combined with prior knowledge gained from different sources and from early development to establish a DOE. This DOE not only investigates the impact of critical parameters on CQAs and KPAs, but also targets process robustness.

- **Optimization and determination of reference conditions:** DOE results are used to create prediction models that allow understanding of factor effects and interactions. Optimal conditions are then identified using desirability functions. Reference conditions are finally optimized for robustness using overlay plots.

- **Determination of design space:** Based on simulations, the design space is defined using as criterion an upper limit for the simulated defect rate. Simulations within the design space are also used to gain more insight into how the different responses contribute to the predicted defect rate. Finally, this section shows how process knowledge within the design space can be advantageously combined with a simple univariate study to integrate the incubation time into the design space.

- **Univariate studies:** The way to study the possible impact of the enzyme batch is discussed along with the limitations linked to this specific investigation.

6.6.2. Experimental Design

Factors to be investigated in a multivariate study

The three high-risk process parameters that were identified by risk assessment analysis (see previous section) are investigated in a multivariate study:

- pH
- enzyme concentration
- incubation temperature

The other key parameters (incubation time and enzyme batch) are investigated in univariate studies.

3106   **Figure 6-3: Overview of the Statistical Approach Applied to Define the Design Space**

Prior knowledge – Early development	→	Phase 1 and 2 batches
		100 U/ml – 35°C – pH 8.4

↓

Risk assessment	→	Critical parameters
		enzyme cc – Temperature – pH

↓

Design of experiment

↓

Prediction models	→	Understanding factor effects
Response surfaces – interaction plots		and interactions

↓

Optimization using desirability functions	→	Optimal conditions
		100 U/ml – 37°C – pH 8.3

↓

Optimization using overlay plots	→	Reference conditions
Find a more robust zone, on average		150 U/ml – 33.5°C – pH 8.3

↓

Simulations over the experimental space	→	Confirmation of reference
Check the proportion of batches that meet all the specs		conditions

↓

Determination of design space	→	Graphical design space
Criterion: defect rate ≤10% (based on simulated results)	→	Tabulated design space
		120–200 U/ml
		31.5–35°C
		pH 8.1–8.5

3107

3108   ***Type of design***
3109   At this late stage of process development, robustness is key and should be integrated into the
3110   optimization strategy. The experimental approach described in this section is therefore aimed at
3111   identifying optimal as well as robust extraction conditions. It is intended to determine the impact of
3112   process parameters on the variability of the output responses to select the combination of
3113   parameters that minimize variability while achieving the target responses.

3114

3115   Three approaches to robust design are commonly used: Taguchi, Dual Response, and Tolerance
3116   Analysis (compared in *Taylor, W.A (1996) Comparing three approaches to robust design: Taguchi*
3117   *versus Dual Response versus Tolerance Analysis, presented at 1996 Fall Technical Conference,*
3118   *http://www.variation.com/anonftp/pub/ta-3.pdf*). Among these, Dual Response Modeling was
3119   considered the most appropriate with respect to enzyme extraction optimization, chiefly because it
3120   is the only approach that addresses robustness versus unidentified sources of noise. Dual Response
3121   Modeling uses Response Surface Methodology (RSM): it is assumed that each studied response can
3122   be expressed as a mathematical function (second order polynomial) of the different factors
3123   investigated, thereby allowing calculation of the responses over the experimental space. The
3124   experimental structure of the Dual Response Modeling applied in the Quality by Design case study is
3125   illustrated in Figure 6-4:

3126 • A face-centered composite design is used; each studied factor (pH, temperature, and enzyme
3127   concentration) is tested at three levels (see table in Figure 6-4). The ranges investigated are
3128   based on early development results and prior process/product knowledge (enzyme brochure,
3129   literature data, Ps stability data) as detailed in Section 6.3.1 above. Based on this prior
3130   knowledge, a trade-off between horrificase activity and Ps stability should normally be found
3131   within these wide ranges covering both optimal enzyme operating ranges and Ps stability ranges.

3132 • Repeats of the central point (triplicates) and of the entire factorial structure (duplicates) are
3133   performed and used to calculate the standard deviation of each response at these different
3134   places of the experimental domain (Figure 6-4). The repeats are done on different broths to
3135   account for broth-to-broth variability. The result is an economical, robust design compared to
3136   other experimental structures in which each point is repeated in duplicate or triplicate.

3137 • The standard deviations are integrated in the model as secondary responses that will be used to
3138   optimize process robustness (minimize the impact of uncontrolled factors/noise).

3139

3140 **Figure 6-4: Experimental Structure Selected to Optimize Extraction Parameters**

3141 Conditions marked with a black dot are repeated on different broths (duplicates at the vertices and
3142 triplicates at the central point).

3143

Factor	High	Middle	Low
Temperature	37	28.5	20
pH	8.8	8.4	8.0
Enzyme cc	200	112.5	25

3144
3145   **Fixed parameters**   **- incubation time**
3146   **- enzyme batch**
3147   **- mixing conditions**
3148
3149

3150 ***Design implementation***
3151 The 25 extraction conditions of the DOE were tested in random order at lab scale (starting from 0.5 L
3152 fermentation broth), and the resulting extracts were purified using a scaled-down process. Special
3153 care was taken to reproduce as closely as possible the conditions of the commercial scale process:

3154 • All steps: carried out at the same temperature as the large-scale process.

3155 • Vessels and agitation systems for enzymatic treatments and precipitations: same geometry,
3156   same sample volume/headspace ratio, same impeller type and impeller/vessel diameter ratio.

3157 • Filtration steps: same sample volume/filter area ratio, scale-down factor applied to flow rate.

3158 • Tangential flow filtration (TFF): same membranes (material, molecular weight cutoff, channel
3159   configuration, and path length), same sample volume/filter area ratio, same feed and retentate
3160   pressures, retentate flow rate proportional to scale-down factor, same sanitization procedures.

3161 • Chromatographic steps: same sample/resin volume ratio, same bed height, same linear flow
3162   rate, buffer volumes proportional to column scale-down factor, same packing conditions and
3163   sanitization procedures.

3164

3165 The lab scale process was qualified as representative through comparison of process parameters, in-
3166 process data (clearance of contaminants, step yields), and Ps attributes obtained with the scaled-
3167 down process and at commercial scale.

3168
3169
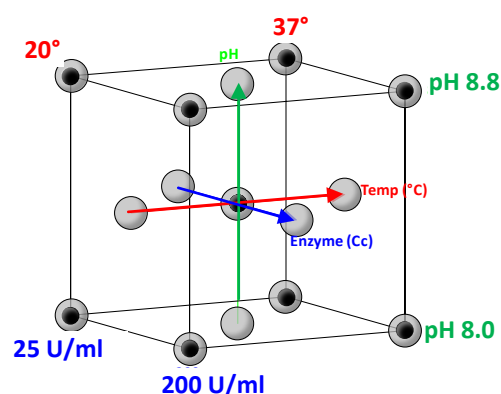
3170 ***Studied responses***
3171 The five responses that were studied to optimize the extraction conditions are discussed in Section
3172 6.2.2. Four numerical outputs reflecting response variability are also analyzed using the standard
3173 deviations of the repeats as new outputs:
3174
3175 Responses (CQAs and KPAs)
3176 •   Residual peptidoglycan content   (% w/w)
3177 •   Ps size   (kDa)
3178 •   Ps O-acetyl content   (mol/mol Ps)
3179 •   Ps extraction yield   (%)
3180 •   Filterability after extraction   value=1 if filterable and 0 if not filterable
3181   *(filterability criterion: > 15 L/m² filter area)*
3182
3183 Associated variability (SD = standard deviation)
3184 •   SD residual peptidoglycan content   (% w/w)
3185 •   SD Ps size   (kDa)
3186 •   SD Ps O-acetyl content   (mol/mol Ps)
3187 •   SD Ps extraction yield   (%)
3188
3189 ### 6.6.3.   Optimization and Determination of Reference Conditions

3190 ***Prediction model creation***
3191 For each response, a reduced polynomial model is determined to reproduce output variation using a
3192 selection of factor effects and interactions. Based on an analysis of variance (ANOVA), factors and
3193 interactions having a $\geq$ 10% probability to influence the response are selected.
3194
3195 ***Analysis of response surfaces: factor effects and interactions***
3196 Using the prediction models, responses can be calculated over the entire experimental domain and
3197 represented as response surfaces to understand how the process parameters impact specific
3198 attributes and create variability in these attributes. An example of such prediction graphs is
3199 illustrated in Figure 6-5 for residual peptidoglycans. Full prediction results for the other responses
3200 can be found in the attached Excel file.
3201
3202
3203   DoE Dual resp and   DoE Dual resp and
3204   results v2.xlsx   results v2.xlsx
3205
3206 The response surfaces reveal that the selected product and process attributes are impacted by pH,
3207 temperature, and enzyme concentration as detailed below. The data are also used to identify factor
3208 interactions that can be best visualized on *interaction plots* as exemplified for Ps size (Figure 6-6).
3209
3210 Impact of process parameters on residual peptidoglycan content (Figure 6-5):
3211 •   Optimum (lowest content) at pH 8.4 reflects horrificase optimum pH.
3212 •   Improvement at higher temperatures and enzyme concentrations. The temperature effect is
3213   consistent with horrificase optimum temperature (36°C).
3214 •   Variability is higher at lower temperatures that are suboptimal for enzyme activity.
3215
3216

3217   **Figure 6-5: Predicted Response Surfaces of Residual Peptidoglycan (PG) Content as a Function of**
3218   **pH and Temperature at 3 Enzyme Concentrations**

25 U/ml   112.5 U/ml   200 U/ml

Enzyme concentration
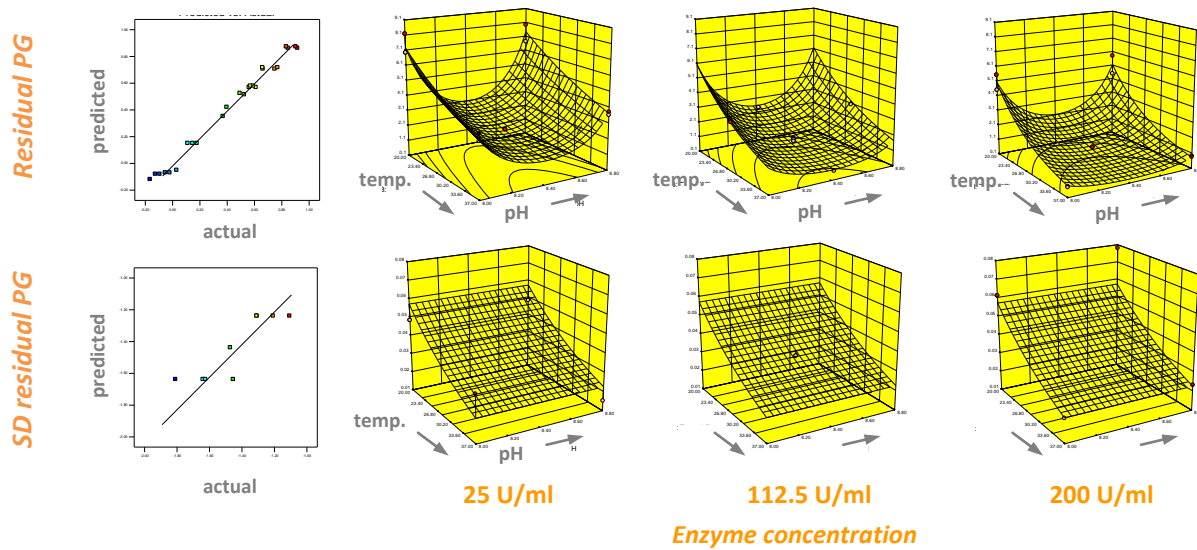
3219

3220   Impact of process parameters on Ps size:

3221   • Size is fairly stable at low temperatures and pH, but decreases at higher temperatures and pH as
3222   a result of hydrolysis of the phosphodiester bond, which is prone to cleavage in alkaline
3223   conditions.

3224   • This hydrolysis at high temperature and pH also impacts size variability.

3225   • Interaction between pH and temperature is significant on Ps size and its associated variability, as
3226   evidenced by interaction plots (Figure 6-6).

3227   **Figure 6-6: pH-temperature Interaction Plots Show a Strong Interaction Between These Two**
3228   **Parameters in the Case of Ps Size and Its Associated Variability**
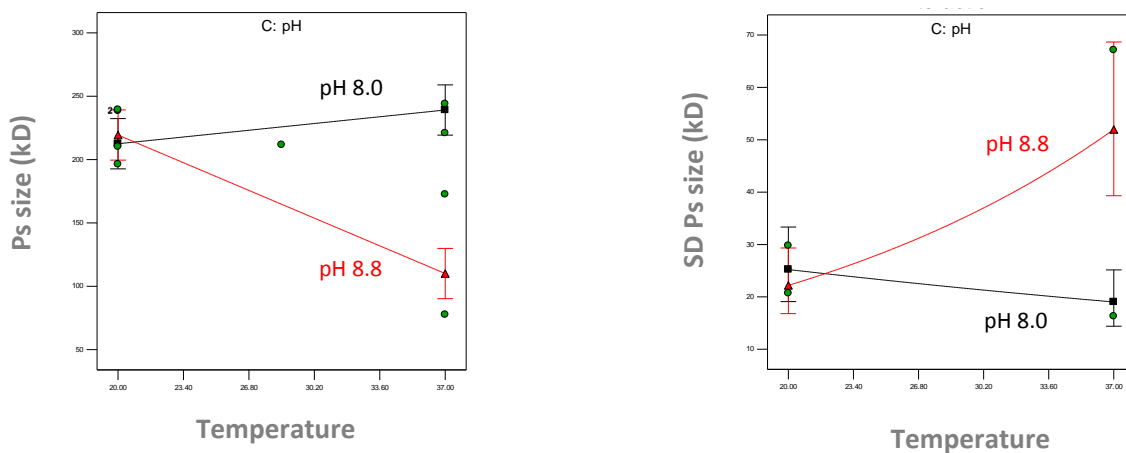
3229

3230   Impact of process parameters on O-acetyl content:

3231   • There is no impact from any of the factors over the entire experimental space. Any combination
3232   of the factors within the experimental domain leads to the expected value.

3233

3234   Impact of process parameters on step yield:

3235   • Maximum yield is obtained at pH 8.4 reflecting the horrificase pH optimum.

3236   • Yield is improved at higher temperatures, although to a lesser extent than residual
3237   peptidoglycan. The temperature effect is consistent with horrificase optimum temperature
3238   (36°C).

3239   • Yield is improved by enzyme concentration between 25 and 112.5 U/ml.

3240   • Variability decreases at higher enzyme concentrations.
3241
3242   Impact of process parameters on filterability:
3243   • Filterability is lowest at low temperatures and pH, conditions in which horrificase is expected to
3244   be less efficient at digesting peptidoglycans and breaking the cell wall open. As a consequence,
3245   filter clogging is observed. Filterability also decreases at high temperature and pH, but in this
3246   case it is caused by a precipitate that starts to form under these conditions.
3247   • Filterability is improved by increasing enzyme concentration to 25 - 112.5 U/ml.
3248
3249   ***Response optimization***
3250   Multi-response optimization frequently involves trade-offs: in most cases, one attribute is indeed
3251   optimized at the expense of another one. The desirability function, first introduced by Harrington in
3252   the mid-1960s (*Harrington, E.C., Jr. (1965) The Desirability Function, Industrial Quality Control* ***21,***
3253   *494-498*), is a widespread approach to balance multiple responses. A desirability function measures
3254   the adequacy of each response to the objective: it is defined by the developer and ranges from 0
3255   (unacceptable response) to 1 (the response fits the objective). In this case, the objectives are defined
3256   as follows:
3257   • Minimal residual peptidoglycan content
3258   • Targeted molecular size of 200 kD
3259   • O-acetyl content > 1.6 mole/mole RU
3260   • Maximal Ps yield
3261   • Filterable extract
3262   • Minimal response variability
3263   • Minimal enzyme concentration to reduce process costs
3264
3265   For each response, desirability is calculated over the experimental space. These desirability functions
3266   are then computed into one single desirability function (geometric mean of the individual
3267   desirabilities), which takes the entire selected product and process attributes into account and can
3268   thus be viewed as a global satisfaction index, enabling the conversion of the multi-response problem
3269   into a single response. The value of this overall desirability is 1 if all the objectives are met and 0 if at
3270   least one response is unacceptable.
3271
3272   Starting from the predicted individual responses, desirability can be predicted over the experimental
3273   domain. Its representation as 3D-response surfaces or 2D-isoresponse plots (Figure 6-7) can be used
3274   to identify optimal conditions and evaluate the relative impact of the different factors. In this case,
3275   the optimal parameter combination is achieved for the following conditions:
3276

Optimal conditions based on desirability response surfaces	
Enzyme concentration	100 U/ml
Temperature	37°C
pH	8.3

3277
3278   It must be noted that integrating the enzyme cost in desirability does not compromise any of the
3279   other CQAs/KPAs.
3280
3281

3282 **Figure 6-7: 3D-Response Surface (A) and 2D-Isoresponse Plot (B) of Desirability as a Function of**
3283 **Enzyme Concentration and Temperature at pH 8.3.**

3284 *Enzyme cost was taken into account to calculate desirability. The arrows point to the optimal*
3285 *conditions.*
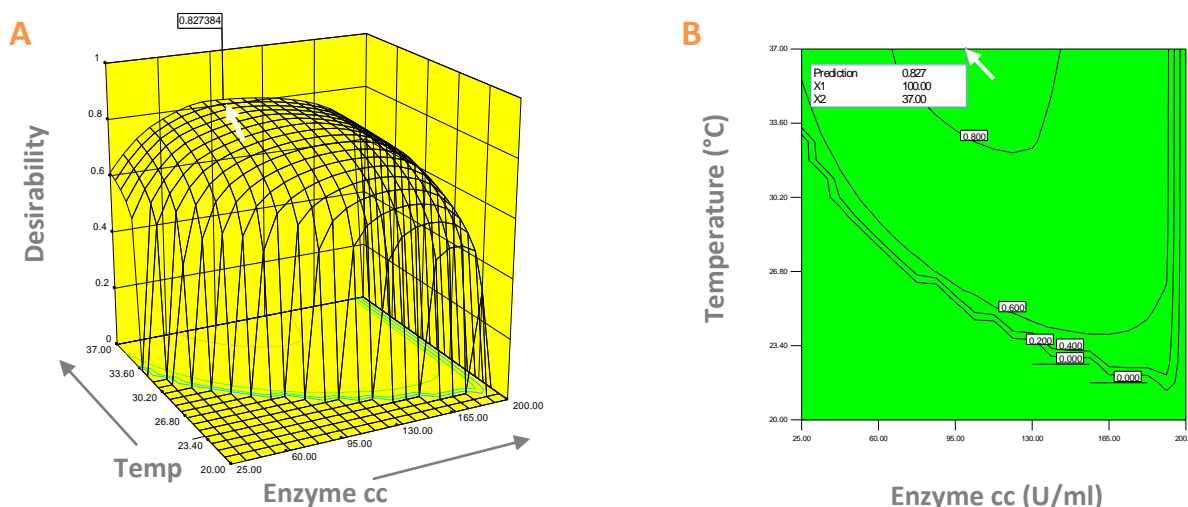
3286
3287 ***Robustness***

3288 To avoid the selection of a satisfying but very sensitive combination of extraction parameters, the
3289 experimental space is studied from a robustness point of view. Ideal conditions should result in the
3290 desired attributes, but should also be located in the middle of a large area of conditions leading to
3291 acceptable responses. This area will allow departures from reference conditions (voluntarily or not)
3292 without affecting the process and product outputs.
3293
3294 A target range is specified for each response and for its associated coefficient of variation
3295 (Table 6-12).
3296
3297 **Table 6-12: Target Ranges for Studied Responses**

Response	Target range	
	Response	Coefficient of variation (CV)
Residual peptidoglycan content	< 2%	< 15%
Ps molecular size	150–300 kD	< 20%
Ps O-acetyl content	> 1.6 moles/moles RU	< 10%
Ps yield	> 75%	< 15%
Filterability	1	NA

3298
3299 Based on the prediction models, these target ranges are displayed simultaneously on an *overlay plot,*
3300 enabling discrimination among areas where all the criteria are met and those where one or more
3301 criteria are out of specification (Figure 6-8A):
3302 • Green areas: all target values are met.
3303 • Yellow areas: predicted responses comply with the target ranges, but one or more confidence
3304 interval(s) are out of range.
3305 • White areas: one or more criteria are not met.
3306

3307   The overlay plot in Figure 6-8A shows that the optimal conditions are poorly situated in terms of
3308   process robustness. The combination of selected parameters is indeed located at the edge of the
3309   experimental domain with respect to temperature and close to borderline conditions with respect to
3310   enzyme concentration. Hence, the possibility of being close to unfavorable conditions cannot be
3311   ruled out. The optimal temperature (37°C) is of particular concern in this respect for two reasons:

3312   •   The Ps hydrolyzes readily above 37°C in mild alkaline conditions.

3313   •   Horrificase starts to denature at temperatures $\geq$ 38°C.

3314
3315   Because of these limitations, tests of additional conditions in an augmented DOE exploring
3316   temperature above 37°C were not considered. A better optimum that results in the desired process
3317   and product attributes, but is located in a more robust area of the design space should be evaluated.
3318   This can be done by decreasing the temperature and increasing the enzyme concentration as
3319   illustrated in Figure 6-8B. Thus, in this case, robustness is improved at the expense of enzyme cost.

3320

Reference conditions based on overlay plots, optimized for responses and robustness

Enzyme concentration	150 U/ml
Temperature	33.5°C
pH	8.3

3321

Figure 6-8: 2D-Overlay Plot

Ps compliance with specifications as a function of enzyme concentration and temperature at pH 8.3.
Optimal conditions (white arrow) are located at the edge of the experimental domain (A). Reference
conditions were therefore adapted to achieve a better robustness (B).
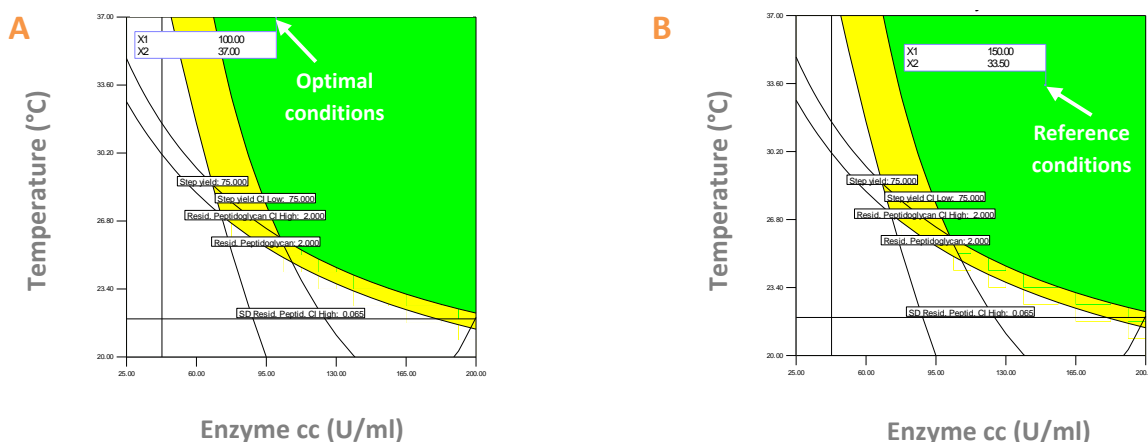
Predicted results with associated 95% confidence intervals and coefficient of variation can be
calculated for these reference conditions (Table 6-13).

Table 6-13: Predicted Process Results at Reference Parameters

Response	Prediction	Lower 95% CI	Upper 95% CI	Predicted CV
Residual peptidoglycan content	0.79	0.73	0.86	6.4%
Ps molecular size	220	197	242	12.3%
Ps O-acetyl content	1.86	1.78	1.93	8.2%
Ps yield	91.5	84.9	98.2	8.2%
Filterability	1	1	1	NA

6.6.4. Determination of the Design Space

From predictions to simulations
Determination of the reference conditions was based on predicted responses and associated
variability, which are actually predicted *averages*. For instance, a predicted molecular size of 200 kD
means that 50% of future size responses will be below 200 kD and 50% above 200 kD. While the
predicted results should, at the very least, meet the acceptance criteria *on average*, the proportion
of future responses meeting the specifications (Table 6-14) is equally important information, of
particular relevance to delineate a design space.

Table 6-14: Subset of Specifications Selected to Define the Design Space

Response	Specification
Residual peptidoglycan content	< 2%
Ps molecular size	150–300 kD
Ps O-acetyl content	> 1.6 moles/moles RU
Filterability	1

3345   To this end, a global model (e.g., Seemingly Unrelated Regression) synthesizing all individual
3346   prediction models is used. Monte-Carlo simulations, which reproduce process/measurement
3347   variability, can then be performed to mimic a huge number of experiments at numerous places of
3348   the experimental domain. Finally, the proportion of simulated results complying with the
3349   specifications can be graphically represented to generate a *3D-robustness surface plot* or its
3350   associated contour plot (Figure 6-9A).

3351

3352   In this case, the enzyme-temperature domain was subdivided into 20 x 20 intervals and 10,000
3353   simulations were calculated for each response in each of these enzyme-temperature conditions at
3354   the reference pH (8.3). Conditions in which at least 90% of simulated results fall within the
3355   acceptance criteria listed in Table 6-14 are indicated in green in Figure B. Reference conditions (red
3356   arrow) are located within the optimal area with a prediction of 99% of future results meeting all the
3357   specifications.

3358

3359   **Figure 6-9: Robustness Surface (A) and Contour Plots (B) Showing the Proportion of Simulated**
3360   **Results Meeting the Specifications as a Function of Temperature and Enzyme Concentration at pH**
3361   **8.3**

3362   *Conditions in which at least 90% of simulated results fall within the specifications are shaded in*
3363   *green. Reference conditions are indicated by the arrows.*

3364
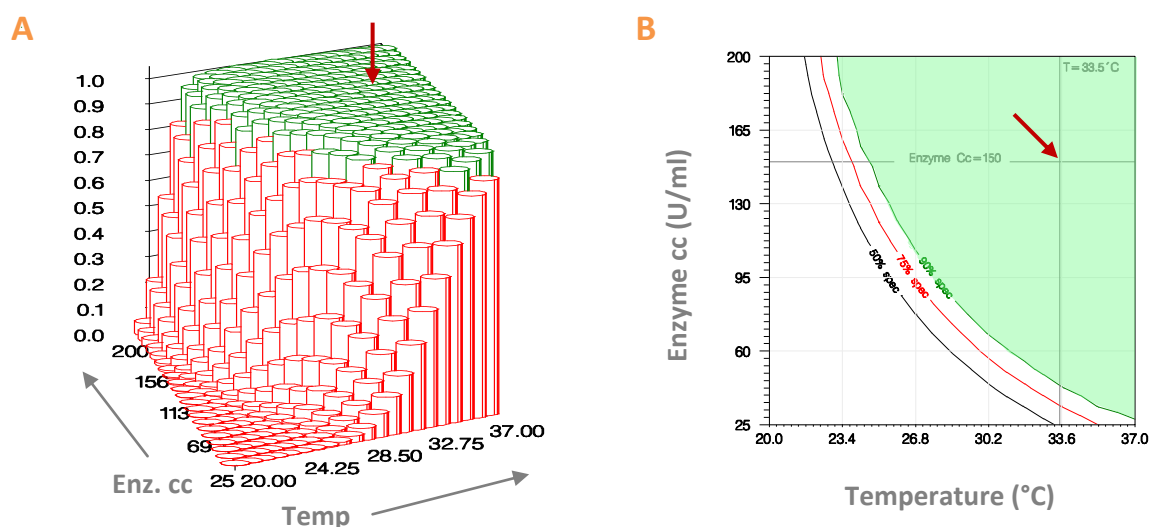
3365
3366   The arrows indicate the reference conditions.

3367

3368   **Design space (enzyme concentration, pH, and temperature)**
3369   The design space, within which process parameters can deviate from reference conditions without
3370   leading to a critical increase in defect rate, can be determined graphically using simulations. Setting
3371   an upper limit of 10% for the defect rate, the design space (enzyme concentration x temperature) at
3372   pH 8.3 corresponds to the green area on the contour plot of Figure 6-9, which is only a slice of the
3373   design space. The same approach must indeed be repeated at other pH's to get a more complete
3374   visualization of the design space, as illustrated in Figure 6-10A. With three parameters as in this case,
3375   the design space could still be represented in three dimensions or under the form of different slices.
3376   With four or more parameters, however, graphical representation becomes increasingly complex. A
3377   more practical, intuitive alternative is to define the design space as a combination of ranges that can
3378   be easily tabulated. To this end, an iterative algorithm is used to determine the largest subdomain
3379   inscribed in the design space with constraints on minimal temperature and pH ranges ($\Delta T \geq 3°C$ and
3380 $\Delta pH \geq 0.4$). The obtained cuboid design space is represented as rectangular slices on the contour
3381   plots of Figure 6-10B. It can be seen that the practical aspects linked to this tabulated design space
3382   are gained at the expense of its size. Regarding the upper limit of 10% defect rate used as criterion
3383   to define the design space, it should be kept in mind that the approach is based on predictions

3384   associated with an uncertainty of 5%. Therefore, targeting a lower defect rate would reflect an
3385   excessive confidence in the prediction models and could lead to a situation in which the uncertainty
3386   over the predicted responses would exceed the targeted defect rate. A too stringent defect rate
3387   could also lead ultimately to a narrow, unrealistic design space characterized by unaffordable
3388   operating ranges that are not in line with the accuracy of the standard equipment.
3389   On the other hand, targeting a defect rate that is too high would extend the design space with
3390   conditions of little added value, corresponding to highly variable CQAs/KPAs. This is reflected by the
3391   steep red zone on Figure 6-9A, as opposed to the green flat surface delineated by the 90% cut-off
3392   defect rate limit and selected because of the robustness of the different responses toward process
3393   parameters.
3394
3395   **Figure 6-10: (A) Robustness Surfaces Showing the Proportion of Simulated Results Meeting the**
3396   **Specifications as a Function of Temperature and Enzyme Concentration at pH 8.1, 8.3, and 8.5. (B)**
3397   **The Graphical Design Space, Represented As Green Areas, Is Significantly Larger Than the**
3398   **Tabulated Design Space (Rectangles)**
3399
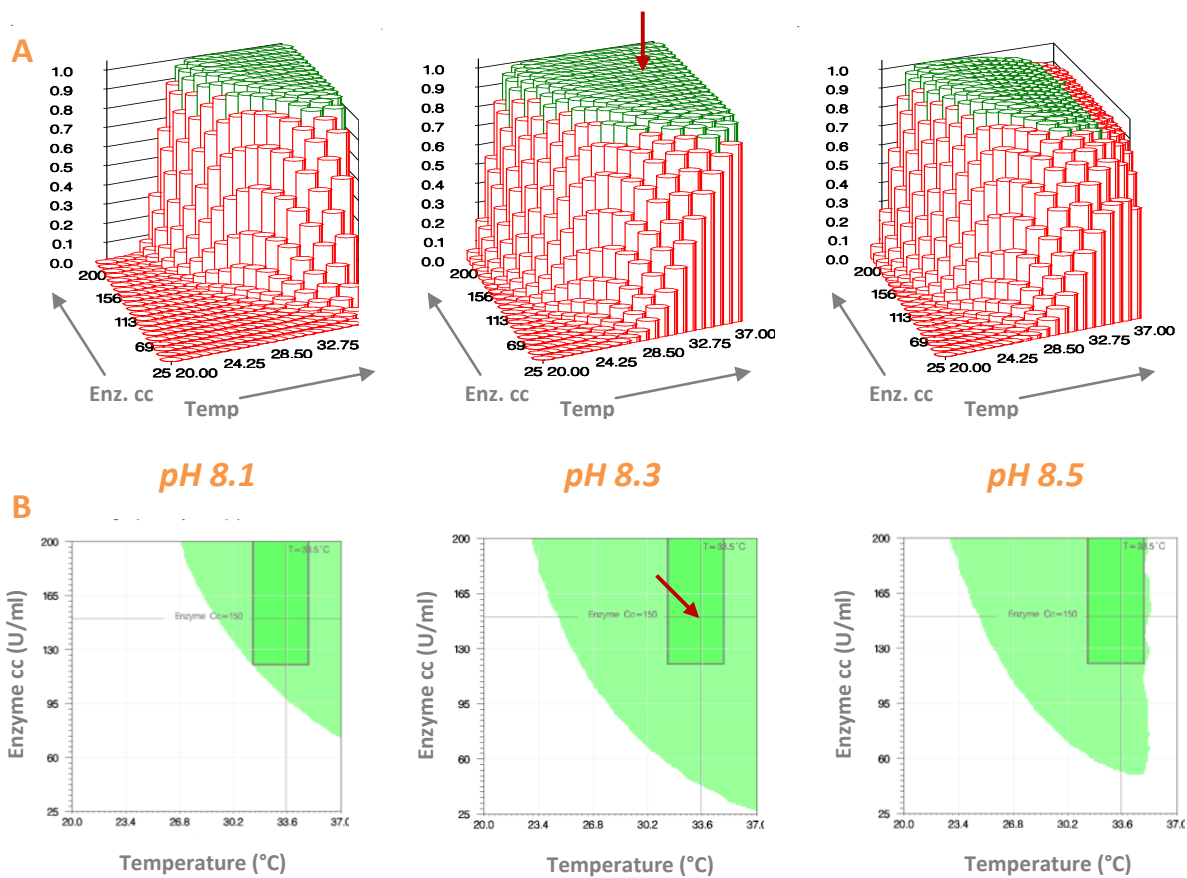
3400
3401

Design space based on simulated results, targeting maximum 10% defect rate	
Enzyme concentration	120–200 U/ml
Temperature	31.5–35°C
pH	8.1–8.5

3402

3403 The tabulated design space can then be studied in further detail to acquire more process and
3404 product knowledge within the defined ranges. A useful tool in this respect is the *defect profiler*;
3405 relying once again on Monte-Carlo simulations, the defect rates for the different responses and the
3406 overall defect rates are graphically displayed diverging from the reference values as a function of
3407 each parameter (Figure 6-11). Such representations allow visualizing simultaneously the respective
3408 contributions of each response to the overall defect rate.
3409
3410 However, it should be kept in mind that the defect profiler is a univariate graphical representation.
3411 Hence, Monte-Carlo simulations are generated with process parameters randomly located in the
3412 design space, assuming a uniform distribution for each factor. These simulations therefore include
3413 the most unfavorable combinations of parameters. The individual and overall defect rates are then
3414 calculated from the simulated results at reference conditions and within the design space (Table
3415 6-15); the overall defect rate at reference conditions amounts to 0.88%. It is slightly higher (1%) on
3416 average all over the tabulated design space and reaches a maximum of 8.08%.
3417
3418 Table 6-15 also confirms that Ps molecular size is the attribute that accounts for the major part of
3419 the defect rate, followed, locally, by residual peptidoglycans. The contribution of O-acetyl, if any, is
3420 marginal, and filterability is not a constraint. This type of information should be of great help to
3421 refine risk assessment and to design an appropriate control strategy.
3422
3423 **Figure 6-11: The Defect Profiler Shows Defect Rates of Simulated Results as a Function of Enzyme**
3424 **Concentration, Temperature, and pH.**

3425 *Defect rates refer to specifications of Table 6-15.*
3426
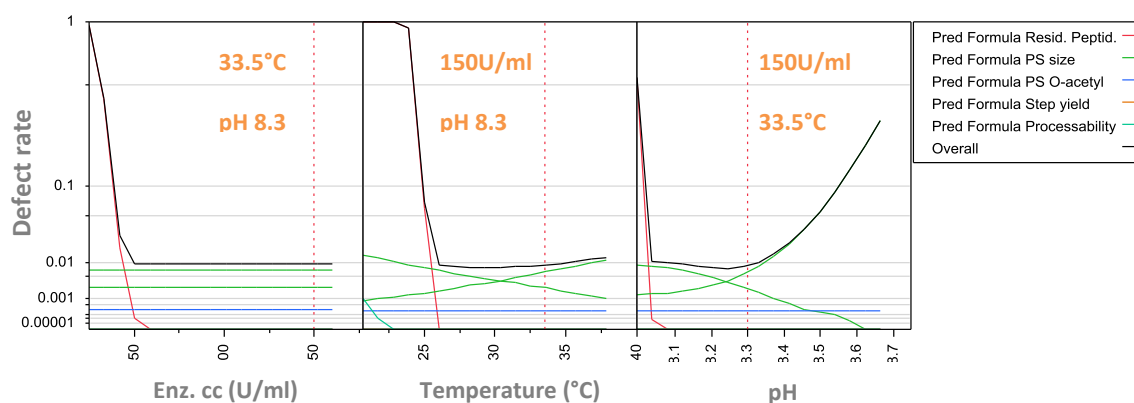
3427
3428
3429 **Table 6-15: Predicted Robustness Results at Reference Conditions and Into Design Space**

Parameters	Reference conditions	Design space	–
Enzyme concentration (U/ml)	150	120–200	–
Temperature (°C)	33.5	31.5–35	–
pH	8.3	8.1–8.5	–
Defect rates	**Defect rate at ref. conditions**	**Average defect rate into design space**	**Maximum into design space**
Residual peptidoglycan	0%	0.001%	0.54%
Ps size	0.85%	0.98%	8.06%
Ps O-acetyl	0.02%	0.02%	0.02%
Filterability	0%	0%	0%
All	*0.88%*	*1.00%*	*8.08%*

3430   ***Adding a parameter to the design space: incubation time***
3431   The time course of extraction was already investigated during early development (see Section 6.3) to
3432   define the incubation time (12 hours). Since the initial temperature and enzyme concentration were
3433   modified according to the results of the robustness DOE, the impact of incubation time is first re-
3434   explored in the new reference conditions:

3435   • enzyme concentration   150 U/ml   *(initial conditions : 100 U/ml)*
3436   • temperature   33.5°C   *(initial conditions : 35°C)*
3437   • pH   8.3   *(initial conditions: 8.4)*

3438
3439   Each incubation time is tested at lab scale on three different fermentation broths, and two
3440   responses are studied: the Ps extraction yield as determined by HPAEC-PAD and filterability of the
3441   extract. As shown in Figure 6-12, two to four hours are required to achieve a Ps recovery of 75% in
3442   the extract, but it takes eight hours to ensure that all three extracts tested are filterable. The
3443   incubation time could therefore range from eight to 24 hours, which is advantageous in terms of
3444   organizational flexibility. A safety margin of two hours, however, is applied to the upper and lower
3445   limits, restricting the range to 10 to 22 hours.

3446
3447   **Figure 6-12: Time Course of Extraction Step: The Target Yield Is Achieved Before the Filterability**
3448   **Criterion (Arrows)**

3449   ✔ *= filterable extract -* **x** *= nonfilterable extract*
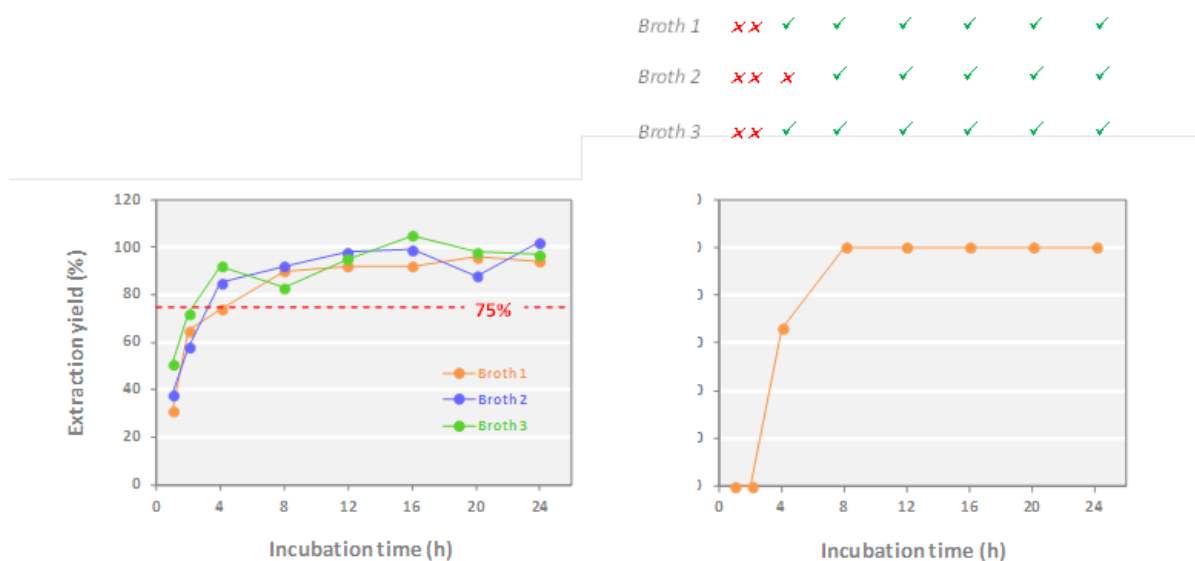
3450
3451   To validate this range in the design space, the lower and upper incubation times are combined with
3452   worst-case conditions deduced from prior knowledge and from the design space limits:

3453   • 10-h incubation combined with lowest pH (8.1), lowest temperature (31.5°C), and lowest
3454   enzyme concentration (120 U/ml) (i.e., conditions in which the reaction velocity is at a minimum
3455   and could thus lead to low extraction yields, poor filterability, and out-of-specification (OOS)
3456   levels of residual peptidoglycan).

3457   • 22-h incubation combined with highest pH (8.5) and highest temperature (35°C) (i.e., conditions
3458   in which the Ps is most prone to hydrolysis). In this case, the enzyme concentration shouldn't
3459   have any impact and can be used at its reference concentration (150 U/ml).

3460
3461   If both extracts are filterable and the Ps yields exceed 75%, the full process is applied to check that
3462   the purified Ps complies with all CQAs and KPAs. If this is actually the case, and assuming that factor
3463   effects and interactions are not impacted by the incubation time, it suggests that the 10–22-h
3464   incubation range is applicable all over the design space.

3465   In this approach, process and product knowledge captured from DOE studies is leveraged and
3466   combined with a simple univariate study to add a dimension to the design space with a limited
3467   number of experiments.
3468

> **Design space based on simulated results, targeting maximum 10% defect rate**
>
> | | |
> |---|---|
> | Enzyme concentration | 120–200 U/ml |
> | Temperature | 31.5–35°C |
> | pH | 8.1–8.5 |
> | Incubation time | 10–22 h |

3469
3470   ## 6.6.5. Univariate Studies

3471   ***Incubation time***
3472   The univariate study of the incubation time was integrated in the design space study and is therefore
3473   included in the section dedicated to the design space.
3474
3475   ***Enzyme batch***
3476   The study of this parameter is hampered by the limited availability of different enzyme batches at
3477   the time of process development (only two batches available). Indeed, to identify a possible batch-
3478   to-batch effect (three sigma) with a power >90%, a minimum of three batches are required and five
3479   repeats should be performed with each batch (Table 6-16); this would be unaffordable in terms of
3480   workload even if the batches were available.
3481
3482   Impact of the enzyme batch is therefore assessed through continuous monitoring as new batches
3483   are made available. If, despite passing all the QC tests, an enzyme batch is suspected to negatively
3484   impact CQAs/KPAs, its behavior could be checked at the vertices of the design space as described in
3485   the continuous improvement section for the shift to recombinant enzyme.
3486

3487   **Table 6-16: Power to Detect a Three-Sigma Difference between Lots** (F-test from a random one-
3488   way analysis of variance, α=5%)

Nb. Lots

Nb. Repeats/lot \	2	3	4	5	6	7	8	9	10
2	43%	65%	79%	88%	94%	96%	98%	99%	99%
3	63%	84%	93%	97%	99%	99%	100%	100%	100%
4	70%	89%	96%	99%	100%	100%	100%	100%	100%
5	74%	92%	97%	99%	100%	100%	100%	100%	100%
6	77%	94%	98%	99%	100%	100%	100%	100%	100%
7	79%	95%	99%	100%	100%	100%	100%	100%	100%
8	81%	95%	99%	100%	100%	100%	100%	100%	100%
9	82%	96%	99%	100%	100%	100%	100%	100%	100%
10	83%	96%	99%	100%	100%	100%	100%	100%	100%

Nb. Lots

Legend: ● <80% ● >80% ● >90%
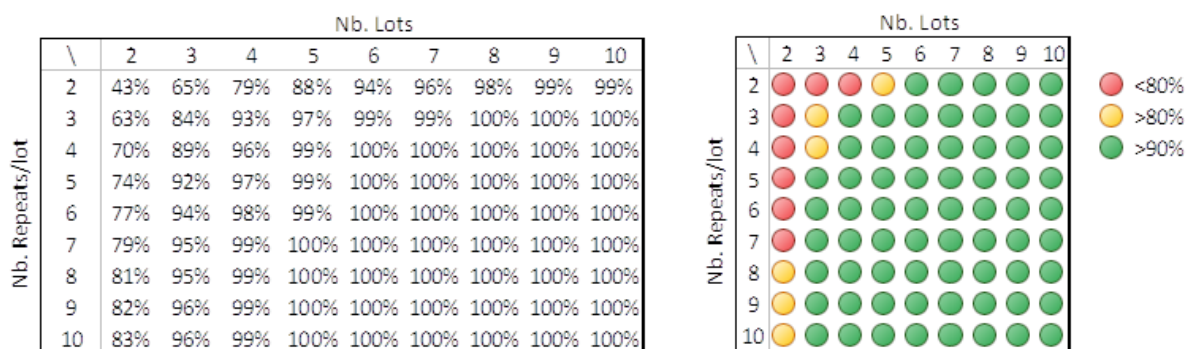
3489

6.7.   Polysaccharide Extraction Scale-Up

Knowledge and mechanistic understanding of the process serve as a foundation for developing a strategy for scale-up to manufacturing. Quality by Design tools and methodology help facilitate a systematic knowledge gain and process understanding. This knowledge is then coupled with thorough understanding of manufacturing-scale equipment (capabilities and limitations) to segregate process parameters considered important into scale-dependent and scale-independent parameters.

In the extraction step, parameters such as enzyme concentration, incubation time, and incubation temperature may be considered scale-independent parameters as long as confirming data using the full-scale equipment could be cited. With currently available technologies to ensure accurate reagent charges, ability to achieve a homogenous solution, and robust temperature control, maintaining control at manufacturing scale would not require any additional study. Acceptable ranges for these parameters would still need to be defined based on lab-scale studies, and ability to control these parameters at manufacturing scale would need to be confirmed. Confirmation also would be needed to demonstrate that dissolution characteristics of enzyme and other reagents are not sensitive to the type of mixing. This would be done through small-scale studies to evaluate dissolution rates using varying degrees of mixing (e.g., stir bar vs. overhead mixer).

In the event where the product is sensitive to shear or reaction rates are faster than a few seconds, parameters such as mixing, reagent addition methods, and variability in pH may be classified as scale-dependent parameters. Sensitivity to various types of mixing may need to be studied depending on mechanistic understanding of the process step and kinetics of reaction. In the extraction step, the type of mixing may be important to control shear on the molecule or prevent aggregation while ensuring good mixing. Reagent addition methods (dip-tube design, location, etc.) may impact reaction rates in cases where the kinetics of reaction is faster than the time it takes to achieve a homogenous solution. A reaction that takes place in a fraction of a second may require an exact scale-down version of manufacturing-scale equipment for development studies. In extraction for a 12-hour enzyme reaction, addition methods may not be as critical and may not require special equipment for development studies.

Lastly, variability in pH at manufacturing scale may negatively impact process performance. Ability to control pH at manufacturing scale would need to be well understood, including variability introduced by the pH measurement system. In the extraction step, enzyme efficiency may be optimal at the target pH and diminish quickly for a pH lower or higher than target pH. Assuming that manufacturing-scale equipment and pH control strategy are able to achieve control over only ±0.2 pH units and lab-scale process was developed by controlling pH within ±0.1 pH units, additional lab-scale studies may be required to show acceptable performance over this wider range of pH. Ideally, lab-scale process should be demonstrated over a pH range of ±0.3 pH units, slightly wider than the ability to control at manufacturing scale. See Figure 6-13 for an example of this data.

3531   **Figure 6-13: Residual Peptidoglycan in Purified Ps vs. pH during Extraction at Lab Scale**
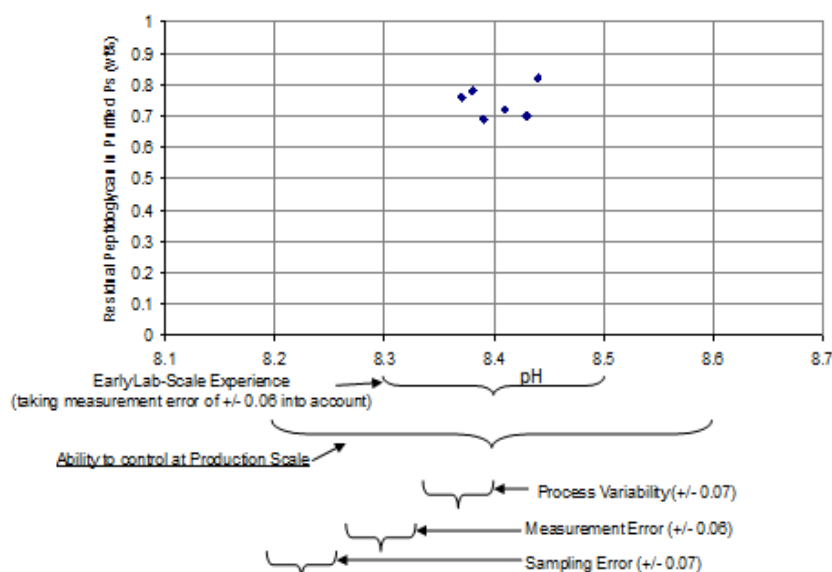
3532
3533   In an unlikely event where a wider pH range of ±0.3 pH units does not show acceptable process
3534   performance, additional development work and process changes may be required to ensure
3535   successful scale-up to manufacturing.
3536
3537   In an ideal scenario, knowledge related to the ability to control at production scale should be
3538   incorporated early in process development and generate data to support a wider pH range. In other
3539   words, design space work done early would be sufficient and no additional work would be required
3540   for scale-up. But in a typical process development scenario, it may be difficult to perform a large
3541   number of DOE studies early on to evaluate the impact of pH, and it may be desirable to tightly
3542   control pH around a known optimum to minimize risk of failure and stay on track for Phase 1 or 2
3543   clinical timelines.
3544
3545   As the program progresses and probability of scale-up increases, a risk assessment exercise should
3546   be planned to identify scale-dependent parameters and ability to control them at production scale.
3547   These design reviews involving process, manufacturing, and equipment experts early in the
3548   development process will ensure "right the first time" DOE design. They will minimize the number of
3549   experiments required at lab scale for Phase 3 process development while ensuring high probability
3550   of success at manufacturing scale. Early design reviews also help confirm applicability of the scale-
3551   down model and facilitate work to qualify the model.
3552
3553   Parallel processing of the same starting material through lab, pilot, and manufacturing-scale
3554   equipment would be one way to confirm applicability of the scale-down model. These experiments
3555   would be evaluated through appropriate CQA, product, and process characterization testing (data
3556   not shown).
3557
3558   In summary, early design space work using Quality by Design methodology can help ensure sufficient
3559   data is collected to properly define the manufacturing process and list of important parameters to
3560   be controlled. This early characterization work helps minimize the number of additional small-scale
3561   studies required during scale-up and tech-transfer activities. It also helps ensure that manufacturing-
3562   scale equipment is designed to best fit the process.

6.8.   Polysaccharide Extraction Post-Licensure Change

***Shift to recombinant enzyme expressed in* E. coli**

During the life cycle of a commercial product, changes in raw materials (e.g., source, vendor) often occur. Manufacturers must have processes in place to accommodate these changes without compromising product quality based on established critical quality attributes. A risk assessment is usually performed to assess the impact of a change such as a different raw material on the critical quality attributes. Using risk assessment tools, a severity score can be assigned based on the main and interaction effects (see Section 6.5, Polysaccharide Late Stage Risk Assessment). Based on the outcome of the risk assessment, manufacturers must develop a strategy to evaluate the change.

A change in raw material merits a number of considerations. As raw materials are usually product contact, the safety and consistency of the raw material are essential. Raw material qualification should be part of a company's GMP procedures and change control. The process typically involves qualification/audit of the vendor and qualification of the specific raw material (*Shadle, P.J., BioPharm, February 2004*). Raw material testing is also a key part of change control when a new raw material is introduced into the manufacturing process.

In the current case study, A-VAX, nonrecombinant enzyme (horrificase) that is purified from the bacterium *X. lyticus* is replaced with a new recombinant horrificase that is expressed in *E. coli* as part of a post-launch change. Because horrificase is a critical raw material, a change in expression source requires qualification and testing. It is expected that the vendor manufactures the raw material using a controlled process that is documented and personnel are trained to perform the manufacturing process. The following discussion addresses only the anticipated change in enzyme source. All other steps in the extraction process will be performed as developed, and thus no changes in impurity levels (e.g., DNA) are expected.

Raw material testing is performed to ensure that the new enzyme acts as expected in the vaccine manufacturing process. This qualification includes a comparison of the performance of the original enzyme (nonrecombinant purified from *X. lyticus*) with the new enzyme (recombinant purified from *E. coli*) against performance criteria that have been established for the specific unit operation (release of the capsular polysaccharide from *X. horrificus)*. For the new enzyme, testing of different lots (or batches) is performed to ensure consistency of the new raw material (refer to ICH guidance Q7: Good Manufacturing Practice Guide for active pharmaceutical ingredients (API)). Verification that the new enzyme also meets the specifications stated in the vendor's certificate of analysis is performed and involves evaluation of the enzyme activity and purity as noted in the specifications provided by the manufacturer.

In this case study, the change in enzyme was made because the recombinant enzyme had better purity compared with the nonrecombinant horrificase Table 6-17.

3603   **Table 6-17: Horrificase Batch Specifications**

Attribute	Specification (non recombinant)	Specification (recombinant)	Method
Purity	>90%	>95%	RP-HPLC
Specific activity	>5,000 U/mg	>5,000 U/mg	Turbidimetric assay Ref. lot: horrificase (manufacture A) as standard
Absence of contaminant glycosidase activity	Pass (no size decrease of ref. Ps in predefined conditions)	Pass (no size decrease of ref. Ps in predefined conditions)	SEC-RI Ref. lot: Ps bulk

3604
3605   The impact of this raw material change can be evaluated using a traditional or enhanced approach.
3606   The traditional approach relies on "confirm and verify," and the process would be run at a small
3607   scale using the setpoints (input parameters) previously established. The "output parameters" are
3608   measured and must meet the responses (CQAs) established. For A-VAX, the specifications are shown
3609   in Table 6-18. Finally, comparability studies would be performed to assess the conformance and
3610   behavior of the Ps bulks at commercial scale (see Comparability Section X.Y).
3611
3612   **Table 6-18: CQAs and Methods for Drug Substance (Extraction Step)**

Parameter	Specification	Method
Peptidoglycan content (%, w/w)	< 2	H-NMR
Ps size (kDa)	150–300	SEC-MALS
Ps O-acetylation (mol/mol Ps)	≥1.6	HPLC
Ps purity (%, w/w)	≥80	H-NMR
Ps yield (%)	>75	HPAEC-PAD

3613
3614   **Enhanced approach**
3615   The enhanced approach relies on application of product and process knowledge from the DOE used
3616   to determine the design space for the nonrecombinant horrificase (Table 6-19). Rather than
3617   checking the equivalence of the current and new enzymes at reference process conditions, the
3618   enhanced approach addresses whether the design spaces for the two enzymes overlap. To this end,
3619   a mini-DOE is performed at lab scale (0.5 L) to evaluate the behavior of the recombinant horrificase
3620   at the vertices (extremes) of the design space determined for the nonrecombinant horrificase. The
3621   lab-scale model was qualified as representative of the commercial-scale process during development
3622   with the nonrecombinant enzyme (see "Design Space" Section 6.6). It is assumed that the
3623   representativeness of the lab-scale model can be extended to the recombinant enzyme. This
3624   assumption relies on a risk assessment exercise based on product and process knowledge (not
3625   reported in this case study). Recall that the validity of the lab-scale model was verified using a
3626   commercial-scale batch produced with the nonrecombinant enzyme (extraction and purification at
3627   lab-scale run in parallel from the same commercial batch and comparison of in-process, QC, and
3628   characterization data).

3629   The aspects of process performance and product quality are addressed as follows (Figure 6-14):

3630   • Process performance (equivalence of KPAs): Extraction yield and filterability of the extract are
3631   checked at reference conditions and eight conditions representing the extremes of the design
3632   space. The clarified extract is not further processed.

3633   • Product quality can be assessed only on the purified Ps: The full purification process is applied
3634   (in duplicates) to clarified extracts obtained in two worst-case conditions of the design space.
3635   The resulting Ps is submitted to the full QC and characterization plan, including accelerated
3636   stability testing. Worst-case conditions are identified through risk analysis based on product and
3637   process knowledge:

3638   – Condition 1 is the worst case for enzyme activity. It corresponds to the lowest enzyme
3639   concentration and shortest incubation time combined with the lowest pH and temperature
3640   (suboptimal conditions for enzyme activity).

3641   – Condition 8 is the worst case for Ps stability. It corresponds to the longest incubation time
3642   combined with the highest pH and temperature (risk of Ps hydrolysis).

3643

3644   **Table 6-19: Reference Conditions and Design Space for Extraction Step (Nonrecombinant**
3645   **Horrificase)[a]**

Parameter		Design space range	Reference cond.
Enzyme concentration	(U/ml)	120 – 200	150
Temperature	(°C)	31.5 – 35.0	33.5
pH		8.1 – 8.5	8.3
Incubation time	(h)	10 – 22	12

3646
3647   a. See "Design Space" Section 6.6.

3648

3649   **Figure 6-14: Experimental Setup to Demonstrate the Design Space Equivalence for Current and**
3650   **New Enzyme. All Experiments Are Performed at Lab Scale.**
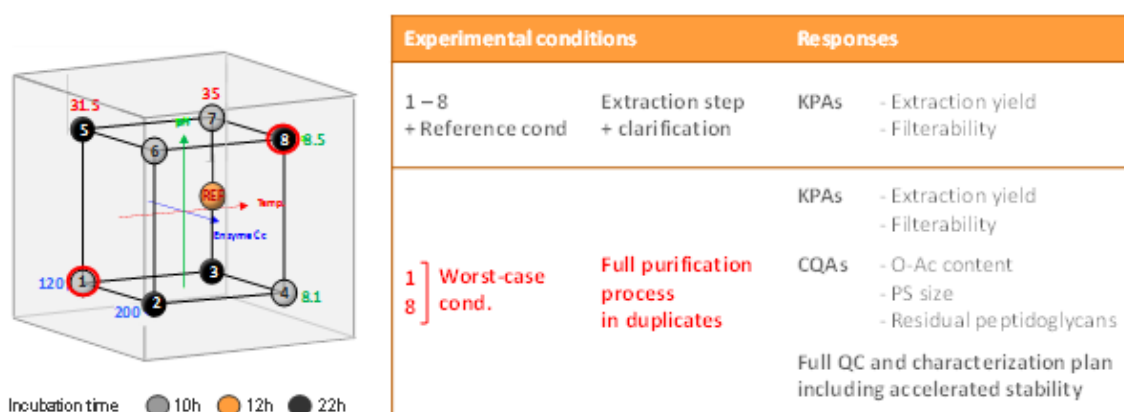
3651
3652

3653   Figure 6-15 (KPAs) and Table 6-20 (CQAs) show the results of the mini-DOE; all the responses meet
3654   the following acceptance criteria:

3655

3656   *For KPAs (Figure 6-15):*

3657   • Extraction yields with the recombinant enzyme fall within ranges in which 95% of the results are
3658   expected with the nonrecombinant enzyme.

3659   • All the extracts are filterable (> 15 L/m² filter area).

3660

3661   *For Ps quality attributes (Table 6-20):*

3662   • All Ps CQAs and other QC data meet the specifications (T=0 and accelerated stability).
3663   • All Ps CQAs, QC, and characterization data fall within ranges in which 95% of the results are
3664   expected in reference conditions with the nonrecombinant enzyme (T=0 and accelerated
3665   stability). For the sake of conciseness, only the three Ps CQAs used as responses in the initial
3666   DOE on the current enzyme are listed in Table 6-20.
3667
3668   It is concluded that the design space defined for the nonrecombinant enzyme applies to the
3669   recombinant enzyme, which is therefore deemed equivalent to the current enzyme.
3670
3671   **Figure 6-15: Extraction Experiment Design and Results Using the Nonrecombinant Enzyme. The**
3672   **extraction and clarification steps are performed at reference conditions and at the eight extremes**
3673   **of the design space with the new enzyme. The responses meet the acceptance criteria: Extraction**
3674   **yields are in the expected ranges, and all the extracts are filterable.**
3675
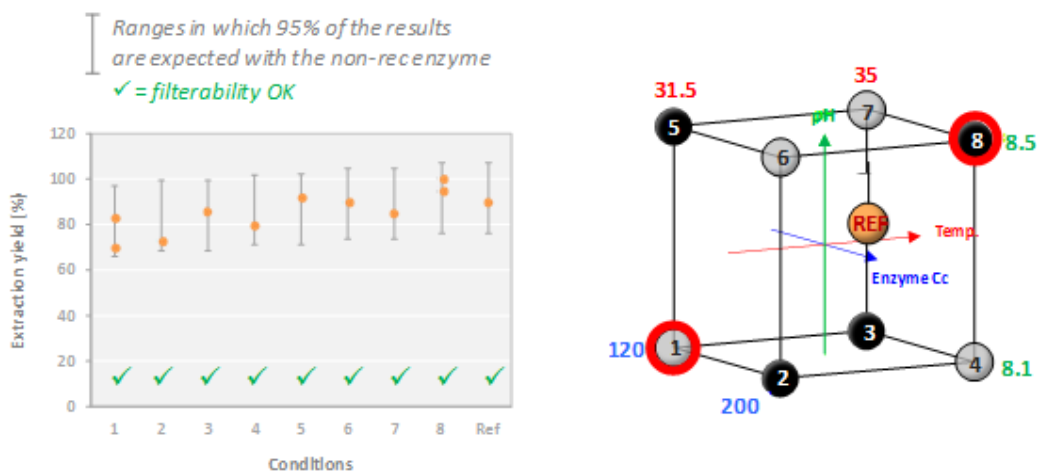
3676
3677
3678   **Table 6-20: Extraction Plus Purification Experimental Results with Nonrecombinant Enzyme. The**
3679   **full process is applied in duplicates to clarified extracts obtained in two worst-case conditions with**
3680   **the new enzyme. The four purified Ps meet the acceptance criteria: They comply with the**
3681   **specifications, and all the attributes fall within the expected ranges.**

CQA	Spec	Expected range*	Cond 1	Cond 8
Ps size	150-300 kD	180 – 260	189 – 248	211 – 191
Resid PG	< 2%	0.3 – 1.0	0.7 – 0.9	0.4 – 0.4
O-ac	> 1.6 mol/mol	1.7 – 2.0	1.92 – 1.89	1.95 – 1.73
All other acceptance criteria were met (QC/characterization data at T=0 and upon accelerated stability)				
* With nonrecombinant enzyme				

3682
3683   In the case of biological products with process improvements that have low-level impact and high
3684   process robustness based on well-defined CQAs and design space, the process is in a state of control
3685   and meets the predetermined quality requirements. As such, the requirement to complete three
3686   validation runs at full scale would not apply, and data from the DOE studies described in the
3687   enhanced approach could be used to support this change. Continued process verification is sufficient
3688   to show that at full scale, the purified Ps extracted with the new enzyme complies with all CQAs and
3689   KPAs and is comparable to the Ps produced with the current enzyme. In this case, establishing the

3690   comparability is facilitated by the high degree of physico-chemical characterization that can be
3691   achieved on polysaccharides. The enhanced approach is outlined in Figure 6-16. As explained in the
3692   regulatory section, a comparability protocol can be filed to seek regulatory approval. Comparability
3693   would be demonstrated at small scale; i.e., demonstration of design space equivalence between the
3694   current and the new enzyme, including the processing of two small scale lots to purified
3695   polysaccharide utilizing two worst-case conditions (see Figure 6-15 and Table 6-20). As laid out in
3696   Figure 6-16, full quality control including characterization and accelerated stability data are
3697   generated on the material at lab scale. This regulatory package should be satisfactory to seek
3698   regulatory approval; no commercial scale data are deemed necessary as the small scale model was
3699   demonstrated representative of commercial scale. Continuous process verification data on
3700   commercial scale lots, confirming process consistency within pre-set control limits, would be
3701   available post-registration and can be reviewed by the authorities as part of the Company's Quality
3702   Management System.
3703
3704   **Figure 6-16: Overview of the Enhanced Approach**
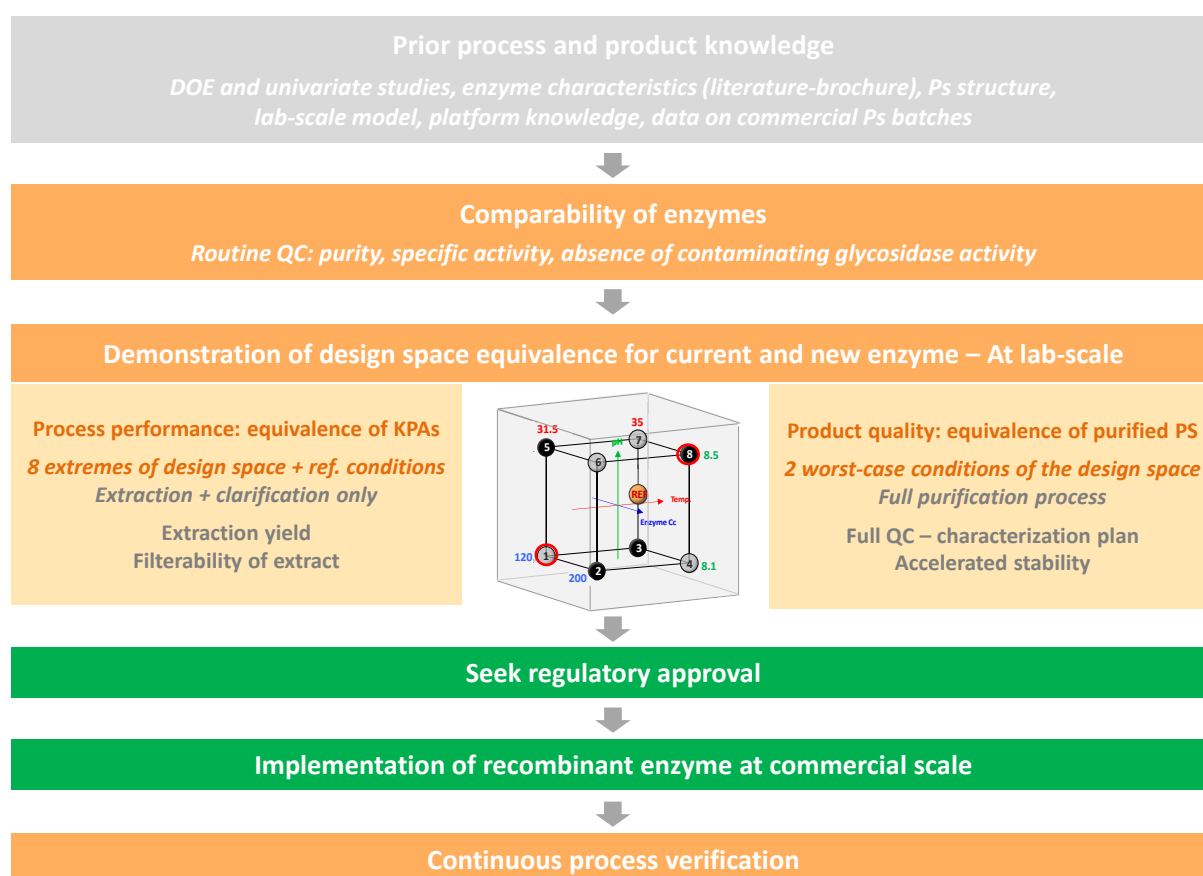
3705
3706   In a worst-case scenario where the recombinant horrificase did not perform as observed in the
3707   previous design space for the nonrecombinant horrificase, the results would be exploited to extend
3708   the DOE with relevant conditions to recalculate a new design space. Wherever possible, prior
3709   knowledge should be used to reduce the work. The new design space must provide a process that
3710   yields a purified Ps that complies with all CQAs and KPAs and should correspond to operating ranges
3711   that are compatible with the existing equipment.
3712
3713   Whether the design spaces for the current and new enzymes are equivalent or not, the enhanced
3714   approach offers several advantages in terms of process understanding and control. In a traditional
3715   approach, the current reference conditions would be applied to the new enzyme on three
3716   consistency batches at commercial scale, and the success criteria would be met if the three Ps
3717   batches comply with the usual QC requirements.
3718   The enhanced approach, however, provides information on process robustness and determines if
3719   the new enzyme is more sensitive than the current enzyme to the process parameters. In addition,

3720   in the event of problems with performance at scale, the enhanced approach provides important
3721   information for how to adapt the process parameters. In the case that the design spaces are not
3722   equivalent, the results of the mini-DOE can help orient an extended DOE and ultimately delineate a
3723   new design space – and perhaps new reference conditions – ensuring better process robustness and
3724   control.
3725
3726   Thus, in both cases (design spaces are equivalent or not), the enhanced approach reduces the failure
3727   risk of the first Ps batches produced at commercial scale with the new enzyme, assuming that the
3728   lab-scale model is predictive.
3729
3730   **Regulatory Filing Strategy**
3731   To utilize product knowledge captured in the design space to achieve a lowered change reporting
3732   category at the time of change implementation (at a later time), the design space pertinent to
3733   assessing future changes must be captured in the regulatory filings and approved as a sanctioned
3734   approach for regulatory change management. To accomplish this in US and EU filings, a protocol
3735   would need to be placed into the regulatory filings for each of the changes envisioned in the future
3736   that would merit the effort of seeking a lowered regulatory reporting category. In the arena of
3737   downstream processing, this could include a change in the type of process step (e.g., change in
3738   tangential flow cartridge, chromatography resin, change in critical raw material, change in process
3739   parameters).
3740
3741   The change in source of horrificase (nonrecombinant to recombinant) as presented in this case study
3742   is an anticipated change. A DOE approach would be used to determine whether the polysaccharide
3743   extraction process performs in the existing design space or whether a new design space is needed.
3744   To support the process change, the data from these studies would be used, as well as data from
3745   comparability studies performed to assess the conformance and behavior of the Ps bulks and
3746   compared against reference batches. The purified Ps bulk must meet all CQAs and KPAs established.
3747
3748   The initial US filing would be in the form of a "Comparability Protocol" (CP), and the initial EU filing
3749   would be in the form of a "Change Management Protocol" (CMP). These filings would require
3750   approval prior to their use in assessing a change (i.e., the US filing would be a Prior-Approval
3751   Supplement, and the EU filing would be a Type II variation). The protocol may be incorporated either
3752   at the time of the initial filing of the product for marketing approval or added after initiation of
3753   commercial marketing during later product life cycle management through the use of a post-
3754   approval update to the regulatory filings (see "Regulatory" section for more detail). In instances
3755   where a change control matrix has been established within the product marketing application, the
3756   initial filing of the update would also include the revised overall change control matrix table.
3757
3758   The protocol (CP or CMP) adds value for the sponsor by providing an agreement with the regulatory
3759   health authorities on the content of the filing that supports the change in advance of making the
3760   change. This mitigates the risk of delayed regulatory approval and provides additional control over
3761   timing and speed of implementing change for product distribution.
3762
3763   The initial protocols captured in the regulatory filing would fully describe how the change would be
3764   evaluated prior to distribution at commercial sale. The filing would contain a description of the
3765   change and the protocol for product comparability assessment, including prospectively defined
3766   acceptance criteria. The design space data would be provided as background and used to justify the
3767   acceptance criteria that are proposed for the evaluation of product comparability.

3768   The regulatory health authorities would evaluate the filing, and once they approve, it should be
3769   granted a lowered category for reporting. The categorization will depend on the degree to which the
3770   regulatory health authorities find the information sufficient to provide them with confidence that
3771   the change will be assessed in a manner that minimizes the potential for adverse impact on product
3772   safety, purity, potency, and effectiveness.
3773

3774   In general, the US FDA would lower the second report to the CBE30, CBE, or annual report reporting
3775   category level; and the European Union would be expected to reduce the second report to a Type
3776   1A$_{IN}$ or IB variation. The reporting category for the second filing would be proposed in the initial
3777   filing, and the specific second filing category found acceptable to the regulatory health authorities
3778   would be defined in the approval notification.
3779
3780   At the time of implementing the change, the assessment of change would have to be performed
3781   without significant deviation using the specific protocol that was approved by the regulatory health
3782   authorities, and reported using the method specified in the protocol approval notification received
3783   from the regulatory health authorities. Deviations from the protocol should be justified and
3784   discussed with regulatory health authorities to ensure that they do not see the potential for
3785   upgrading the change to a prior approval or Type II submission.
3786

3787   ## 6.9.   Virus-Like Particle Freezing Process Description

3788   ### 6.9.1. Process Overview

3789   The virus-like particles (VLPs) are purified after disrupting the *E. coli* cells in the harvested
3790   fermentation broth. Purification consists of a combination of filtration, chromatographic, enzymatic,
3791   and ultrafiltration steps. The purified VLP solution is frozen and stored at -70°C before conjugation
3792   with activated polysaccharides.
3793   The downstream process flowsheet and the purpose of each step are summarized in Figure 6-17.
3794

3795   ### 6.9.2. Unit Operation Selected

3796   For the sake of conciseness, purified VLP solution freezing is the only VLP downstream step that will
3797   be covered in this case study.
3798

3799   ***Step description***
3800   Purified VLP solution is transferred to containers for freezing and storage at -70°C.

3801   •   VLP solution is dispensed into containers that can withstand the freezing process as well as
3802   physical handling in the frozen state while maintaining integrity.

3803   •   The VLP solution is frozen by placing the containers in a -70°C blast freezer. Afterwards, the
3804   containers are transferred to -70°C freezers for long-term storage.

3805   •   The VLP solution in the containers will eventually be thawed and filtered at 0.2 microns prior to
3806   use in the conjugation process.

3807   ***Rationale for selecting the freezing step as an example***

3808   •   The step is likely to impact the key CQA of average VLP size, an indirect measure of the extent
3809   of aggregation.

3810   ***Subset of CQAs and KPAs used in example***
3811   VLP solution freezing conditions most likely impact the following CQA and KPA, which will be
3812   considered in the example:
3813

3814   **CQA**

3815   •   VLP size: Aggregation of the VLPs may influence the average VLP size and therefore the average
3816   size of the resulting Ps-VLP conjugate.

3817   **KPA**

3818   • VLP concentration following thaw and filtration (yield): Because aggregation can lead to VLP
3819   losses upon filtration of the thawed VLP solution. Measured by UV or BCA protein assay.

3820   **Figure 6-17: Virus-Like Particle Flowsheet and Objectives of the Different Steps**

Fermentation harvest	
↓	*Transfer to downstream*
Cell disruption	→ Releases VLPs
↓	
DNase treatment	→ Digests residual nucleic acids
↓	
Clarification by centrifugation	→ Removes cells and cell debris
↓	
Cation exchange chromatography	→ Removes proteins, host cell impurities
↓	
Hydroxyapatite chromatography	→ Removes proteins and nucleic acids
↓	
Anion exchange chromatography	→ Removes proteins
↓	
Detoxification	→ Removes endotoxin
↓	
Ultrafiltration 100kD Concentration + diafiltration	→ Buffer exchange and concentration
↓	
0.22µm filtration	→ Control bioburden
↓	
Freezing at -70°C	
↓	
Bulk Virus-Like Particles	

3821

3822   ## 6.10. Virus-Like Particle Freezing Early Process Development

3823   Following purification, the purified VLPs are transferred to storage containers, frozen, and stored at
3824   -70°C. During downstream conjugation, the bulk containers are thawed at 2–8°C prior to use.
3825   Although the product is stable at the listed temperatures, limited information is available to
3826   characterize the impact to product quality of the freezing and thawing process.
3827

3828   Literature *(S.D. Webb, J.N. Webb, T.G Hughes, D.F. Sesin, and A.C. Kincaid, "Freezing*
3829   *Biopharmaceuticals Using Common Techniques and the Magnitude of Bulk-Scale Freeze*
3830   *Concentration," Biopharm 15(5) 2-8 (*2002)) suggests that freezing processes can affect the
3831   properties of proteins and other biopharmaceutical intermediates via various mechanisms. One
3832   mechanism, cryo-concentration, has been evidenced through data showing a greater than eight-fold
3833   increase in bulk Bovine Serum Albumin (BSA) concentration and a 20-fold range of BSA
3834   concentrations within frozen 1-liter bottles *(S.D. Webb, et. al.).* During cryo-concentration, salts and
3835   other large molecules diffuse from the ice front that forms as the bulk solvent freezes. Slower
3836   freezing kinetics will increase the degree of cryo-concentration, as the solutes have more time to
3837   diffuse.
3838
3839   Early development: target storage conditions
3840   The scale and container for early development work were chosen to minimize freezing path length
3841   and potential reactions with materials of construction. This work was done in a 1 mL glass cryovial.
3842   Freezing and thawing rates at this scale will be much greater than the practical freezing rate at final
3843   manufacturing scale. The scale/container was chosen to represent "ideal" rates of change (i.e.,
3844   minimization of container path length). A very small container (1 mL) was selected to maximize rates
3845   of freezing and thawing. Analytical confirmation (size by dynamic light scattering (DLS) and
3846   concentration using BCA as the referenced standard) during the freeze/thaw developmental work
3847   confirmed suitability of frozen storage conditions and that the VLP was stable through the freezing
3848   and thawing process.
3849
3850   Seven 1 mL glass cryovials were filled to 800 μL with VLP; one cryovial was placed in a 2–8°C
3851   refrigerator (control), and six cryovials were placed in a -70 °C freezer. After five days of storage, the
3852   vials were thawed at 2–8°C and tested for VLP size using DLS and concentration using BCA as the
3853   reference standard.
3854
3855   Results from the early development work indicate that there were no appreciable changes in VLP
3856   size or concentration following the freeze-thaw process. Measurements of size and concentration
3857   were within 3% of the control value, indicating no significant changes in the attributes.
3858
3859   Early Development: Establishment of Glass Transition Temperature to Determine Storage Conditions
3860   Controlled temperature units (CTUs) typically have a tolerance of +/- 10–15°C around the setpoint. It
3861   is essential that the VLP is stored at a temperature where natural CTU temperature oscillations do
3862   not cause constant transition across the $Tg^{'}$. Additionally, because the bulk will be kept in inventory
3863   for ~10 years, storage conditions will be chosen so that the VLP is below the $Tg^{'}$.
3864
3865   Results from differential scanning calorimetry (DSC) are presented in Table 6-21. An example of the
3866   DSC plot is shown in Figure 6-18. The average $Tg^{'}$ value by DSC analysis for three lots of VLP was
3867   calculated as -40.8°C.
3868
3869   Standard freezer design requirements are intended for storage at -20, -40, or -70°C. Ideally, the VLP
3870   would be frozen at -20 or -40°C; however, the $Tg^{'}$ determined by DSC indicates that selection of a
3871   -20°C freezer would be above the glass transition temperature and a -40°C freezer would cause
3872   continuous oscillations across the transition temperature because of freezer cycling. The $Tg^{'}$ data
3873   suggests -70°C storage is more appropriate for the VLP.
3874

3875   **Table 6-21: VLP in 200 mM NaCl, 30 mM Histidine pH 7.2**

Description	N	Tg' (deg C)	Onset (deg C)	Heat flow reduction (deg C)
VLP Run 1	1	-38.62	-42.29	-21.9
VLP Run 2	2		-40.99	-21.71
VLP Run 3	3		-39.26	-21.49
		AVERAGE	**-40.85**	**-21.7**

3876

3877   **Figure 6-18: Example Glass Transition Temperature and Heat Flow Onset for VLP**
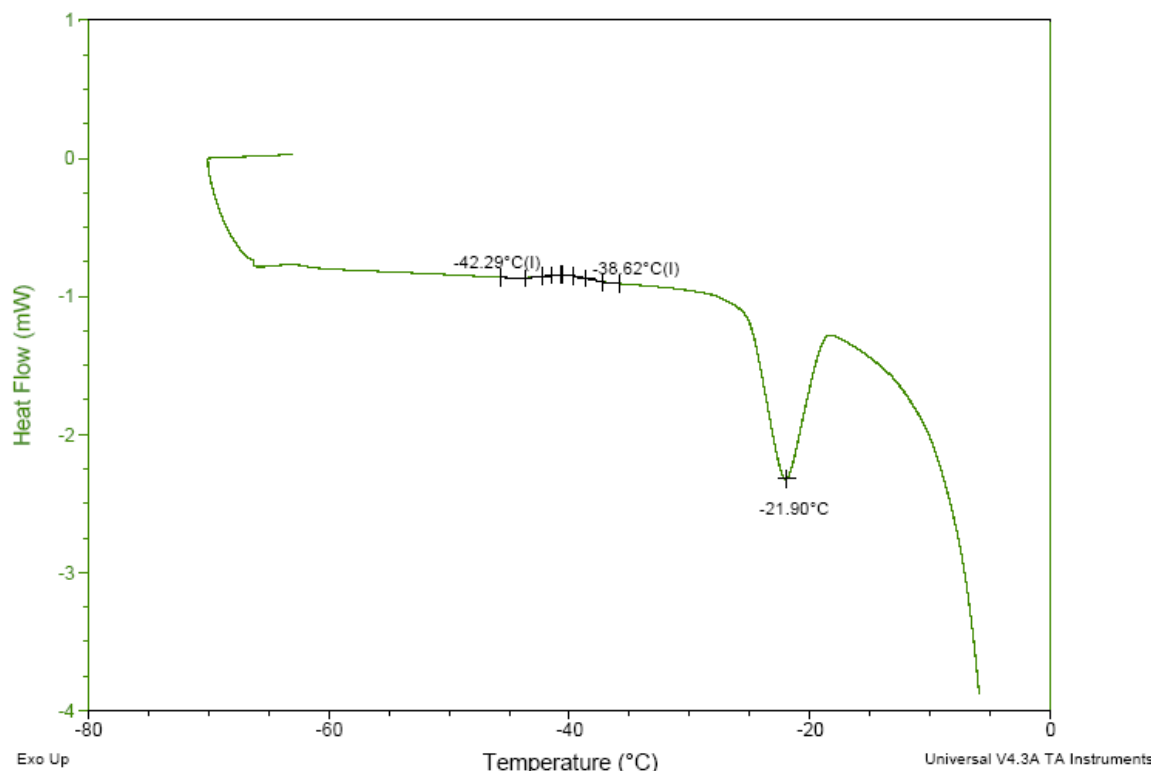
3878
3879

3880   ## 6.11. Virus-Like Particle Freezing Risk Assessment

3881   Because the VLP will be stored as a bioburden-reduced bulk, sterilized containers will be required.
3882   Additionally, container closure integrity (CCI) must be demonstrated to prevent potential extrinsic
3883   contamination during the container life cycle. Following container selection, freezing conditions, and
3884   determination of fill volume, torque specifications and CCI for the closure will be established as part
3885   of a separate validation study. All of these will be taken into account when selecting the final VLP
3886   container.
3887
3888   At a VLP concentration of ~ 1 g/L, approximately 100 L of purified bulk will be generated per lot. It is
3889   assumed that minimizing path length is critical to prevent impact on the bulk attributes during
3890   freezing. The appropriate container size will minimize the number of containers while maximizing
3891   the fill volume (typically 60–80% of container volume). This balance also will consider the greater
3892   path length with increasing container size. The bottle cannot be so large that the kinetic rate of
3893   freezing/thawing as a result of path length impacts the bulk attributes.
3894
3895   A cause-and-effect matrix risk assessment (Table 6-22) was performed to categorize the operating
3896   parameters that may impact VLP attributes during freezing and thawing. The parameters were

3897 placed into two groups: (i) parameters warranting experimental evaluation and (ii) parameters that
3898 are considered low risk and would not require evaluation. The category (ii) parameters would
3899 employ ranges based on prior knowledge. Each process parameter was assessed based on the
3900 potential impact on VLP size and VLP concentration.
3901
3902 The scoring of process parameters and quality attributes is described in Section **Error! Reference
3903 source not found.** and outlined in Table 6-30. The cumulative score is determined by *Σ(Impact of
3904 parameter x weight of quality or process performance attribute).* The cumulative score represents
3905 the relative importance of the parameter on VLP storage considerations. Parameters with scores
3906 exceeding 50 were considered to be high risk with the potential to impact product quality or process
3907 performance and were candidates for further experimental evaluation. Those with scores less than
3908 50 were considered low risk and were not further evaluated.
3909
3910 **Table 6-22: Cause-and-Effect Matrix for VLP Storage Conditions**

Parameter	Quality Attribute Weight				Cumulative Score
	VLP Size		VLP Concentration		
	Impact	*Weight*	*Impact*	*Weight*	
Container Size	7	7	7	7	98
Fill Volume	1	7	1	7	14
Rate of Thawing	5	7	7	7	84
Rate of Freezing	7	7	7	7	98
Material of Construction	5	7	5	7	70
Initial Temperature	1	7	1	7	14
Initial [VLP]	1	7	5	7	42

3911

6.12. Virus-Like Particle Freezing Design Space

3913 Using the cause-and-effect matrix cumulative scores, three design criteria were assessed during
3914 developmental work: the rates of freezing and thawing and the container size. A full-factorial DOE
3915 (n=3, 3 levels) covering three freezing and thawing conditions and three container sizes was
3916 conducted for various materials of construction.
3917
3918 Freezing levels were on dry ice, in a -70°C upright freezer, and at 0.1°C/min. The 0.1°C rate of
3919 change was mediated through a temperature-controlled chamber (TCC). Rheostat control was used
3920 to adjust the TCC between -80 and 8°C to achieve the predefined freezing rate. The three freezing
3921 rates represent fast, medium, and slow freezing, respectively. Thawing was initiated two days after
3922 freezing. Table 6-23 lists the full-factorial design conducted per container.
3923
3924 Thawing levels were evaluated using a 30°C water bath, 2–8°C CTU, and 0.1°C/min, representing
3925 fast, medium, and slow thawing, respectively. Following thawing, samples were kept at 2–8°C before
3926 testing. Container size was modeled by scaling the final targeted containers (1 L, 2 L, and 3 L) to
3927 cryovials of increasing size. Samples were filled to 0.80 mL in a 1 mL cryovial, 1.09 mL in a 5 mL
3928 cryovial (26% increase in path length), and 1.27 mL in a 10 mL cryovial (37% increase in path length),
3929 illustrated in Figure 6-19.
3930
3931 All samples were tested against the 1 x 1 mL cryovial control. Since a cryovial is much smaller than
3932 the final manufacturing container, concentration and size effects may not be observed. The intent of
3933 varying path length during developmental work was to determine if any sensitivity exists when

3934   tested at a minimized scale. If attribute changes related to changing path length are observed at a
3935   small scale, the opportunity for freezing the VLP in larger containers may be limited.
3936
3937   **Figure 6-19: Increasing Path-Length Modeling Varying Container Sizes**
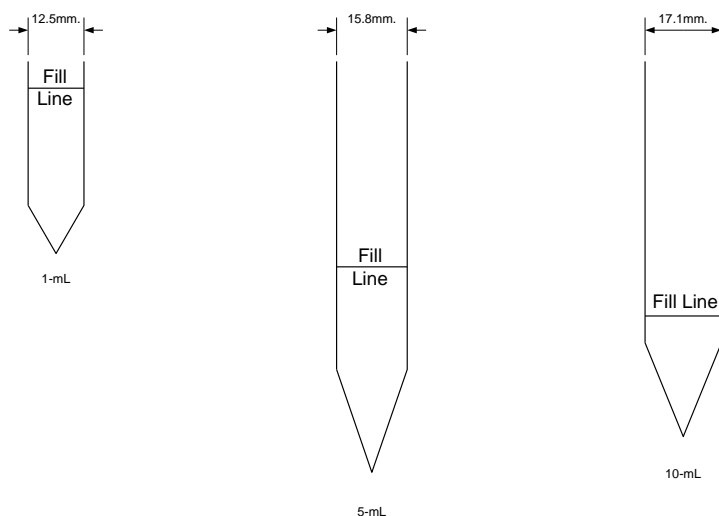
3938
3939
3940   Because material of construction also scored high, a variety of materials were also evaluated
3941   experimentally. Because the $Tg^{'}$ studies indicated frozen bulk storage would be required, a subset of
3942   materials was chosen because of the materials' thermostability at -70°C and previously
3943   demonstrated CCI validation. The three materials selected were polypropylene, perfluoroalkoxy
3944   (PFA), and fluorinated ethylene propylene (FEP).
3945
3946   An additional FMEA (not shown) was conducted to identify failure modes during the freezing
3947   process. The highest-scoring RPN out of that assessment resulted from pulling a "half-frozen"
3948   container out of the freezer, thawing, and then re-freezing it. A one-factor-at-a-time study was
3949   conducted to evaluate multiple freeze/thaws. The results of this study showed no statistically
3950   significant ($p < 0.05$) differences against an unfrozen control.
3951
3952   **Table 6-23: Freeze-Thaw Study Arm Description**

Factor	High	Middle	Low
Thawing	30 °C	2–8 °C	0.1 °C/min
Freezing	Dry ice	-70 °C	0.1 °C/min

3953   Fixed parameter: fill volume
3954
3955   Data analysis identified PFA as the material showing the least change in VLP attributes. The
3956   analytical summary of PFA results is presented in Table 6-24.
3957
3958   The VLP was insensitive to freezing or thawing rates and container size within the bounds of the
3959   study at all but one condition. When the VLP was frozen at the slowest and thawed at the fastest
3960   kinetic rates, there was a statistically significant increase in VLP size ($P < 0.05$).
3961
3962   The experimentally evaluated design space encompassed a broad range of kinetic rates. Although no
3963   failure limits were identified within the selected ranges, the design space would suggest there is an
3964   impact on VLP size when a slow rate of freezing is combined with a high rate of thawing, regardless
3965   of path length. This interaction was not seen when the main effects were evaluated for each
3966   individual condition. Additionally, the effect was noted only for VLP size.
3967

3968   **Table 6-24: Percent Change Against 2–8 °C Reference for PFA Container DOE**

Fill volume	Freezing	Thawing	% change after freeze/thaw against 2–8 °C reference	
			VLP size	VLP conc.
+	+	+	+ 2.7	+ 2.5
+	+	(1)	+ 1.0	+ 0.2
+	+	-	+ 0.3	- 1.9
+	(1)	+	+ 2.1	+ 2.1
+	(1)	(1)	- 2.1	- 2.7
+	(1)	-	+ 1.9	- 2.2
+	-	+	+ 8.2	+ 0.4
+	-	(1)	- 0.4	+ 0.0
+	-	-	- 0.1	+ 1.0
(1)	+	+	- 0.5	+0.1
(1)	+	(1)	+0.1	+0.2
(1)	+	-	+0.0	+0.5
(1)	(1)	+	-0.4	+2.4
(1)	(1)	(1)	-0.2	- 0.5
(1)	(1)	-	+ 2.7	+ 2.5
(1)	-	+	+ 13.0	+ 0.2
(1)	-	(1)	+ 0.3	- 1.9
(1)	-	-	+ 2.1	+ 2.1
-	+	+	- 2.1	- 2.7
-	+	(1)	+ 1.9	- 2.2
-	+	-	+0.3	-2.1
-	(1)	+	-1.8	+0.2
-	(1)	(1)	- 0.5	+0.3
-	(1)	-	-0.7	+.08
-	-	+	+ 9.6	+1.4
-	-	(1)	-1.4	+2.1
-	-	-	+1.3	+2.2

3969   (1) represents center, (+) represents high, (-) represents low
3970
3971   Lab-scale model:
3972   Six batches of VLP drug substance lots in 200 mM NaCl, 30 mM histidine (pH 7.2) were aseptically
3973   transferred to autoclaved, 1 L PFA bottles with c-flex tubing and closures. A 1 L container was
3974   selected to determine if the early development work was reproducible at a larger scale. After filling,
3975   the PFA bottles were placed in an upright freezer (<-60°C) for at least 15 hours. Frozen VLP lots were

3976   thawed either in an approximately 20°C water bath with periodic swirling or in an approximately 4°C
3977   cold vault without swirling.
3978
3979   Samples taken from VLP lots before and after the freeze/thaw cycle were assayed using DLS for size
3980   and BCA for concentration. The ID number and fill weight of each lot are listed in Table 6-25. Also
3981   listed in Table 6-25 is the approximate thaw temperature used for each VLP lot.
3982
3983   **Table 6-25: ID Numbers, Fill Weights, and Thaw Temperatures used in 1 L PFA Freeze/Thaw Studies**

VLP ID number	Fill weight (g)	Approximate thaw temperature (°C)
VLP 1	507	20
VLP 2	293	20
VLP 3	455	4
VLP 4	429	20
VLP 5	510	20
VLP 6	443	4

3984
3985   The percentage (%) change in VLP size and concentration after the freeze/thaw in 1-L PFA bottles is
3986   listed in Table 6-26. Based on the results shown in Table 6-26, there were no statistically significant
3987   changes in properties measured by the DLS or BCA assays ($p < 0.05$).
3988
3989   **Table 6-26: Percent Change in VLP Properties after Freeze/Thaw**

VLP ID number	% change after freeze/thaw	
	VLP Size	VLP conc.
VLP 1	- 2.7	- 2.5
VLP 2	- 1.0	- 0.2
VLP 3	- 0.3	1.9
VLP 4	- 2.1	- 2.1
VLP 5	2.1	2.7
VLP 6	- 1.9	2.2

3990
3991   Results of the 1 L PFA bottle scale-down confirmed that the VLP attributes remain unchanged when
3992   compared with the early development work. The 500 mL fill in a 1 L PFA bottle will be used to model
3993   the rate of freezing. This rate will be used to specify the large-scale design requirements. Because
3994   thawing rates have not shown an impact on conjugate attributes at 1 mL and 500 mL scale, a fixed
3995   2–8°C thaw will be used for the final process.
3996
3997   Static freezing temperature profiles
3998   Experiments were performed to determine the freezing profiles of 500 mL of VLP buffer (200 mM
3999   NaCl, 30 mM histidine, pH 7.2) in a 1 L PFA bottle. Studies were conducted within a <-60°C upright
4000   static freezer (Forma Scientific). A single bottle filled with room-temperature buffer was placed in
4001   the middle of the second shelf from the top (in the four-shelf freezer). Temperatures were collected
4002   during the freezing process. Three independent experiments were performed, each collecting
4003   temperatures at three different positions along a horizontal plane in the PFA bottle.
4004

4006   Figure , thermocouples 1, 3, 4, 6, 7, and 9 were positioned 1.5 cm from the vertical wall of the bottle;
4007   thermocouples 1, 2, and 3 were positioned 1 cm from the bottom of the bottle; and thermocouples
4008   7, 8, and 9 were positioned 1.5 cm below the buffer surface. The 500 mL buffer volume was
4009   measured to have a liquid height of 7.8 cm in the 1 L PFA bottle. Thermocouples were equally
4010   spaced along the horizontal plane at 1.5 cm apart.
4011
4012   Temperatures were recorded for thermocouple positions 1 through 3 for freezing experiment 1,
4013   positions 4 through 6 for freezing experiment 2, and positions 7 through 9 for freezing experiment 3.
4014
4015   **Figure 6-20: Position of Thermocouples**

4016   Experiment 1: Thermocouple Positions 1 through 3,
4017   Experiment 2: Thermocouple Positions 4 through 6, Experiment 3: Thermocouple Positions 7 through 9
4018
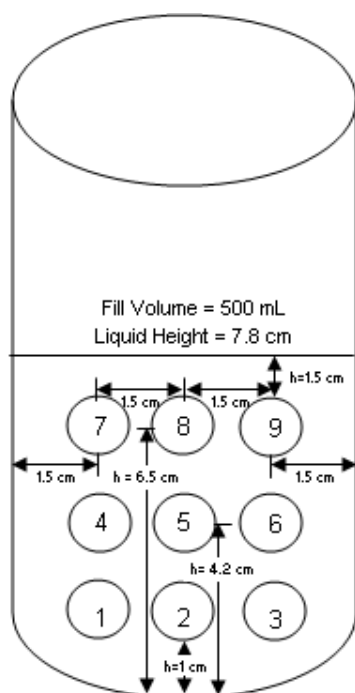
4019
4020
4021   Refer to Figure 6-21 for the static freezer temperature profiles for a single set of experimental
4022   conditions (worst-case freezing positions shown). Experiment 1 evaluated the bottom-most
4023   container plane (positions 1–3). Experiment 2 evaluated the mid-plane (positions 4–6). Experiment 3
4024   evaluated the top-most plane (positions 7–9). Thermocouple position 5 (experiment 2, position 5)
4025   was identified as the worst-case location for freezing, and the maximum pull-down time to the onset
4026   of the glass transition temperature, -41°C, was determined to be 3.7 hours. Each thermocouple
4027   position within the 1 L bottle reached -70°C after six hours of storage. Because the 500 mL lab-scale
4028   work showed no impact to VLP attributes at the same rate of freezing, the 3.7-hour pull-down time
4029   was used to set the large-scale user requirements.
4030
4031

4032   **Figure 6-21: Experiment 2 Temperature Profiles: Thermocouple Positions 4 through 6**
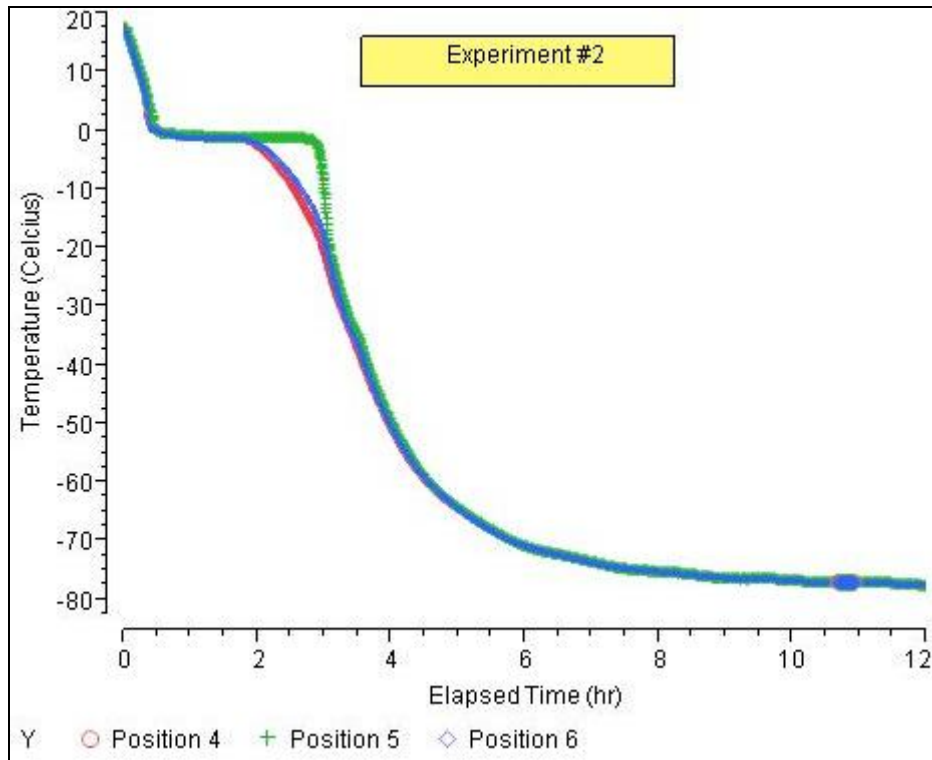
4033

4034
4035   These studies were completed in 1 L PFA bottles (d=92 mm). The rate of freezing at the 1 L scale is
4036   presented as a worst case and will be used to justify the maximum allowable drop-down to the glass
4037   transition temperature onset in larger-capacity bottles. Table 6-27 indicates the bottle specifications
4038   for the 1 L, 2 L, and 3 L narrow-mouth PFA bottles. Because path length is critical in freezing
4039   phenomena such as cryo-concentration, maintaining or reducing the pull-down time of 3.7 hours for
4040   the 1 L bottle (worst-case condition) assures that the overall rate of freezing is faster than the 1 L
4041   scale-down study.

4042
4043   For the final manufacturing facility, the blast freezer user requirements specify a pull-down time of
4044   3.7 hours for a 3 L bottle with a 146 mm diameter or a 2 L bottle with a 125 mm diameter. Since a
4045   maximum of 100 L purified bulk will be generated per batch, approximately 65 containers will be
4046   generated. The blast freezer and associated trolley should be designed to allow all 65 containers to
4047   be frozen at once.

4048
4049   **Table 6-27: Narrow-Mouth PFA Bottle Specifications**

Part No.	Neck ID (mm)	Filled Capacity (mL)	Body Diameter (mm)
1 L bottles	25.5	1,060	92
2 L bottles	36.5	2,080	125
3 L bottles	26.5	3,350	146

4050
4051   Cryo-concentration and blast freezer evaluation:
4052   In a liquid nitrogen blast freezer, a fine spray of liquid nitrogen is directed on the product containers.
4053   Two internal "turbulence fans" circulate the cold gas generated by the evaporation of the liquid
4054   nitrogen. This freezing method takes advantage of both a high temperature gradient (ΔT) for the
4055   entire freeze cycle and an increased overall heat transfer coefficient achieved by the convection
4056   enhanced by the turbulence fans.

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4057   Because the pull-down time specified in the static freezing experiment is not achievable in a
4058   conventional <-60°C upright freezer, a blast freezer will be used.
4059
4060   The maximum pull-down time of 3.7 hours was used to set blast freezer design criteria.
4061
4062   The 2 L production-scale containers were filled with 1.6 L of VLP in 200 mM NaCl, 30 mM histidine,
4063   pH 7.2. Increasing to a 3 L bottle increases the diameter by 15%. The 2 L bottle was chosen because
4064   of blast freezer design considerations. The addition of 15% in bottle diameter would drive the
4065   purchase of an additional 8.5 kW of condensing requirements. This increase would result in an
4066   additional upfront capital cost of ~35%. It is always possible to increase the height of the 2-L bottle
4067   and maintain the path length. The containers selected here are currently available from an approved
4068   vendor and were selected to minimize additional vendor qualification activities.
4069
4070   During blast freezer operational qualification (OQ), the unit was temperature mapped using
4071   minimum and maximum bottle loads within the freezer trolley. This study was to identify which
4072   position in the chamber was the fastest and slowest to reach the glass transition temperature.
4073
4074   Following blast freezing to -70°C, one bottle from the center of each shelf and the slowest and
4075   fastest freezing locations was physically cut into three discs (top, middle, and bottom). The top and
4076   middle discs were cut into nine segments. The bottom disc was cut into two concentric circle
4077   segments. After the segments were thawed, the conductivity and VLP size of each sample were
4078   tested to determine whether stratification or cryo-concentration had occurred at the final design
4079   condition and to determine if there were any impacts on the VLP size. As expected, the maximum
4080   observed conductivity and size difference was at the center of the bottle. The difference at the
4081   center was within 5% and 3% of the average conductivity and size values, respectively (acceptance
4082   criteria is < 10% and < 5% against control for conductivity and size, respectively. These results
4083   confirm cryo-concentration was successfully minimized upon scale-up.
4084
4085   Establishing a design space for VLP bulk storage requirements demonstrated that the VLP attributes
4086   of size and concentration can be preserved within the ranges tested. The ranges were used to select
4087   the final container and design the final freezer requirements needed to maintain the maximum pull-
4088   down rate. The design space data also showed that the VLP attributes were relatively unaffected
4089   within the ranges tested. Use of the blast freezer and a 2 L PFA container, regardless of the thawing
4090   rate, will be acceptable during the final manufacturing process. Table 6-28 shows the target and
4091   acceptable ranges based on the design space.
4092
4093   **Table 6-28: Target and Acceptable Ranges for VLP Freezing Design Space**

Parameter	Target	Acceptable Range
Material of construction	Perfluoroalkoxy (PFA)	N/A
Container diameter (mm)	125	+/- 20 mm
Fill volume (L)	1.6	+/- 0.5 L
Average rate of freezing (°C/min)	- 0.64	</= - 0.64
Average rate of thawing (°C/min)	0.03	</= 0.03

4094
4095   **Post-licensure change**
4096   Changes in material availability are a common occurrence during a product life cycle. If the current
4097   2 L container is no longer available, a change will be required to continue manufacturing activities. If
4098   a comparable 2 L PFA container is not available, any container within the acceptable diameter range
4099   can be considered. The design of the blast freezer was chosen to achieve frozen conditions at all
4100   container locations using a 125 mm-diameter container. A decrease in size below the target

4101 diameter would decrease the path length and maintain the acceptable rate of freezing. Increases in
4102 diameter are acceptable; however, modifications to the blast freezer may be required to ensure
4103 acceptable rates of freezing. To support this change, freezing rates would be confirmed using
4104 temperature mapping during the blast freezing process.
4105
4106 If a change in VLP mass is required on a per-container basis, the fill may be increased or decreased
4107 within the acceptable range. Since volume change does not alter path length, the rate of freezing
4108 will not alter at the core locations (worst-case location). If a fill volume change and a container
4109 diameter increase are required, the same consideration for blast freezer design will be evaluated.
4110
4111 If needed, the listed change would occur after initiation of commercial marketing during later
4112 product life cycle management through the use of a post-approval update to the regulatory filings
4113 (see "Regulatory" section for more detail).
4114
4115

4116   6.13. Ps-VLP Conjugation Process Description

4117 **Unit operations selected**
4118
4119 A-VAX provides an enhanced cellular (Th1) and humoral (Th2), antigen-specific, protective immune
4120 response when compared to a natural *X. horrificus* infection. The exact mechanism by which A-VAX
4121 stimulates the cellular and humoral immune response is not known; however, only the Ps-VLP
4122 conjugate can initiate a protective immune response to Ps in the target age group. The effectiveness
4123 of this conjugate in vaccination depends on the activation and conjugation steps since they
4124 determine the chemical structure of the product.
4125
4126 **Process description**
4127
4128 The conjugation process is summarized in Table 6-29.
4129
4130 *Activation*
4131 The rationale for the activation design was to increase the number of polysaccharide chains and
4132 attachment sites, more specifically the number of available aldehyde groups on a polysaccharide
4133 chain that could be used for conjugation. The target mean molecular size for the depolymerized
4134 polysaccharides was based in part on literature precedence, intellectual property, and the target
4135 density of the reducing end sugar groups.
4136
4137 Dissolved polysaccharide is treated with base to reduce the O-Ac content and create more vicinal
4138 diols for oxidation to aldehydes (Figure 6-1). Oxidation is accomplished with sodium meta-periodate.
4139 Conditions were optimized for decreasing polysaccharide chain length to an average MW between
4140 10,000 and 15,000 Da, and for the activated polysaccharide to contain an average concentration of
4141 reducing groups of 30 mol/mol of Ps. Size is monitored at-line by HPSEC. Activation is closely
4142 monitored and controlled: pH is monitored in-line and molecular size is monitored at-line.
4143
4144 *Conjugation*
4145 The conjugation was designed to link aldehyde groups on the activated polysaccharide directly to
4146 amino groups on the VLP via reductive amination. Conjugation was optimized to produce a loading
4147 ratio of activated polysaccharide to VLP of 0.3–0.7 based on the results of animal studies for
4148 maximum immunological response. Reductive amination is accomplished using sodium
4149 cyanoborohydride. The number of available aldehydes is controlled by time and pH of conjugation,
4150 and the conjugation reaction is stopped by a "capping" reaction with sodium borohydride to reduce
4151 unreacted aldehydes to alcohol. Unreacted Ps is separated from the conjugated VLPs using
4152 tangential flow filtration and chromatography unit operations (Table 6-29).

4153
4154 **Subset of CQAs and KPAs**
4155 • Activation and conjugation parameters can be critical as these steps determine the chemical
4156 structure of the product.
4157 • Conjugation performance is linked with the outcome of the activation step.
4158 • Conjugation can Impact downstream steps (e.g., aggregate from conjugation step could result in
4159 fouling of TFF membrane).
4160
4161 CQAs

4162 **Activation**
4163 • Activated Ps size: There is a general relationship between immunogenicity and Ps size. Size is
4164 monitored at-line by HPSEC.
4165 **Conjugation**
4166 • Free Ps: The presence of free unbound Ps could modify the immune response produced by the
4167 immunization with the Ps-VLP. Also, a conjugate vaccine with less unconjugated Ps is preferable
4168 since it contains more active ingredient. Free Ps is monitored by HPAEC-PED.
4169 • Ps-VLP ratio: The ratio of Ps to protein was found to be critical for optimal antibody responses in
4170 other Ps-protein conjugate vaccines. The ratio is calculated from extent-of-conjugation data.
4171 • Ps-VLP size: The molecular size of the conjugate is considered important for the potency of the
4172 targeted product. Ps-VLP size is monitored by dynamic light scattering (DLS).
4173 • Potency: Conjugation reaction completes the formation of the Ps-VLP molecule that is the active
4174 ingredient inducing immunologic response.
4175 KPAs

4176 • Reducing activity after activation: Ps cannot be chemically linked to a protein without first
4177 undergoing activation.
4178 • O-acetyl concentration after activation: It could be linked with the immunogenic epitope of the
4179 Ps. The concentration is calculated by H-NMR or the Hestrin method.
4180 • Activation and conjugation step yields.
4181
4182 *Impact of conjugation on potency*
4183 The premise behind the example in this case study is unique. Though differences in the nature of the
4184 conjugated Ps-VLP product could impact its potency, we cite prior experience and claim that results
4185 of *in vivo* testing of Ps-VLP product made using worst-case conjugation conditions (at extremes of
4186 the targeted design space) show that differences in conjugate structure in this example do not
4187 impact its potency. However, even if this were true, a typical vaccine candidate would not have a
4188 potency assay that had been correlated with human performance as is claimed for four of the
4189 serotypes in this case study. Therefore, a typical vaccine candidate might be handled as the fifth
4190 serotype in this case study, and only minor changes within the design space might be considered
4191 acceptable without clinical confirmation.

4192   **Table 6-29: Process Flow Diagram**

Dissolution of the Polysaccharide Bulk Time 8–12 hr Mix speed 200–250rpm Temperature 2°C–8°C	The powder is dissolved in 75 mM sodium acetate to a concentration of 10 g/L.
↓	
Activation of the dissolved Ps a. Add 80 mM NaOH and incubate 15 min ± 5 min at 35±5 °C, pH 11 b. Adjust pH to 5.5 ± 0.1 with HCl and adjust temp to 15°C ± 2°C c. Adjust the Ps solution to 25 mM sodium meta-periodate, pH 5.5, and stir at 15°C in dark d. Allow the reaction to mix until the mean molecular size is less than 15,000 Da determined by HPSEC e. Quench the reaction by adding 0.5 mL of glycerol per gram Ps.	Ps is depolymerized and oxidized using periodate to introduce terminal reactive aldehydes. Monitoring testing: - Sampling for Ps size (HPLC) - pH
↓	
Concentration and diafiltration of the depolymerized/activated polysaccharide Adjust pH 6.3 ± 0.1 (pH adjusted with 1N NaOH and 1N HCl) and concentrate to 20g L. Diafilter against PBS and 0.1M PIPES (MWCO 1000 Da).	Remove activation reactants/residuals and exchange buffer for preparation of conjugation.
↓	
Conjugation of depolymerized/activated polysaccharide (DAPS) to VLP a. Target 10 gL-1 VLP and 20 gL-1 DAPS b. Adjust pH to 8.0–8.5 c. Add NaCNBH4 at excess 10–20 mg mL-1 d. Mix 18–24 hr @ 200 ± 50 rpm @ 15–35 °C e. Dilute with saline 1:2 f. Add NaBH4 at excess 10–20 mg mL-1 g. Mix 15–25 min@ 200 ± 50 rpm	
↓	
Tangential Flow Filtration Diafilter, 10 vol physiological saline, 50 kDa MWCO membrane.	Remove unreacted components and conjugation residuals.
↓	

Hydroxylapatite Chromatography Elution with phosphate buffer in isocratic gradient.	Remove unreacted components and conjugation residuals.
↓	
Tangential Flow Filtration Diafilter, 15 vol PBS, pH 6.3, 100 kDa MWCO membrane.	
↓	
0.22 µm filtration	

4193

6.14. Ps-VLP Conjugation Early Process Development

6.14.1. Prior Knowledge

4196 General process steps and conditions were defined based on two licensed conjugated
4197 polysaccharide vaccines and general conditions described in literature.
4198
6.14.2. Activation

4200 **Literature reference**
4201 The rationale for developing a depolymerization process of the purified capsular polysaccharide was
4202 to decrease the Ps size and increase the number of activation sites per polysaccharide chain that
4203 could be used for conjugation *(Silveira et al, Vaccine 25 (2007), 7261–7270)*.
4204
4205 The operating ranges mentioned in the literature cover the following ranges:
4206

Process parameter	Min	Max
Sodium meta-periodate concentration (mM)	10	25
Activation time (hr.)	0.5	4
Temperature (°C)	15	40
pH	9	12

4207
4208 However, the literature does not show a consistent relationship between Ps size and
4209 immunogenicity (*C.H. Lee, et al, Vaccine, 27, 2009; T. Carmenate et al, FEMS Immunology and
4210 Medical Microbiology, 40, 2004*).
4211
4212 **Early process development**
4213 To determine the minimum chain length of the Ps that can be used to elicit a specific anti-
4214 polysaccharide immune response in laboratory animals and define a working range for temperature
4215 and sodium meta-periodate concentration, the following was performed with four lots of Ps.
4216
4217 Two levels of temperature and sodium meta-periodate were selected while keeping all other
4218 variables at target values (see process flow diagram in Table 6-29).
4219

Lot	Temperature (°C)	Sodium meta-periodate (mM)
1	15	10
2		25
3	40	10
4		25

4220

4221 The rate of depolymerization was evaluated by sampling at different times and size fractions
4222 evaluated by HPSEC (Figure 6-22).

4223

4224 Based on the results, it can be observed that:

4225 • The rates of the depolymerization reactions are faster at higher concentrations of sodium meta-
4226 periodate (groups 2&4).

4227 • There was no apparent relationship between reaction temperature and the rate of
4228 depolymerization (group 1 vs. 3 and group 2 vs. 4).

4229 • The reducing activity content of the four lots was considered comparable to one another based
4230 on the assay variability (data not show).

4231

4232 **Figure 6-22: Mean Ps MW (Da) by Reaction Time (min)**
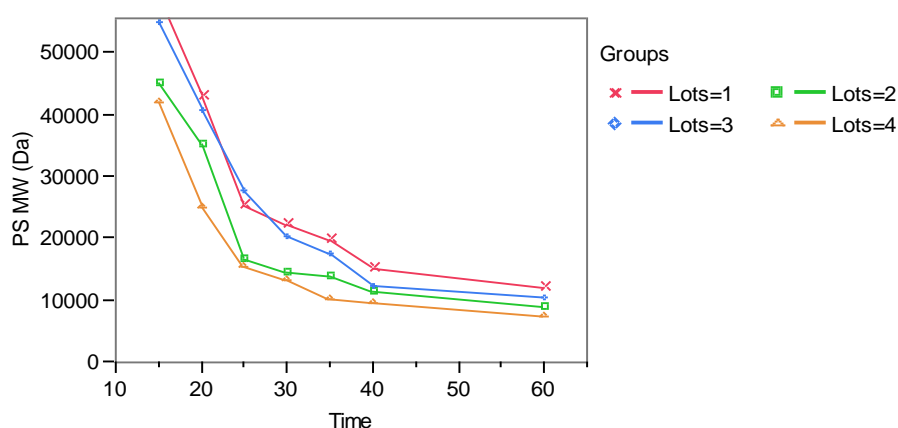
4233
4234
4235 Fractions of small, medium, and large size were conjugated accordingly for further study of their
4236 immunogenicity.

4237

Immunogen	Ps MW (Da)	Titer*
Low	5,000–10,000	4.9
Medium	10,000–15,000	4.3
Large	15,000–25,000	3.5
Initial Ps	> 40,000	1.6

4238 * Mean ELISA titers were calculated using arbitrary unit of ELISA (EU/mL).

4239

4240 Although these studies were successful in confirming that all of the conjugates developed greater
4241 response than the initial polysaccharide, no significant response was observed among the Ps size
4242 tested. Also, determining the minimum chain length requirement to elicit immunogenicity of the
4243 polysaccharide-protein conjugate in lab animals is a risk because these relatively short chain lengths
4244 may not necessarily be the optimal chain length that maximizes the immune response in humans.
4245 Thus, taking into account literature precedence, intellectual property, and the target density of the
4246 reducing end sugar groups, a final range of 10,000 to 15,000 Da was defined.

4247   In addition, the primary structure of the depolymerized Ps purified from the reaction was evaluated
4248   by NMR spectroscopy. The ^{1}H NMR spectra of DAPS presents the same assignments as the Ps,
4249   showing that the polysaccharide structure remains unchanged. However, after de-O-acetylation and
4250   periodate treatment, chemical shifts are present that correspond to the novel end groups. These
4251   chemicals shifts are consistent with the aldehydic group.

4253   As a result, the following conditions were defined.

Temperature (°C)	35 ± 5
Sodium meta-periodate (mM)	25
Ps size (Da)	10,000–15,000

6.14.3. Conjugation

4257   The process that was evaluated during the design phase attempted to yield more than one reactive
4258   site per polysaccharide chain, and this in turn led to multi-site attachment of the Ps to the VLP.
4259   Furthermore, the process while maintaining antigenic consideration must also be applicable to
4260   conjugate the five different serotypes.

Literature reference
4263   Typically, the ratio DAPS:VLP could change among the serotypes, leading to adjustments in the DAPS
4264   and VLP concentration. In addition, increasing the VLP concentration while keeping the DAPS
4265   concentration constant normally results in an increase in VLP-VLP cross-link, which has a potential
4266   impact on filterability. Also, conjugation reaction could be affected by the charge density associated
4267   with each serotype polysaccharide and the reactivity of the amino groups of VLP *(Joshi et al,*
4268   *Carbohydrate Polymers 75 (2009), 553–565)*.

Early process development
4271   Concentration ratios from about 1:2 to about 2:1 were used for the other serotypes. Based on that
4272   previous experience, 2:1 conditions for the Ps:VLP concentrations were selected. To define pH
4273   conditions, different pHs were evaluated at lab scale while keeping constant other reaction
4274   conditions. Conjugate molecules were further purified by dialysis.

Ps molecular weight (MW)	Ps:VLP concentration	pH	Free Ps (%)	Conjugate ratio (0.3–0.7)
10,000–15,000	2:1	7.0	10.2	0.28
10,000–15,000	2:1	7.5	9.5	0.32
10,000–15,000	2:1	8.0	11.3	0.53
10,000–15,000	2:1	8.5	10.8	0.49

4277   Working in a pH range of 8.0 to 8.5, there did not appear to be a significant impact on either the
4278   polysaccharide-to-protein ratio or the extent of free Ps. The other attributes met their criteria. As a
4279   result, the following conditions were defined.

Conjugation pH	8.0–8.5
Concentration ratio Ps:VLP	2:1

6.15. Ps-VLP Conjugation Early Process Risk Assessment

4284   A cause-and-effect matrix (C&E) was the risk assessment (RA) tool used to identify processes
4285   parameters for creation of the design space. The C&E matrix provides a mechanism to assess process
4286   parameters (inputs) against quality and process attributes (outputs) to prioritize parameters for
4287   experimental studies. However, the matrix does not provide manufacturing control boundaries
4288   (process parameter ranges) to assess the potential severity impact of the factors assessed.
4289
4290   The goals of the C&E matrices are to capture the current knowledge and the relationships among
4291   inputs and outputs, to prioritize areas for further study and experimental design, and to evaluate the
4292   completeness of the process understanding.
4293
4294   The key deliverable is the prioritization of high-risk process parameters for designed process
4295   characterization experiments. As knowledge of the commercial manufacturing process and facility
4296   becomes available, facility control and procedural capabilities may also be evaluated with failure
4297   modes and knowledge gaps identified.
4298
4299   **Cause-and-effect matrices**
4300   To create a cause-and-effect matrix, the following steps are necessary:
4301   3.   Create a process flow map (prerequisite as described above).
4302   4.   Define focus areas/unit operations (prerequisite).
4303   5.   Identify and rank attributes (quality and process) for each focus area/unit operations.
4304   6.   Identify and rank the relationship between process parameters and attributes.
4305   7.   Calculate cumulative parameter scores.
4306
4307   The CQAs for the final drug substance and drug product should be determined prior to the creation
4308   of a C&E matrix.
4309
4310   Each process parameter (input) is assessed based on the potential impact on the outputs of a
4311   particular focus area, including quality attributes or process performance attributes. The inputs are
4312   process parameters that can be people, equipment, measurements, process, materials environment
4313   etc., while the outputs are VLP-poly conjugates, aggregates, biopotency, endotoxins, free VLP, free
4314   poly, excess reagents, contaminants, product degradants, step yield, etc. A subset of CQAs was
4315   considered for risk assessment (e.g., free Ps, Ps/VLP ratio, Ps-VLP size, potency). The objective is to
4316   establish the functional relationship between quality attributes (y) and process parameters (x). Each
4317   quality attribute is assigned a "weight" score based on its potential impact on product quality,
4318   safety, or efficacy (Table 6-30).
4319
4320   For example, QAs that are deemed to be critical will fall into the 10 or 7 scores, while QAs that are
4321   borderline regarding criticality would score a 5 (Table 6-30).
4322
4323   A cumulative score is then calculated for each parameter using Equation 6-1.
4324
Equation 6-1: Cumulative Score for Parameter in C&E Matrix

4325   *Cumulative score = ∑ (Impact of parameter x weight of quality or process performance attribute)*
4326   The cumulative scores in the (C&E) matrix are used to identify the process parameters and the
4327   experimental approach for process understanding studies. The maximum cumulative score will vary
4328   by focus area and will depend on the number of attributes scored.
4329
4330   The cumulative score represents the relative importance of a process parameter for the focus area
4331   (or unit operations), so parameters with high scores could potentially be of high risk to product
4332   quality or process performance and should have supporting process understanding. The process

4333 parameter prioritization for experimentation is subject to the team's interpretation and may be
4334 governed by statistical approaches, prior knowledge, or specific product safety concerns.
4335

4336 For those parameters requiring study, a combination of univariate and multivariate experimental
4337 studies may be performed to identify significant effects and to characterize the process design
4338 space. The justification for parameters requiring no new studies may be complemented by the
4339 consideration of prior knowledge established for the same or related products (platform data) or of
4340 literature information.
4341

4342 The process parameters evaluated in the risk assessment for the activation and conjugation steps in
4343 Table 6-31 and Table 6-32, respectively, were identified based on prior experience.
4344

4345 **Table 6-30: Scoring of Process Parameters and Quality Attributes**

Process Parameters		Attributes[1]	
Impact Score	Ranking Criteria	Weight Score	Ranking Criteria
10	Strong relationship known based on available data and experience	*10*	Established or expected direct impact on safety and/or efficacy of product.[2]
7	Strong relationship is expected	*7*	Moderate or indirect impact on safety and/or efficacy. Direct impact on efficiency.
5	Not-so-strong relationship expected or unknown	*5*	Low or unlikely impact on product safety and/or efficacy. Moderate or indirect impact on efficiency.
1	Known to not have a relationship	*1*	No impact to product safety and/or efficacy. Low or unlikely to impact efficiency.

4346
4347 [1] Process performance attributes may have no direct impact on product quality, safety, or efficacy but are assessed where
4348 they are important indicators of focus area function or performance consistency. Examples include step recoveries and
4349 overall yield.
4350 [2] May include efficiency/process attributes, but most efficiency attributes are not a 10 unless they significantly impact
4351 product viability.
4352

4353 **Table 6-31: Cause-and-Effect Matrix for Activation of Polysaccharide**

	Reducing activity	[O-Ac]	Activated poly size	Yield	Total score
Quality attribute scores	10	7	7	7	
Parameter					
Activation temp	10	7	10	5	254
Activation pH	10	10	7	5	254
Activation time	10	7	10	5	254
Poly concentration	1	5	5	1	87
Total grams of poly added	1	5	5	1	87
Concentration of meta-periodate [1]	5	5	1	1	87

	Reducing activity	[O-Ac]	Activated poly size	Yield	Total score
Addition rate of meta-periodate	1	5	1	1	59
Activation reaction agitation rate	1	5	1	1	59
Ratio of glycerol to poly for quenching	1	1	1	1	31
Quenching reaction time	1	1	1	1	31
Post-quench hold temperature	1	1	1	1	31
Post-quench hold time	1	1	1	1	31

4354 [1] Parameter known to not have an impact on activated Ps size at the range to be used in this process based on prior
4355 experience.
4356
4357 The highlighted scores signify grouping of parameters with similar scores. In this example,
4358 parameters with scores of 254, highlighted in red in the C&E table, are deemed to be of high priority
4359 for process characterization studies. The color grouping of parameters is based on the natural breaks
4360 in the scores. For example, parameter scores of 87 are highlighted yellow, and the remaining
4361 parameters with scores from 59 through 31 are not highlighted. The parameters highlighted in
4362 yellow have lower cumulative scores and have ample prior knowledge/literature, thus do not require
4363 further studies. The parameters in the no-shaded box were deemed to be of low risk, and no further
4364 study was undertaken.
4365 **Table 6-32: Cause-and-Effect Matrix for Conjugation**

	Free Ps	Ps/VLP ratio	Ps-VLP size	Yield	Potency	Total score
Quality attribute scores	10	10	10	7	10	
Parameters						
VLP and poly concentration	10	10	10	10	10	470
Conjugation reaction incubation temp	10	10	10	10	7	440
Agitation rate during VLP addition	10	5	5	10	7	370
NaCNBH4 excess ratio	10	10	5	5	7	370
VLP addition rate	5	1	1	5	5	155
Conjugation reaction time	1	1	1	1	5	87
Conjugation reaction agitation rate	1	1	1	1	1	47
NaBH^4 excess ratio	1	1	1	1	1	47

	Free Ps	Ps/VLP ratio	Ps-VLP size	Yield	Potency	Total score
Capping reaction time	1	1	1	1	1	47
Capping reaction temp	1	1	1	1	1	47

4366

4367 The process parameters identified (highlighted in red in the C&E table) after RA for further study are:
4368 activation temperature, time, and pH for the activation of polysaccharide step. For the conjugation
4369 reaction: VLP/poly stoichiometry, incubation temperature, agitation rate during VLP addition, and
4370 NaCNBH$_4$ excess ratio. All of these parameters were selected for their relative high scores when
4371 compared with the other parameters assessed in their respective unit operations. The parameters
4372 highlighted in yellow have lower cumulative scores and have ample prior knowledge/literature, thus
4373 do not require further studies. The parameters in the no-shaded box were deemed to be of low risk,
4374 and no further study was undertaken.

4375

4376 ## 6.16. Ps-VLP Conjugation Late Stage Risk Assessment

4377 The second-round RA is conducted prior to process validation. For this evaluation, the large-scale
4378 manufacturing process normal operating ranges (NORs) are known or estimated based on prior
4379 experience. The DOE studies have identified potential NORs and proven acceptable ranges (PARs)
4380 within which consistent process performance and acceptable product quality are expected.
4381 The FMEA is conducted to evaluate the drug substance manufacturing processes and the potential
4382 impact on process performance and product quality.
4383 The goals of the FMEA are focused on assessing the potential severity impact in relation to
4384 manufacturing process, site capabilities, and operational experience. Other outcomes from the
4385 second RA include process parameter risk identification/mitigation and potential parameter
4386 criticality classification.

4387

4388 **Failure Modes Effects Analysis (FMEA)**
4389 The principles of FMEA were previously described in Section 6.5.

4390

4391 Table 6-33 and Table 6-34 describe FMEA analyses performed to identify critical process parameters
4392 and potential actions to mitigate their criticality for the activation and conjugation steps,
4393 respectively.

4394 **Table 6-33: Activation Step FMEA Scores**

Process parameter	NOR	Failure mode	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Ps concentration (g/L)	5-15 g/L	Ps concentration < NOR	Mixing conditions during dissolution of the bulk powder (agitation 200-250 rpm & time 8-12 hr)	Possible impact on free Ps and ratio Ps/VLP if correlation with reducing activity is confirmed		9	3	3	81	Ensure dissolution consistency	Mixing ranges are to be validated concurrent with process validation batches. Also a monitoring test before activation step to control Ps concentration may be added.
			Moisture content of the purified polysaccharide bulk powders is variable			9	1	1	9		Moisture test for Ps release and validated Ps container closure
Temperature (ªC)	30-40°C	Overheating	Heating transfer issues		Possible yield impact due to suboptimal level of reducing activity	5	3	1	15	Vessel design was considered during scale-up definition.	
			Equipment-dependant failure		Possible yield impact due to suboptimal level of reducing activity	5	3	3	45	Cover by equipment & instruments qualification.	Temperature monitored during activation. Tank Maintenance plan.
pH	10-12	pH outside NOR	NaOH preparation	May impact Ps size. Degree of de-Oacetilation is pH dependent		7	3	5	105	Range is suitable for control of the Ps size.	pH monitoring during activation
			NaOH addition			7	3	3	63	Cover by equipment & instruments qualification.	

Process parameter	NOR	Failure mode	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Time (min)	10-12	Under time limit	Human error		Deviation below this range may impact overall yield by decreasing the level of reducing activity	5	1	1	5		Kaizen criteria in SOP description to reduce risk of human error.

4395

4396

4397 **Table 6-34: Conjugation Step FMEA Scores**

Process parameter	NOR/PAR	Failure	Cause	Effect on quality attributes	Effect on process attributes	Severity	Occurrence	Detectibility	Risk score	Rationale	Action if required
Ps concentration (g/L)	5-15 g/L	Concentration range outside PAR	Mixing conditions during dissolution of the bulk powder (agitation 200-250 rpm & time 8-12 hr), error in analysis	Possible impact on free Ps and ratio Ps/VLP if correlation with reducing activity is confirmed		9	3	3	81	Ensure dissolution consistency.	Mixing ranges are to be validated concurrent with process validation batches. Also a monitoring test before activation step to control Ps concentration may be added.
Temperature (ªC)	30-40°C	Overheating	Heating transfer issues Equipment-dependant failure		Possible yield impact due to suboptimal level of reducing activity	5	3	1	15	Vessel design was considered during scale-up definition.	
					Possible yield impact due to suboptimal level of reducing activity	5	3	3	45	Cover by equipment & instruments qualification.	Temperature monitored during activation. Tk Maintenance plan.
pH	10-12	pH outside PAR	NaOH preparation	May impact Ps size		7	3	5	105	Range is suitable for control of the Ps size.	pH monitoring during activation
			NaOH addition			7	3	3	63	Cover by equipment & instruments qualification.	
Time (min)	20-Oct	Under time limit	Human error?		Deviation below this range may impact overall yield by decreasing the level of reducing activity	5	1	1	5		

4398

6.17. Ps-VLP Conjugation Design Space

6.17.1. Objective

Given that activation and conjugation steps were considered most significant in potentially impacting CQAs of A-VAX based on prior knowledge, a multivariate experimental design was employed to understand the effect of process parameters on those steps.

To allow an optimal and economic transition between the screening phase and optimization phase, experiments have the following objective and structure:

a. Screening design: Parameters and ranges are selected based on risk assessment and prior knowledge with the objective to identify main effects on the selected attributes. Two levels of fractional-factorial central composite design plus two central points are used. Each parameter was represented at the levels (minimum and maximum) indicated below. As a result, main effects are identified between the parameters and the attributes.

b. Optimization design: Augment the screening results by adding axial and central points considering only those parameters with an effect on attributes. The final design matrix is a fractional-factorial central composite design combined with central points and axial points, where one parameter is set at an extreme level while the other parameters are set at their central point level ($\alpha=\pm1$). Thus, experimental-based ranges can be defined to ensure CQA acceptability.

Multivariate techniques such as partial least square can handle large numbers of variables simultaneously, while DOE deals with a limited numbers of variables because of limited experimental runs.

The use of fewer experimental runs, particularly during the screening phase, could underestimate the impact of any particular parameter on the evaluated attributes. To reduce this risk whenever possible, prior knowledge will be used to select parameters.

Also, the results obtained through these DOE studies can be used as complementary information when the process is established, allowing a better understanding of its inherent complexity.

All experiments were performed at lab scale considering scalable requirements.

DOE definition and analysis were performed using the software package: JMP v7.0 (SAS).

6.17.2. Activation Step

Factors
Four critical process parameters were identified as design factors based on the risk assessment analysis.

Ranges (Table 6-35) were selected based on prior knowledge and realistic manufacturing operating ranges.

4440   **Table 6-35: Activation Parameters**

Parameters	Unit	Min (-1)	Max (+1)
Ps concentration	g/L	5	15
Temperature	°C	30	40
pH	pH unit	10	12
Time	Min	10	20

4441
4442   **Attributes**
4443
4444   The activation process responses or attributes (Table 6-36) were selected based on risk assessment
4445   analysis.
4446
4447   **Table 6-36: Activation Attributes**

Attributes	Category	Unit	Min	Max	Analytical Procedure
Reducing activity	KPA	mol/mol Ps	18	30	BCA (using glucose as a reference)
O-Ac	CQA	mol/mol Ps	–	1.8	H-NMR/Hestrin
Ps size	CQA	Da	10,000	15,000	HPSEC-MALS-RI
Ps yield	KPA	%	75	–	High-pH HPAEX-PAD

4448
4449   **Screening design**
4450   To identify which parameters have significant effects on the selected attributes, a two-level factorial
4451   design including two central points was employed in which each parameter was represented at the
4452   levels (minimum and maximum) indicated above.
4453
4454   Taking into account the previous knowledge gained through production of other conjugate vaccines and
4455   the risk assessment, a fractional-factorial design was chosen; it ignores interactions among parameters
4456   (resolution III) to minimize the number of runs. Only parameters with high significant levels will be
4457   selected for optimization studies.
4458
4459   Table 6-37 shows the results obtained after the first set of experiments.
4460

4461 **Table 6-37: Activation Screening Design Matrix and Results**

Run	Temperature (°C)	pH	Time (min)	Ps concentration (g/L)	O-Ac	Reducing activity (mol/mol Ps)	Ps size (Da)	Ps yield (%)
1	40	12	10	5	0.14	34.77	13598.89	59.15
2	30	10	10	15	1.27	12.23	16616.15	93.7
3	30	10	20	5	0.25	32.64	17195.17	78.12
4	30	12	10	5	0.34	32.69	12685.52	60.76
5	40	10	10	15	1.32	13.66	16182.89	90.59
6	35	11	15	10	0.8	23.89	14879.44	74.11
7	30	12	20	15	0.89	18.72	13178.31	74.5
8	35	11	15	10	0.64	22.02	15135.85	76.56
9	40	10	20	5	0.1	34.53	14548.39	69.18
10	40	12	20	15	0.94	19.26	14328.87	67.12

4462
4463 The analysis of variance was performed for all attributes. Table 6-38 shows for each studied attribute
4464 the p value and the estimate value for each of the parameters.
4465
4466 Parameters that were significant at a 95% confidence interval (p-value < 0.05) were selected for further
4467 evaluation.
4468
4469 However, the estimated value of each parameter could also be used to support the selection of
4470 parameters. For example, the temperature effect on yield is not significant, but the effect is large
4471 enough to be further evaluated.
4472
4473 For this exercise, only results on reducing activity will be discussed.
4474
4475 **Table 6-38: Summary of Results for Screening Design on Activation Step**

Attribute	Temperature (°C)		pH		Time (min)		Ps concentration (g/L)	
	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate
O-Ac	0.3954	-0.03125	0.0662	-0.07875	*0.0213*	-0.11125	*<0.0001*	0.44875
Reducing activity (mol/mol Ps)	0.1572	0.7425	*0.0179*	1.5475	*0.0214*	1.475	*<0.0001*	-8.845
Ps size (Da)	0.7249	-127.0138	*0.0110*	-1343.876	0.9535	20.91125	0.4418	284.78125
Ps yield (%)	0.1432	-10.13	0.8379	-1.2575	0.3822	5.59	0.9789	-0.1625

4476

4477   **Results**

4478   Table 6-39 shows sorted parameter estimates for reducing activity. It can be seen that Ps concentration,
4479   activation time, and pH have p values <0.05 and thus are significant for reducing activity levels.
4480
4481   The activation temperature results are neither significant at 0.05 nor have high estimated value;
4482   therefore, temperature is not expected to have a significant impact on reducing activity.
4483
4484   **Table 6-39: Sorted Parameter Estimates for Reducing Activity (Screening)**

Term	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
Ps (g/L) (5,15)	-8.845	0.446542	-19.81		<.0001
Activation pH (10.12)	1.5475	0.446542	3.47		0.0179
Activation Time (10.20)	1.475	0.446542	3.30		0.0214
Activation Temperature (°C)(30.40)	0.7425	0.446542	1.66		0.1572

4485
4486   After removing the insignificant term (activation temperature), a model fit was performed (Figure 6-23).
4487   The ANOVA table shows that the model as a whole is significant (p= 0.0001).
4488
4489   **Figure 6-23: Model Fit and ANOVA for Reducing Activity**

Actual by Predicted Plot

Predicted P<.0001 RSq=0,98
RMSE=1,4368
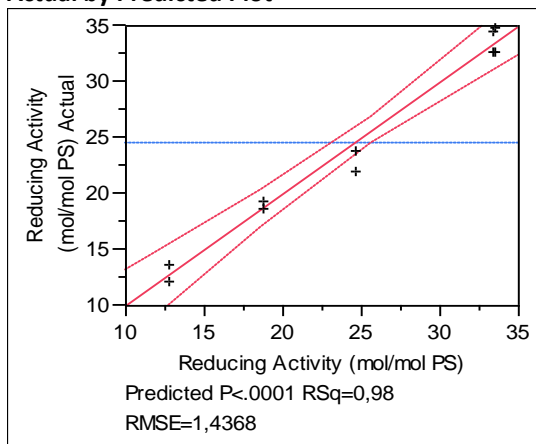
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-Ratio
Model	3	662.43525	220.812	106.9614
Error	6	12.38644	2.064	**Prob > F**
C. Total	9	674.82169		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-Ratio
Lack of Fit	1	5.520490	5.52049	4.0202
Pure Error	5	6.865950	1.37319	**Prob > F**
Total Error	6	12.386440		0.1013
				Max RSq
				0.9898

4490
4491   The following figure displays a set of predicted values for reducing activity for the extremes of the
4492   parameter ranges (vertices of a cube). It can be seen that some process conditions could lead to values
4493   outside the criteria for reducing activity (18-30 mol/mol Ps). Thus, process ranges for the selected
4494   parameters must be adjusted to meet the criteria for reducing activity.
4495
4496

4497   **Figure 6-24: Box Plot on Reducing Activity**
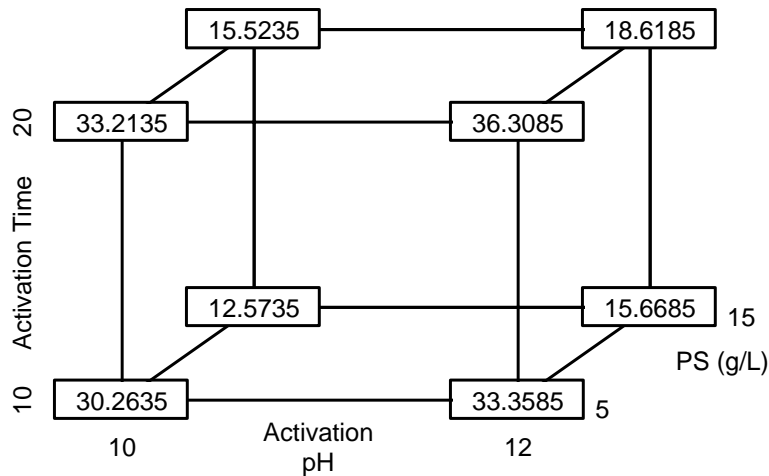
4498
4499
4500   **Conclusion on screening design**

4501   The results for the screening design show the following conclusions:

4502   •   There is no apparent relationship between temperature and the attributes in the evaluated range;
4503   therefore, it is considered to not be a critical process parameter. In addition, because of the high
4504   estimated value obtained for yield, the target value could be further optimized.

4505   •   Significant interaction among activation time, pH, and Ps concentration on the evaluated attributes
4506   was found. Thus, these parameters must be considered as critical process parameters and their
4507   ranges adjusted to guarantee process robustness.

4508   •   There is a significant impact of pH and Ps concentration on yield; however, caution must be taken to
4509   optimize yield based on these parameters as they have an impact on a CQA.

4510
4511   **Optimization design**

4512
4513   Results obtained during the screening phase show that some process conditions could lead to values out
4514   of acceptance criteria for reducing activity (18-30 mol/mol Ps). They also allow identification of the
4515   process parameters that have significant impact on reducing activity.

4516
4517   Taking into account the screening results, an augment design is proposed to test intermediate process
4518   conditions and also to evaluate second-order interactions.

4519
4520   The final design (Table 6-40) matrix is a fractional-factorial central composite design. It combines four
4521   central points and six axial points where one parameter is set at an extreme level while the other
4522   parameters are set at their center point ($\alpha=\pm1$). The values of the parameters are given in Table 6-37.
4523
4524

4525   **Table 6-40: Activation Optimization Design Matrix**

Run	pH	Time (min)	Ps concentration (g/L)
1	1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	1	-1	-1
5	-1	-1	1
6	0	0	0
7	1	1	1
8	0	0	0
9	-1	1	-1
10	1	1	1
11	0	0	0
12	0	0	0
13	1	0	0
14	-1	0	0
15	0	1	0
16	0	-1	0
17	0	0	1
18	0	0	-1

4526
4527
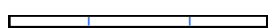
4528 **Results**

4529

4530 A preliminary evaluation of the parameters and their interactions is performed to identify the strongest
4531 effects.

4532

4533 Table 6-41 shows that only Ps concentration has a significant effect on reducing activity (p-value <
4534 0.005). However, the estimate values are comparable between parameters and parameter interactions.
4535 Specifically, the second-order interaction "Ps concentration*activation time" has a comparable value of
4536 estimate and a borderline p-value. The results suggest that Ps concentration and the second-order
4537 interaction "Ps concentration*activation time" should be further evaluated (Ps concentration must be
4538 included as it is involved in the second-order interaction).

4539

4540 **Table 6-41: Contrasts for Reducing Activity (mol/mol Ps)**
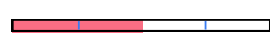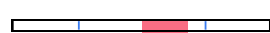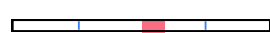
Term	Contrast	Plot of t-ratio	Length t-ratio	Individual p-value
Ps (g/L)	-5.69005		-4.37	*0.0039*
Activation Time	2.00799		1.54	0.1286
Activation pH	0.04472		0.03	0.9726
Ps (g/L)*Ps (g/L)	-2.01731		-1.55	0.1275
Ps (g/L)*Activation Time	2.21743		1.70	0.0982
Activation Time*Activation Time	0.98267		0.75	0.4271
Ps (g/L)*Activation pH	-1.81718		-1.40	0.1651
Activation Time*Activation pH	-1.80525		-1.39	0.1671
Activation pH*Activation pH	2.03553		1.56	0.1246
Ps (g/L)*Activation Time*Activation pH	0.20767		0.16	0.8814

4541

4542 The ANOVA analysis shows that the model as a whole is significant (data not show). However, only Ps
4543 concentration has p-values <0.05 and thus is significant (Table 6-42).

4544

4545 **Table 6-42: Sorted Parameter Estimates for Optimization Design**

Term	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
Ps (g/L) (5.15)	-7.634	1.716786	-4.45		0.0006
Activation Time (10.20)	2.694	1.716786	1.57		0.1389
Ps (g/L)*Activation Time	1.5475	1.919425	0.81		0.4336

4546

4547 Despite the fact that only Ps concentration was found to be significant, a new analysis was performed. It
4548 considered both Ps concentration and activation time because of the high estimated value obtained for
4549 activation time (2.694). Second-order interactions are considered negligible.

4550

4551 The ANOVA analysis for the resulting model is significant at p-values <0.05.

4552

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4554   **Figure 6-25: Model Fit and ANOVA for Reducing Activity**

Actual by Predicted Plot

Reducing Activity (mol/mol PS)
Predicted P=0,0006 RSq=0,54
RMSE=5,6145
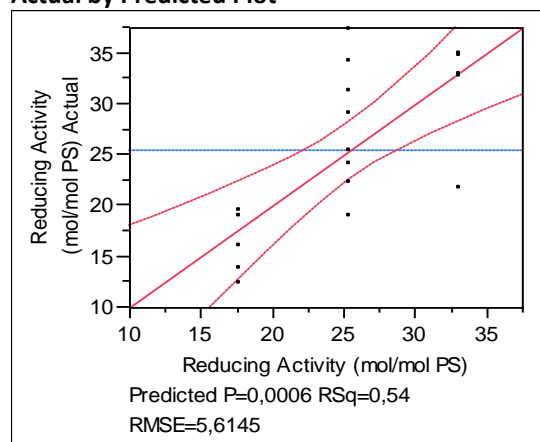
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-ratio
Model	1	582.7796	582.780	18.4876
Error	16	504.3641	31.523	**Prob > F**
C. Total	17	1087.1437		0.0006

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	1	73.25142	73.2514	2.5487
Pure Error	15	431.11267	28.7408	**Prob > F**
Total Error	16	504.36409		0.1312
				Max RSq
				0.6034

4555

4556   The sized Ps has a MW of ~10–15 kD, which corresponds to six to ten repetitive units.

4557   According to the *X. horrificus* serotype 2 capsular polysaccharide structure, after the activation step,
4558   three activated sites per repetitive unit are expected, resulting in multipoint attachment to the VLP
4559   (Figure 6-1).

4560   However, the 2 OH on Glc could also be oxidized to render five activated sites per unit. This could lead
4561   to increased Ps-VLP conjugation sites, which may have an undesirable impact on Ps/VLP ratio and Ps-VLP
4562   size. Therefore, the range for reducing activity has been defined as 18-30 mol/mol Ps.

4563   In an attempt to increase confidence about the degree of multipoint attachment of the Ps-VLP, the
4564   target value for reducing activity was defined as 24 mol/mol Ps.

4565   Using the desirability function where a value of 1 represents 24 mol/mol Ps, the target values for
4566   activation time and Ps concentration are estimated as 11.4 min. and 9.65 g/L, respectively (Figure 6-26).

4567

4568   **Figure 6-26: Desirability Function for Reducing Activity vs. Activation Time and Ps Concentration**
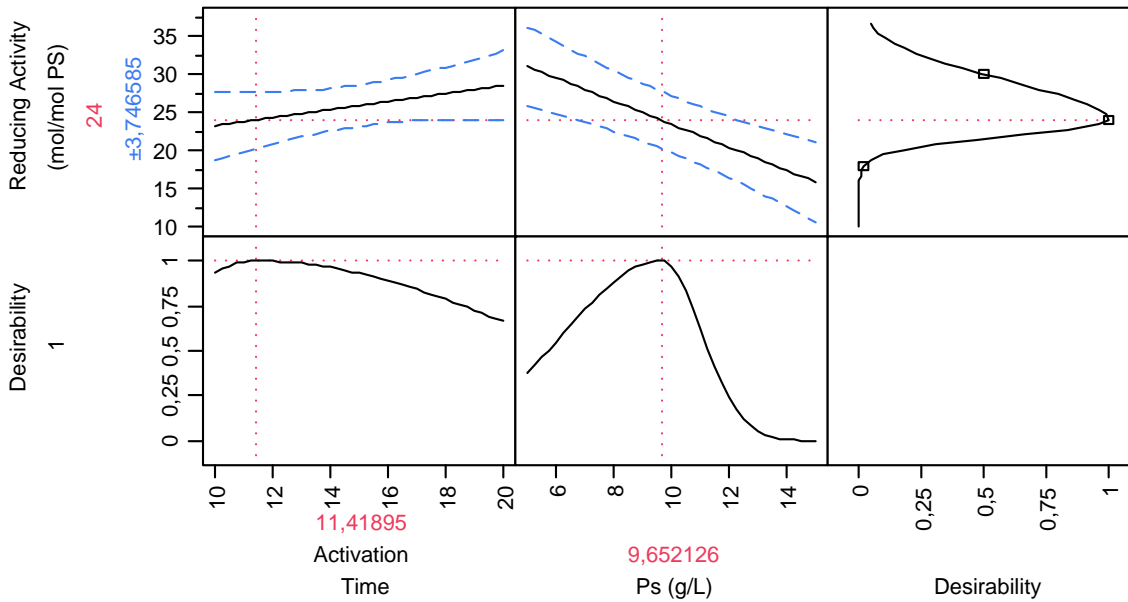
4569
4570

4571   When the reducing activity is plotted against the activation time and the Ps concentration, it can be
4572   observed that between Ps concentrations of 9 and 13 g/L, the variation of time within the established
4573   range does not lead to out-of-specification values of reducing activity (Figure 6-27).
4574
4575   Thus, the Ps concentration range can be narrowed from 5–15 g/L to 9–13 g/L with a target value of 11
4576   g/L. The range could even be tightened to 9.0–12.0 g/L to prevent a low level of reducing activity.
4577
4578   **Table 6-43: Inverse Prediction for Reducing Activity**

Reducing Activity (mol/mol Ps)	Predicted Ps (g/L)	Lower Limit	Upper Limit	1-Alpha
18	14.8456467	12.6040951	20.1995286	0.9500
24	10.9158589	9.0153857	13.4045828	
30	6.9860711	3.1346763	8.9016371	

4579
4580

4581   **Figure 6-27: Reducing Activity Values Plots vs. Ps Concentration and Time**
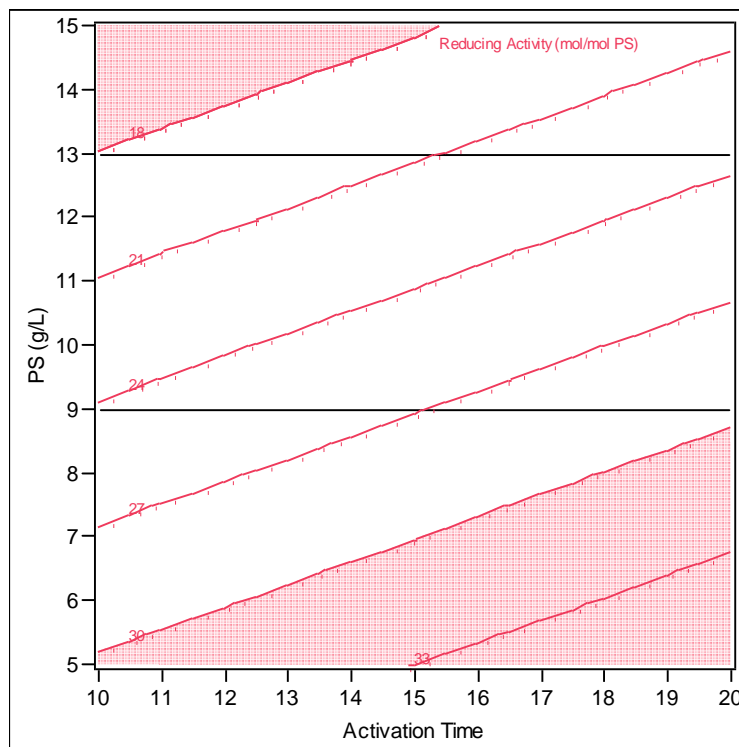
4582
4583
4584   **Conclusion on activation**

4585

4586   The pH range was also adjusted because it has a correlation to Ps size. However, because of the on-line
4587   HPSEC monitoring of the Ps size during sodium meta-periodate treatment, no further tightening of the
4588   pH ranges was considered necessary. No adjustment was found necessary for time and temperature
4589   ranges. Based on these conclusions, the design space for activation is defined as follows:

4590

4591   **Table 6-44: Process Parameter Ranges for Activation Step**

Parameters	Unit	Min	Max
Ps concentration	g/L	9.0	12.0
Temperature	°C	30	40
pH	pH unit	11	12
Time	min	10	20

4592

4593   6.17.3. Conjugation Step

4594   **Parameters**

4595

4596   Five critical process parameters were identified as design factors based on the risk assessment analysis
4597   (Table 6-45). Incubation time for the conjugation step has been identified as a process improvement
4598   opportunity and therefore is included in the design. Ranges were selected based on prior knowledge
4599   and realistic manufacturing operability.

4600

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4601   **Table 6-45: Conjugation Parameters**

Parameters	Unit	Min	Max
VLP concentration	g/L	8	12
DAPS concentration	g/L	15	25
Incubation temperature	°C	15	35
Agitation rate during VLP addition	rpm	150	250
NaCNBH4	mg/mL	10	20
Incubation time	hr	12	24

4602
4603   **Attributes**
4604
4605   The product and process attributes were selected (Table 6-46) based on the risk assessment analysis.
4606   Yield is included for a comprehensive evaluation of the design space.
4607
4608   **Table 6-46: Conjugation Attributes**

Attributes	Category	Unit	Min	Max	Analytical Procedure
Free Ps	CQA	%	–	10	High-pH HPAEX-PAD
Ps/VLP ratio	CQA	–	0.3	0.7	HPLC/BCA protein assay
Ps-VLP size	CQA	nm	20	50	DLS
Ps-VLP yield	KPA	%	50	–	HPAEC-PAD or ELISA

4609
4610

4611   **Screening design**
4612
4613   A two-level factorial design including two center points was employed. Each parameter was represented
4614   at two levels (minimum and maximum) in ten runs (Table 6-47). The result is a resolution-three
4615   screening design. All the main effects are estimable, but they are confounded with two-parameter
4616   interactions as was mentioned in the screening design for the activation step. The runs were performed
4617   in random order, and results are displayed in Table 6-48.
4618
4619   Table 6-47: Conjugation Screening Design Matrix

Run	DAPS (g/L)	VLP (g/L)	Incubation temperature (°C)	Agitation during VLP addition (rpm)	NaCNBH$_4$ (mg/mL)	Time (hr)
1	25	12	35	250	20	24
2	25	8	15	250	20	12
3	15	8	15	250	10	24
4	15	12	15	150	20	12
5	15	8	35	150	20	24
6	25	12	15	150	10	24
7	25	8	35	150	10	12
8	20	10	25	200	15	18
9	15	12	35	250	10	12
10	20	10	25	200	15	18

4620
4621

4622   **Table 6-48: Conjugation Screening Design Results**

Run	Free Ps (%)	Ps/VLP ratio	Ps-VLP size	Yield (%)
1	11.58	0.59	54.36	53
2	12.78	0.49	31.31	45
3	7.58	0.24	27.57	44
4	7.13	0.28	48.32	35
5	8.31	0.26	26.85	57
6	10.19	0.25	59.2	35
7	13.33	0.58	32.84	53
8	9.4	0.49	41.21	47
9	7.35	0.22	46.24	58
10	11.24	0.40	37.73	56

4623
4624   The analysis of variance was performed for all attributes. Table 6-49 shows for each studied attribute
4625   which parameters are significant at a 95% confidence interval. However, only results on Ps-VLP size will
4626   be discussed.
4627
4628   The estimated value for attributes could be also used to support the selection of parameters. For
4629   example, the DAPS concentration effect on Ps/VLP ratio is not significant, but the effect is large enough
4630   for further evaluation. A similar situation can be expected for the effect on yield of VLP concentration
4631   and agitation during VLP addition.
4632
4633   **Table 6-49: Summarized Results for Screening Design on Conjugation Step**

Parameter	Free Ps (%)		Ps/VLP ratio		Ps-VLP size		Yield (%)	
	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|	Estimate	Prob>|t|
DAPS (g/L)	2.18875	0.0057	0.11375	0.0730	3.59125	0.0100	-1.08375	0.5923
VLP (g/L)	-0.71875	0.1020	-0.02875	0.5423	11.19375	0.0004	-2.13875	0.3234
Conjugation incubation temperature (°C)	0.36125	0.3258	0.04875	0.3292	-0.76375	0.3025	7.64375	0.0244
Agitation during VLP addition (rpm)	0.04125	0.9020	0.02125	0.6473	-0.96625	0.2141	2.50125	0.2618
NaCNBH$_4$ (mg/mL)	0.16875	0.6221	0.04125	0.3979	-0.62625	0.3834	0.13375	0.9459
Incubation time (hr)	-0.36625	0.3202	-0.02875	0.5423	1.15875	0.1560	-0.21625	0.9126

4634
4635   **Results**
4636
4637   Table 6-50 shows sorted parameter estimates for Ps-VLP size. Both VLP and DAPS have p-values <0.05
4638   and thus are significant on Ps-VLP size. Also they account for the higher estimated values.
4639
4640

4641   **Table 6-50: Sorted Parameter Estimates for Ps-VLP Size**

Parameter	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
VLP (g/L) (8,12)	11.19375	0.614896	18.20		0.0004
DAPS (g/L) (15.25)	3.59125	0.614896	5.84		0.0100
Incubation time (Hs) (12.24)	1.15875	0.614896	1.88		0.1560
Agitation during VLP addition (rpm) (150.250)	-0.96625	0.614896	-1.57		0.2141
Conjugation incubation temperature (°C) (15.35)	-0.76375	0.614896	-1.24		0.3025
NaCNBH$_4$ (mg/mL) (10.20)	-0.62625	0.614896	-1.02		0.3834

4642

4643   After removal of the insignificant terms (incubation time, agitation during VLP addition, conjugation
4644   incubation temperature, and NaCNBH$_4$ concentration), a model fit was performed (Figure 6-28). The
4645   ANOVA table shows that the model as a whole is significant (p= 0.0001).
4646
4647

4648   **Figure 6-28: Model Fit and ANOVA for Ps-VLP Size**

Actual by Predicted Plot

P<.0001 RSq=0,97 RMSE=2,2389
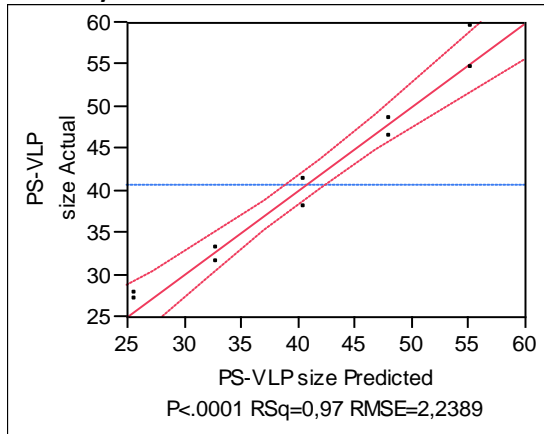
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-ratio
Model	2	1105.5769	552.788	110.2770
Error	7	35.0891	5.013	**Prob > F**
C. Total	9	1140.6660		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	2	13.728235	6.86412	1.6067
Pure Error	5	21.360850	4.27217	**Prob > F**
Total Error	7	35.089085		0.2891
				Max RSq
				0.9813

4649

4650   Figure 6-29 displays a set of predicted values for Ps-VLP size for the extremes of the parameter ranges
4651   (vertices of the cube). Based on these preliminary results, some process conditions could result in values
4652   outside of the acceptance criteria for Ps-VLP size (20–50 nm). Thus, process ranges for the selected
4653   parameters (VLP and DAPS concentration) must be adjusted to meet the criteria for Ps-VLP size.

4654

4655   **Figure 6-29: Box Plot on Ps-VLP Size**
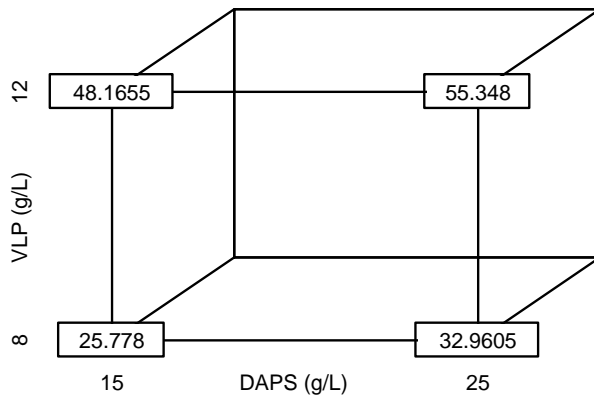
4656
4657

4658   **Conclusion on screening design**
4659
4660   The results for the screening design show the following conclusions:
4661   •   No correlation for $NaCNBH_4$ concentration was found. This is an expected result considering that it is
4662   added in excess.
4663   •   There is no apparent relationship between incubation time and the evaluated attributes.
4664   •   Agitation rate at this scale has no significant effect on the evaluated attributes.
4665   •   A positive correlation of incubation temperature on yield allows for optimizing the process
4666   conditions. Also, VLP concentration and agitation during VLP addition should be taken into account
4667   as they reach high estimated values.
4668   •   Process ranges for VLP and DAPS concentrations require further evaluation because of their
4669   correlation with Ps-VLP size.
4670   •   DAPS concentration has a significant effect on free Ps. Other parameters were found not to be
4671   significant and had low estimated values.
4672
4673   **Optimization design**
4674
4675   **Results**
4676
4677   Considering the screening results, some combination of values for DAPS and VLP concentration could
4678   lead to unacceptable values for Ps-VLP size (20–50 nm). Thus, a reevaluation of the preliminary ranges
4679   was required. An augmented design is proposed based on the screening results. The final design matrix
4680   (Table 6-51) is a full-factorial central composite design of two parameters, including four center points
4681   and four axial points on the face for each design factor ($\alpha=\pm1$). Free Ps is also included in the evaluation
4682   since a correlation with DAPS concentration was found.
4683

4684   **Table 6-51: Optimization Matrix and Results for Conjugation Step**

Run	DAPS (g/L)	VLP (g/L)	Free Ps (%)	Ps-VLP size
1	25	12	11.58	54.36
2	25	8	12.78	31.31
3	15	8	7.58	27.57
4	15	12	7.13	48.32
5	15	8	8.31	26.85
6	25	12	10.19	59.2
7	25	8	13.33	32.84
8	20	10	9.4	41.21
9	15	12	7.35	46.24
10	20	10	11.24	37.73
11	20	10	8.42	39.04
12	20	10	10.22	41.5
13	25	10	13.02	40.7
14	15	10	7.88	39.99
15	20	12	10.65	49.12
16	20	8	9.37	30.66

4685
4686   Table 6-52 shows the sorted parameter estimates for Ps-VLP size. The results confirm the correlation
4687   observed for DAPS and VLP concentration on Ps-VLP size (p-values <0.05), but second-order interactions
4688   were not found to be significant. Also, the estimated values of VLP and DAPS concentration are large,
4689   thus supporting the selection.
4690
4691   **Table 6-52: Sorted Parameter Estimates**

Parameter	Estimate	Std Error	t-ratio	t-ratio	Prob>|t|
VLP (g/L) (8.12)	10.801	0.693654	15.57		<.0001
DAPS (g/L) (15.25)	2.944	0.693654	4.24		0.0017
VLP (g/L)*DAPS (g/L)	1.15875	0.775529	1.49		0.1660
DAPS (g/L)*DAPS (g/L)	0.7320455	1.280743	0.57		0.5802
VLP (g/L)*VLP (g/L)	0.2770455	1.280743	0.22		0.8331

4692
4693   After removal of the insignificant parameters (second-order interactions), a model fit was performed
4694   (Figure 6-30). The ANOVA table shows that the model as a whole is significant (p= 0.0001).
4695

4696   **Figure 6-30: Model Fit and ANOVA for Ps-VLP Size**

Actual by Predicted Plot
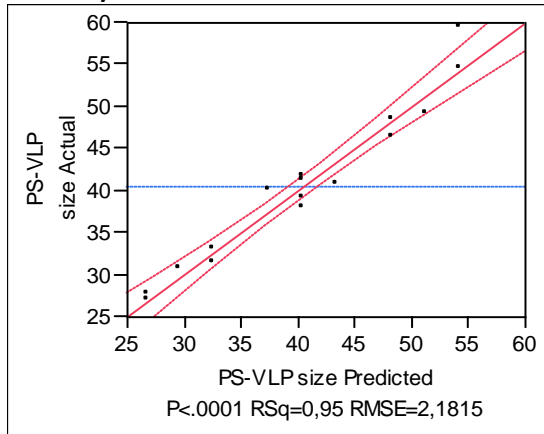
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F-ratio
Model	2	1253.2874	626.644	131.6810
Error	13	61.8644	4.759	**Prob > F**
C. Total	15	1315.1518		<.0001

Lack of Fit

Source	DF	Sum of Squares	Mean Square	F-ratio
Lack of Fit	6	36.837780	6.13963	1.7173
Pure Error	7	25.026650	3.57524	**Prob > F**
Total Error	13	61.864430		0.2474

Max RSq
0.9810

4697

4698   The same analysis was performed on free Ps where DAPS concentration was found to be the only
4699   parameter to have a significant interaction. Figure 6-31 represents the free Ps (blue lines) and
4700   Ps-VLPs size (red lines) results as a function of VLP and DAPS concentrations. To reduce the level of free
4701   Ps (<10%) and maintain the Ps-VLP size within the acceptance criteria (20–50 nm), the process
4702   conditions should be adjusted.

4703

4704   **Figure 6-31: Counter Plots as a Function of VLP and DAPS Concentrations. Shadow Areas Indicate**
4705   **Condition With Results Out of Specifications.**
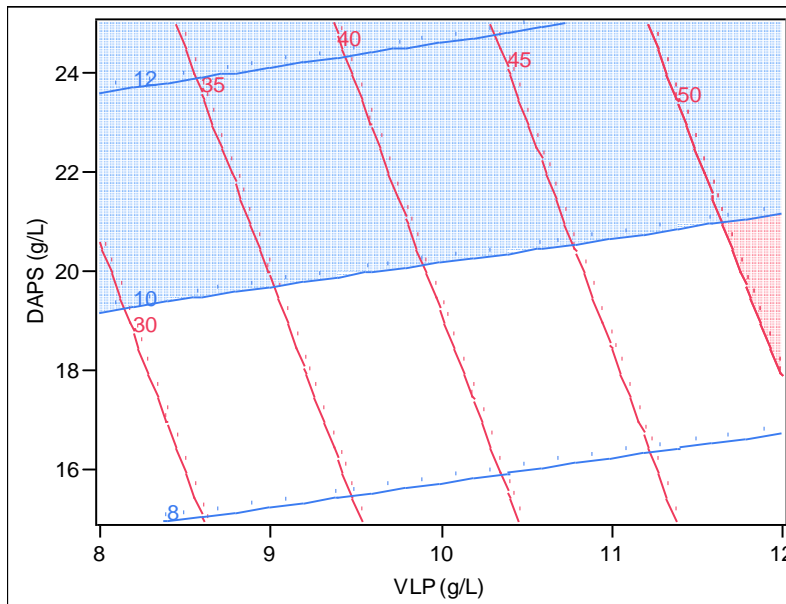
4706
4707

4708   Despite the fact that DAPS concentration has a major impact on free Ps, the following points should be
4709   taken into account to define the range for the process at manufacturing scale:

4710   • Lowering the value of DAPS concentration reduces the level of free Ps; however, process constraints
4711   such as large working volumes should be considered.

4712   • 90% free Ps removal is expected to be obtained through diafiltration in a tangential flow filtration
4713   mode.

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4714
4715   Based on the inverse prediction values (Table 6-53 and Table 6-54), ranges were defined for VLP and
4716   DAPS concentrations.
4717
4718   **Table 6-53: Inverse Prediction Response on Ps-VLP size**

Ps-VLP size	Predicted DAPS (g/L)	Lower Limit	Upper Limit	1-Alpha
35.000000	10.8033288	0.958786539	14.3103961	0.9500
Ps-VLP size	Predicted VLP (g/L)	Lower Limit	Upper Limit	1-Alpha
35.000000	8.99731506	8.71630246	9.23940811	0.9500

4719
4720   **Table 6-54: Inverse Prediction Response Free Ps (%)**

Free Ps (%)	Predicted DAPS (g/L)	Lower Limit	Upper Limit	1-Alpha
7.700000	15.1365894	12.9104875	16.5716027	0.9500
10.000000	20.2138521	19.1021685	21.3603211	

4721
4722   Considering the preliminary work (see prior knowledge Section 6.14.1), no impact on conjugate potency
4723   is expected while moving within the preliminarily selected ranges. However, to confirm this and provide
4724   a complementary confirmation of the selected ranges for VLP and DAPS, the following extreme
4725   conditions were evaluated.
4726
4727   **Table 6-55: Complementary Evaluation on DAPS and VLP Ranges**

DAPS (g/l)	VLP (g/l)	Free Ps (%)	Ps-VLP size	Potency*
12.9	8.7	6.98	29.21	4.5
14.3	9.2	7.50	32.74	4.9
12.9	9.2	6.87	31.91	5.1
14.3	8.7	7.61	30.04	4.1

4728   * Mean ELISA titers were calculated using arbitrary unit of ELISA (EU/mL).
4729
4730   The results of this study confirmed that the selected ranges have no impact on quality attributes of the
4731   conjugate.
4732
4733

4734   ***Conclusion on conjugation***

4735   Based on the aforementioned, the conditions for the conjugation process design space are defined in
4736   Table 6-56.

4737

4738   **Table 6-56: Process Parameter Suggested Ranges for Conjugation Step**

Factors	Unit	Min	Max
VLP concentration	g/L	8.7	9.2
DAPS concentration	g/L	12.9	14.3
Incubation temperature	°C	30	35
Agitation rate during VLP addition	rpm	150	250
NaCNBH4	mg/mL	10	20
Incubation time	hr	12	24

4739

4740   Also, since no correlation was observed between incubation time and the evaluated attributes, it is
4741   advisable to further evaluate this factor to optimize process cycle time. Though scalable requirements
4742   were employed during the designs, the applicability of the design space should be assessed.

4743

4744   ## 6.18. Ps-VLP Conjugation Scale-Up

4745   ### 6.18.1. Sensitivity of Activation and Conjugation to Mixing

4746   Addition of sodium meta-periodate to the reaction vessel may lead to nonrobust activation outputs
4747   during manufacturing by inducing conformational changes within the polysaccharide ring or creating a
4748   heterogeneous distribution of aldehydes within the Ps backbone. Quality by Design tools can be used to
4749   prevent inconsistent levels of activation or heterogeneous distributions of aldehydes during the
4750   oxidation reaction — manufacturing variability that could impact the conjugation reaction and
4751   ultimately the final drug substance's potency.

4752

4753   Heterogeneous activation may directly impact the conjugation chemistry and the resulting conjugate
4754   attributes including molecular weight, free polysaccharide, unconjugated VLP, and the Ps-to-VLP ratio.
4755   Furthermore, heterogeneous activation may lead to multiple covalent attachments between the Ps and
4756   VLP or may lead to VLPs cross-linked by Ps.

4757

4758   The level of activation achieved and the size reduction of the Ps during the activation reaction may be
4759   influenced by the temperature, pH, and amount of sodium meta-periodate added to the dissolved
4760   polysaccharide solution. The data in Figure 6-32 shows the impact of post-activation average molecular
4761   weight (expressed in kilodaltons) and activation level on a key conjugate attribute: the conjugate
4762   molecular weight. Activation level is represented as a ratio of the mols of aldehyde formed during the
4763   oxidation reaction to the polysaccharide molar mass per repeating unit. The data in Figure 6-32 was
4764   generated from three experiments with all variables held constant except for the quality of mixing
4765   during sodium meta-periodate addition. Although the same amount of sodium meta-periodate was
4766   added to the reaction vessel for each experiment, the resulting reducing activity (activation level) varied
4767   from 8 to 34 mol of aldehyde per mol of polysaccharide as the mixing quality decreased. In addition, the
4768   resulting molecular weight of the activated polysaccharide correspondingly measured 5 to 22
4769   kilodaltons after a fixed reaction time of 15 minutes.

4770

4771   The activated process intermediates from the three activation experiments were then analyzed by H-
4772   NMR to show different distributions of aldehydes along the Ps chain. Such variability in activation level
4773   directly impacted the conjugate attributes. The Ps-VLP conjugates that were generated from the three
4774   activation experiments ranged from 20 to 50 nanometers as shown in Figure 6-32. The conjugate
4775   molecular size has been classified as a CQA, important for potency of the targeted product, and must be
4776   controlled within 20-50 nanometers.
4777
4778   **Figure 6-32: Effect of Polysaccharide Reducing Activity and Molecular Weight Inputs on Ps-VLP**
4779   **Conjugate Molecular Size**
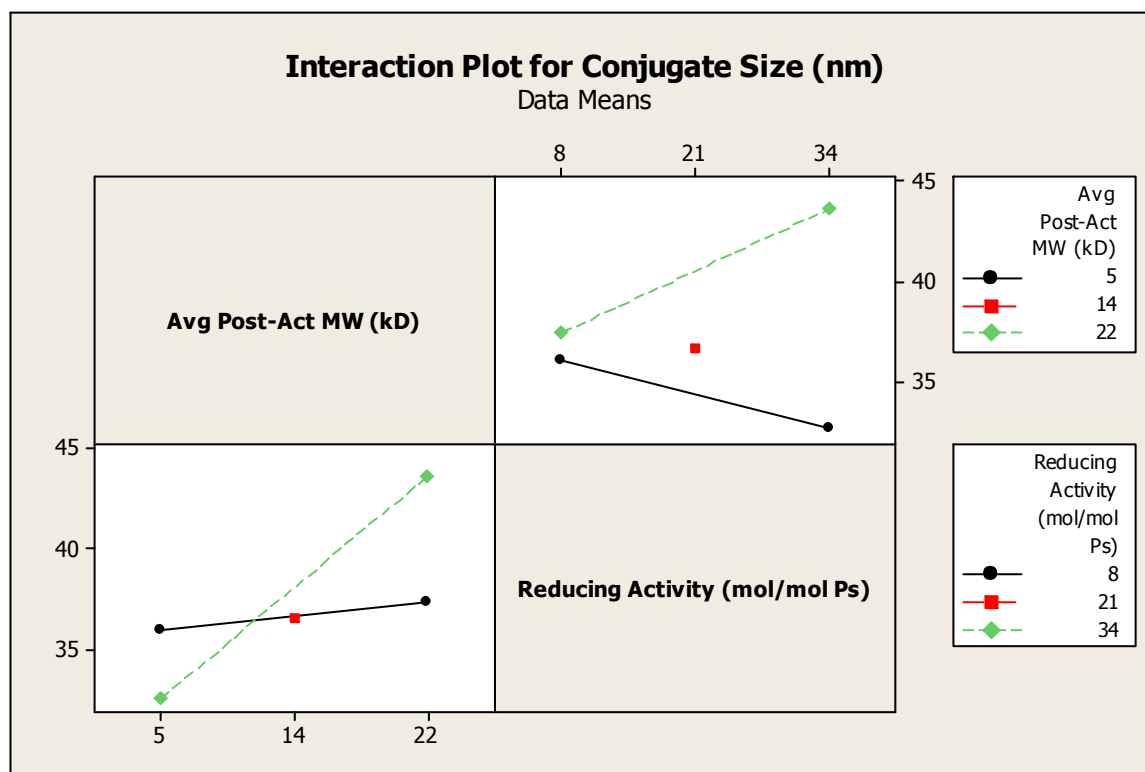
4780
4781
4782   The data in Figure 6-33 shows how the variability in activation level and Ps molecular weight can directly
4783   impact the fraction of conjugated or reacted VLP in the conjugate. The fraction of reacted VLP varied
4784   from 0.45 to 0.85 and was affected by two factors: (1) the different distributions of aldehydes along the
4785   Ps chain and (2) the Ps molecular weight of the activated polysaccharide intermediate. Since the Ps
4786   molecular weight of the activated intermediate is controlled by on-line HPSEC monitoring, the
4787   distribution of aldehydes must be controlled by optimizing the mixing in the activation vessel.
4788
4789

4790   **Figure 6-33: Effect of Polysaccharide Reducing Activity and Molecular Weight Inputs on Percentage of**
4791   **Conjugated VLP in Ps-VLP Conjugate**
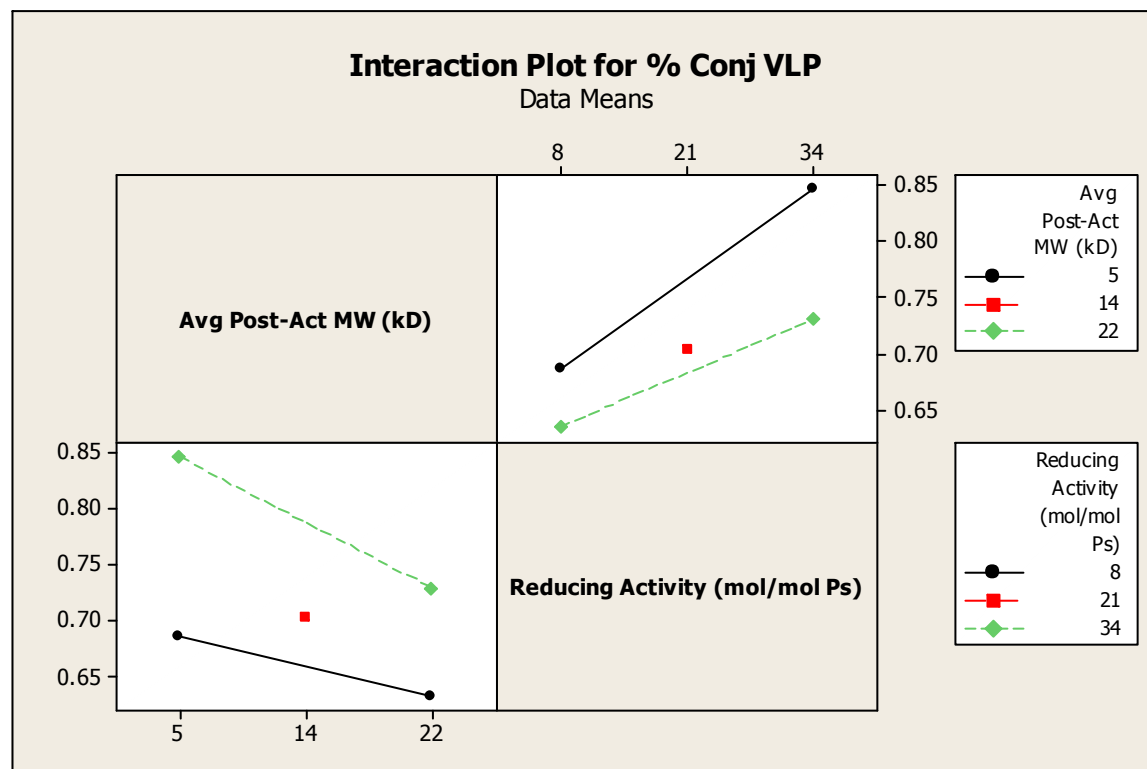
4792
4793

4794   6.18.2. Scale-Dependent Issues

4795   For the chemistry steps of activation and conjugation, process parameters may be classified as either
4796   scale independent or scale dependent. Temperature and reagent concentrations are readily scalable
4797   based on full-scale equipment capabilities and defined as scale-independent parameters for the A-VAX
4798   case study. Lab-scale experiments are still required to determine failure points and define acceptable
4799   ranges for manufacturing. Engineering studies utilizing the manufacturing-scale equipment to determine
4800   parameter controllability are also required. For example, a Kaye validator would be used to ensure that
4801   the temperature distribution in the manufacturing-scale vessel can be maintained within the process
4802   specification.

4803

4804   Although temperature and reagent concentrations are readily scalable, the chemical activation step of
4805   the Ps with sodium meta-periodate has been identified as a mixing-sensitive, scale-dependent reaction.
4806   Activation vessel geometry and impeller design are critical for scale-up. The kinetics of the oxidation
4807   reaction for each of the five serotypes have been quantified on the order of minutes, approximately one
4808   minute for the fastest-reacting serotype ("A") and 20 minutes for the slowest-reacting serotype ("E"),
4809   which has trans-vicinal diols in the Ps structure. Scale-up of mixing is most critical for serotype "A," in
4810   which approximately 2% of the total aldehydes are formed per second during the oxidation reaction.

4811

4812   The activation reaction kinetics suggest that the quality of mixing of the Ps solution during sodium meta-
4813   periodate addition will impact conjugate attributes and ultimately the quality of the drug substance
4814   upon scale-up. By scaling the manufacturing-scale vessel to conserve the mixing successfully
4815   demonstrated at lab scale, these quality implications can be reduced or eliminated.

4816

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4817   The sensitivity of lab-scale mixing on activation is illustrated in Figure 6-34 using an example of power
4818   per volume. By increasing the impeller RPM setpoint during sodium meta-periodate addition, the power
4819   per unit volume also increases. The homogeneity of the aldehyde distribution within the polysaccharide
4820   chain and the average activation level within the polysaccharide chain are directly impacted by the RPM
4821   setting, which influences the axial and radial flow vectors within the vessel. The time constants for the
4822   activation reaction at a molecular level can be calculated to predict scale-up performance.
4823
4824   **Figure 6-34: Effect of Bulk Mixing in Reaction Vessel During Sodium Meta-periodate Addition**
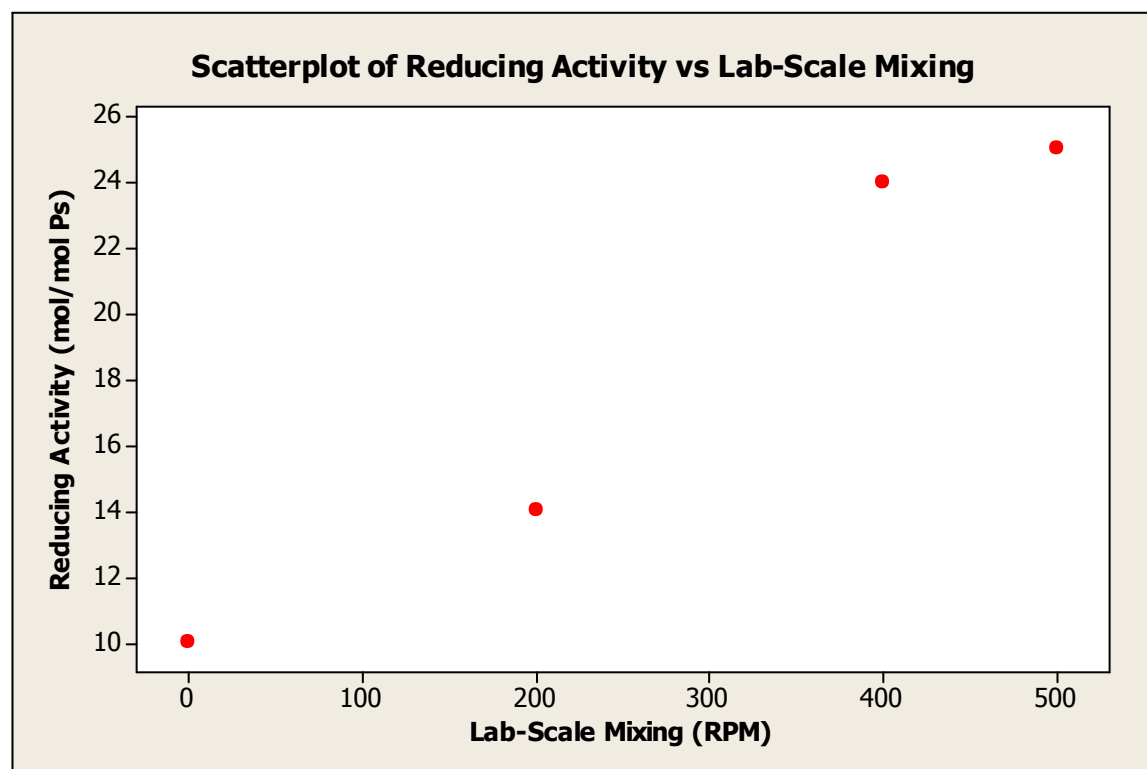
4825
4826
4827   Three mixing regimes must be considered for scale-up of the activation reaction: macro-, meso-, and
4828   micro-mixing. Macro-mixing, or bulk blend time, occurs at the scale of the reactor and is a critical
4829   parameter for suspension of particles larger than 1,000 microns (note that the target activated
4830   polysaccharide chain length is 10 to 15 kDa or less than 10 nanometers). Meso-mixing is a critical
4831   parameter for reagent addition into a stirred tank through a diptube. Turbulent and inertial driving
4832   forces influence how the reagent bolus from a diptube is incorporated into the bulk liquid. Micro-mixing
4833   is a function of kinematic viscosity and energy dissipation at the molecular scale and is maximal near the
4834   impeller.
4835
4836   Micro-mixing and meso-mixing are competing mechanisms. When the time constant for meso-mixing is
4837   smaller than the time constant for micro-mixing, micro-mixing is the limiting mechanism of diffusion in a
4838   reaction, and mixing at the molecular scale (power per volume) is important. When the time constant
4839   for meso-mixing is larger than the time constant for micro-mixing, meso-mixing impacts the reaction
4840   occurring near the impeller, and the final reaction product is sensitive to how reagents are added to the
4841   bulk solution. Process modeling tools, such as Dynochem software, may be used to calculate the local
4842   mixing timescales at the impeller to determine the dominant mixing regimes for the reaction system.
4843   For the reaction system in the A-VAX case study, both meso-mixing and micro-mixing effects were
4844   determined to be most important for scale-up.
4845

6.18.3. Process Model

Scale-up of mixing for the activation reaction from the 0.1 L lab-scale development model to the 100 L manufacturing scale depends on the vessel and impeller geometry. Two ratios must be maintained for a geometric scale-up of the system:

(1) $d_{impeller}/d_{tank}$, where d = diameter

(2) $h_{liquid\_level}/d_{tank}$, where h=height

Recommendations for common ratios of impeller-to-tank diameters and the location of the impeller in the vessel can be found in literature. The guidelines cited in the Handbook of Industrial Mixing by Ed Paul et al. for a liquid-liquid mixing system (which applies to the sodium meta-periodate addition to a Ps solution in this case study) are included in Table 6-57. Note that other impeller equipment designs (e.g., bottom-mounted) may be evaluated.

Table 6-57: Impeller Clearance and Spacing Guidelines

Mixing System	Maximum Liquid Height $h_{liquid\_level}/d_{tank}$	Number of Impellers	Impeller Elevation from Tank Bottom	
			Bottom Impeller	Top Impeller
Liquid-Liquid	1.4	1	$h_{liquid\_level}/3$	
	2.1	2	$d_{tank}/3$	$2\,h_{liquid\_level}/3$

Polysaccharide concentration, activation reaction temperature, and pH are scale-independent parameters and can be controlled within the same ranges at lab scale and manufacturing scale. Therefore, it is assumed for the A-VAX case study that the fluid parameters (density, viscosity, and kinematic viscosity) will remain constant at both scales (data to confirm this assumption could be obtained).

For the A-VAX case study, scaling by power-per-unit volume in the stirred reaction vessel will reduce undesirable effects on activation level and conjugate attributes caused by mixing. Scaling by power-per-unit volume assumes that the feed location is the most turbulent location in the vessel (e.g., not shielded by baffles) and that geometry similarity is maintained. The more precise criterion is to scale by holding constant the local rate of turbulent energy dissipation per unit mass in the region of most intense mixing. For geometrically similar vessels, the local rate of turbulent energy dissipation is proportional to the overall power-per-unit volume. Therefore, for this case study, scaling by power-per-unit volume is specified.

A fundamental understanding of mixing within the Ps reaction vessel is critical for ensuring activation homogeneity, robustness, and consistent process performance upon scale-up. For this process, a feed pipe or diptube is utilized for subsurface addition of sodium meta-periodate to the Ps solution at the region of highest turbulence in the vessel, just above the radial edge of the impeller blade. The parameters for feed addition are critical to maintain the meso-mixing and micro-mixing upon scale-up. The linear velocity of the sodium periodate must be fast enough to prevent backmixing but slow enough to prevent the reagent from jetting past the turbulent impeller zone to the bottom of the vessel.

A test chemistry, such as the ioidide-iodate system proposed by Guichardon et al. (2000), may be used to establish a scale-down mixing model to define manufacturing-scale processing parameters for the fast chemical reactions between the Ps and the sodium meta-periodate. Reagent linear velocity,

4886   impeller type and dimensions, baffling, and power-per-unit volume were optimized in a DOE in the
4887   manufacturing-scale vessel using the test chemistry reagents instead of valuable product.
4888
4889   The scale-down model must be qualified to ensure application of process development results to
4890   manufacturing scale. Parallel activations should be performed in the scale-down system and
4891   manufacturing-scale vessels. The activation kinetics should be characterized at both reaction scales to
4892   demonstrate that the same degree of activation is achieved in the 10–20-minute activation time at both
4893   scales.
4894
4895   For the A-VAX case study, serotype A exhibits the fastest reaction kinetics and will be most sensitive to
4896   mixing during sodium periodate addition. Furthermore, the decrease in O-acetate concentration should
4897   be measured by H-NMR or the Hestrin colorimetric assay before and after the activation reaction at
4898   both reaction scales to confirm the same percentage of decrease. If geometric similarity is maintained
4899   and power per volume is conserved upon scale-up, a comparison of scale at centerpoint conditions
4900   alone is sufficient to qualify the activation scale-down model. Assuming similarity in process
4901   performance as measured by CQA and characterization testing (data not shown here for conciseness),
4902   additional full-scale studies at extremes of the design space are unnecessary.
4903
4904   After activation, scale-up of conjugation can be confirmed by mixing VLP with the depolymerized
4905   polysaccharide (DAPS) at centerpoint conditions. The reaction time, pH, and concentrations of DAPS,
4906   VLP, and sodium cyanoborohydride in the conjugation reaction mixture are scale-independent
4907   parameters that can be controlled within the same range at lab scale and manufacturing scale. Unlike
4908   the activation reaction, the conjugation reaction is less sensitive to mixing because the conjugation
4909   reaction kinetics are characterized to be much slower, on the order of hours instead of several minutes.
4910   Therefore, meso- and micro-mixing do not control the extent of reaction for conjugation. Instead,
4911   macro-mixing is most important for uniform heat transfer throughout the bulk reaction mixture during
4912   the 18- to 24-hour conjugation incubation period.
4913
4914   Since temperature influences the rate of reaction and ultimately the final molecular weight and
4915   conjugate attributes, an engineering study should be performed in the manufacturing-scale vessel to
4916   ensure that the mixing is defined to provide a uniform temperature distribution in the vessel. If the
4917   scale-independent parameters are controlled within acceptable ranges and uniform temperature
4918   distribution is maintained in the conjugation reaction vessel, then the resulting Ps-VLP attributes will be
4919   measured within the design space regardless of scale. Conjugate molecular weight, free Ps,
4920   unconjugated VLP, Ps to VLP ratio, potency, and impurity assays can be used to gauge equivalency of
4921   scale.
4922
4923   Note: Refer to the "Drug Product" Section 7 for additional discussion on mixing scale-up design.
4924
4925

4926   ## 6.19. Ps-VLP Conjugation Post-Licensure Change

4927   ### 6.19.1. Rationale for Change

4928   The conjugation step has a target incubation time of 23+/-0.5 hours, with a proven acceptable range of
4929   18–24 hours. To increase capacity in the manufacturing facility, the incubation time will be reduced to
4930   18.5+/-0.5 hours. The reduction of incubation time will allow an additional capacity of 20% for this
4931   critical vaccine.
4932
4933   ### 6.19.2. Approach

4934   The incubation time is required to ensure the attachment of polysaccharides to the VLP in the presence
4935   of $NaCNBH_4$. The conjugation incubation step has a wide design space, and process characterization data
4936   shows it to be quite robust (Section 0). The DOE studies indicate the incubation time has no impact on
4937   the CQAs (e.g., Ps/VLP ratio, Ps-VLP size, free Ps, and step yield). Therefore, a change in setpoint would
4938   not require an update to the file as it might in a traditional development and filing approach. Step yield
4939   data at 18.5 hours of incubation time will be generated for five lots at manufacturing scale to ensure
4940   there is no reduction in the step yield. In addition, any other CQAs that might be impacted by this
4941   change would be tested for these five manufacturing-scale lots.
4942
4943   As this change is within the filed design space, the proposed change in the incubation time for this step
4944   will be administered by the Change Control process.
4945

7.   Drug Product Section

4946

7.1.   Target Product Profile

4947

4948   A-VAX drug product) is a lyophilized presentation of a pentavalent vaccine containing the capsular
4949   polysaccharide (Ps) of X. horrificus serotypes 1, 2, 3, 4, and 5 individually linked to a recombinant,
4950   noninfectious virus-like particle (VLP). The vaccine is reconstituted with aluminum phosphate adjuvant
4951   prior to immunization. The target profile of the vaccine is shown in Table 7-1.
4952
4953   **Table 7-1: Quality Target Product Profile (QTPP) for A-VAX Drug Product**

Product attribute	Target
Dosage form	Sterile product lyophilized, single use. To be reconstituted with aluminum phosphate diluents.
Dose	50 µg each of polysaccharides from serotypes 1–4 and 5 µg polysaccharide 5, each individually conjugated to VLP and adsorbed to 300 µg aluminum as aluminum phosphate adjuvant following reconstitution.
Label volume	0.5 mL filled (actual fill volume will be greater than the label volume to account for losses)
Concentration	100 µg/mL of active polysaccharide for serotypes 1–4 and 10 µg/mL for serotype 5
Mode of administration	IM
Dose administration	3 doses administered 2 months apart (preferably two, four, and fix months or based on pediatric vaccine schedule)
Dose volume	0.5 mL nominal dose
Viscosity	1–3 cP
Container	Single-dose vial (ISO2R vial, clear, Type I glass), latex-free stopper, and flip-off seal
Shelf life	≥ 3 years at 2—8°C VVM14 required for developing world and emerging-market supply (14 days at 37ºC, and 90 days at 25ºC)
Secondary packaging and shipping	Allowed shipping-excursion temperature 2–40°C for three days in a carton (10 vials/carton)

4954

7.2.   Drug Product Critical Product Attributes

Refer to the *Target Product Profile, Critical Quality Attributes, and Product Risk Assessment* section for drug product CQAs.

7.3.   Initial Formulation and Process Development

Prior to initiation of development studies on the A-VAX vaccine, some assumptions have been made to ensure appropriate formulation and process efforts are resourced effectively.

Drug substance assumptions

- Based on early development work in the downstream drug substance area, the stability of each of the Ps-VLPs has been established. The stability has been based on biophysical analysis and the optimum pH and buffer for the five serotypes established based both on long-term and accelerated stability studies.

- Additional efforts by the downstream drug substance team have led to understanding the freeze-thaw ability, as well as light sensitivity (photostability) of the drug substance, to ensure appropriate process handling parameters were followed during formulation and filling processes.

- Each of the Ps-VLPs can be stored frozen and then thawed without aggregation events. The frozen concentrated drug substance is stored in a similar formulation composition of buffer and excipients as the final drug product and is stored at a final concentration of 1–2 mg/mL.

- All five drug substances have demonstrated acceptable stability during accelerated stress conditions, allowing for formulation and filling activities to be completed at room temperature for up to one week.

Drug product assumptions

- The use of a platform formulation for initial formulation development was implemented. This platform formulation is based on past experience developing conjugated vaccines associated with aluminum-based adjuvants.

- Through initial formulation screening activities at both accelerated and long-term conditions, it was observed that a product that was liquid would not be able to meet the World Health Organization Vaccine Vial Monitor 14 (VVM14) requirements.

- To align with prior knowledge and other marketed vaccines, the drug product will be lyophilized. The final formulation will be designed to enable lyophilization; acceptable glass transition and collapse temperatures will be achieved during in-process conditions and at targeted storage conditions.

- To monitor long-term and accelerated stability, the key stability indicating assay will be based on either nephelometry or an ELISA format. For serotypes 1, 2, 3, and 4, the ELISA-based assay is stability indicating. For serotype 5, the rate nephelometry assay is used, but there is no correlation between in vivo and in vitro. However, for design of experiment (DOE) work, the rate nephelometry assay is utilized.

- To enhance the immunogenic effect, multiple adjuvants were examined in preclinical models and in early stability studies. Through these investigations, an aluminum adjuvant was required for enhanced immunogenicity. Based on the stability profile for the different serotypes, an aluminum phosphate adjuvant was selected with a pI of 5.0 to 5.5.

- Histidine buffer was chosen based on three factors:

| 4999 | | – | pH for maximizing binding to the antigens |

4999   – pH for maximizing binding to the antigens

5000   – Optimal for lyophilization because this buffer minimizes the chances for pH shifts during freezing
5001   and lyophilization

5002   – Stability of the drug substances under frozen conditions and freeze-thaw prior to formulation

5003   • All serotypes do not bind to the aluminum phosphate adjuvant in a similar manner. The formulation
5004   will be designed to optimize binding of all five serotypes. Additionally, it is expected that during
5005   formulation screening and optimization, lyophilization of the Ps-VLPs will not impact their ability to
5006   bind to adjuvant in post-lyophilization and storage.

5007   • The adjuvant will not be part of the drug product matrices that are lyophilized but will be
5008   incorporated into the diluent, and similar binding as observed in liquid will occur.

5009   • Overall adsorption to aluminum occurs within seconds of reconstitution of the drug product with
5010   the diluent and allows for administration soon after reconstitution.

5011

5012   **Process flow for A-VAX vaccine**

5013

5014   Based on the assumptions outlined above, the overall high-level flow diagram for how to manufacture
5015   the A-VAX vaccine is outlined in Table 7-2.

5016

5017   **Table 7-2: Process Flow for Production of the Drug Product**

Step	Process
1	Addition of WFI, buffer, sucrose, and polysorbate to obtain final desired concentration Volume to be between 50% and 60% of final drug product formulation Adjustment of formulation pH to desired condition
2	Mixing of buffer components to ensure homogeneity
3	Thaw of individual antigen components in specified water bath Dilution calculation of antigens to ensure proper amount added to formulation tank Addition of antigens to conjugate blend tank Volume between 50% and 40% of final batch
4	Addition of conjugate blend to final formulation tank Mixing of product to ensure homogeneity Filtration of final formulated bulk through 0.22 um PVDF membranes; two filters in sequence Filtered FFB filled into respective vials and half-stoppered for lyophilization
5	Lyophilization of A-VAX vaccine Sealing and inspection
6	Packaging of A-VAX vaccine Lyophilized A-VAX vaccine combined with aluminum-containing diluent

5018

5019   **Table 7-3: Process Flow for Production of the Aluminum Diluent**

Steps	Process
1	Reception of aluminum adjuvant material
2	Homogenization and transfer of aluminum suspension to sterilization vessel
3	Heat-sterilization of aluminum adjuvant • Sterilization with mixing during 30 minutes at 121,5°C
4	Transfer to storage containers
5	Formulation of aluminum diluent • Re-pooling of aluminum containers in formulation tank • Resuspension of aluminum suspension and dilution with 150 mM NaCl under mixing Transfer to filling tank
6	Filling of aluminum diluent in prefilled syringes Sealing and inspection
7	Packaging of A-VAX vaccine • Combine lyophilized A-VAX vaccine with aluminum-containing diluent

5020

5021   ### 7.3.1. Formulation Process Development

5022   During early development of the formulation for the A-VAX vaccine, initial time and investment were
5023   spent examining multiple formulation conditions in the liquid state. During the early development, a
5024   platform formulation strategy was employed. This platform formulation effort was based on other
5025   programs that have been worked on in the past to expedite development. Through the developmental
5026   efforts, it was determined that the use of an adjuvant would be necessary to ensure a robust
5027   immunogenic response was achieved.
5028

5029   In addition to demonstrating the need for aluminum adjuvant multiple, preclinical immunogenicity
5030   studies would be required to support the desired target product profile.
5031

5032   Completing initial stability studies at both long-term and accelerated conditions clearly indicated that
5033   12-month shelf life stability at 2–8°C could be achieved. This stability followed by decreased stability
5034   under accelerated temperatures of 25 °C and 37°C suggested that to obtain a 36-month shelf life and be
5035   able to penetrate the developing world and emerging markets, the vaccine must be a lyophilized
5036   presentation.
5037

5038   Because of the inability to achieve a liquid formulation, efforts in the formulation centered on using past
5039   lyophilization experience, and they identified an initial formulation consisting of sucrose, histidine, and
5040   polysorbate 80. These formulation excipients have been successfully used in vaccines, and there is
5041   significant prior knowledge on the formulation and potential issues with lyophilization.
5042

5043   The target formulation for the lyophilized pentavalent vaccine containing the capsular Ps of *X. horrificus*
5044   serotypes 1, 2, 3, 4, and 5 individually linked to a recombinant, noninfectious VLP is assumed to be the
5045   following, as shown in Table 7-4.
5046

5047　　**Table 7-4: Assumptions on Platform Formulation for Lyophilized Vaccine**

		Concentration/mL	Amount/dose
Sucrose	Bulking agent/stabilizer	50 mg	25 mg
Histidine	Buffer	10 mM - pH 6.0	
Polysorbate 80	Surfactant	0.01%	0.025mg
Ps 1-VLP	Active	100 µg/mL	50 µg
Ps 2-VLP	Active	100 µg/mL	50 µg
Ps 3-VLP	Active	100 µg/mL	50 µg
Ps 4-VLP	Active	100 µg/mL	50 µg
Ps 5-VLP	Active	10 µg/mL	5 µg

5048

5049   ## 7.3.2. Lyophilization Process Development

5050

5051   After understanding that a liquid platform formulation for A-VAX did not allow the desired target
5052   product profile (TPP) to be achieved, the team devoted efforts to evaluate lyophilization as a means to
5053   achieve the necessary VVM14 required for both the developing world and emerging markets. Upon
5054   reconstitution with aluminum phosphate adjuvant, all five serotypes readily bind to aluminum within
5055   two minutes; this data supported lyophilization as a viable option.

5056

5057   The data became the basis of supporting data that allowed the team to lyophilize the Ps-VLP conjugates,
5058   and then reconstitute the vaccine with the aluminum-containing diluent and achieve similar adsorption
5059   conditions as observed for liquid material following reconstitution and mixing by inverting the vials
5060   three to five times prior to administration.

5061

5062   Although the team did consider lyophilizing the A-VAX vaccine in the presence of aluminum, it has not
5063   been demonstrated to this point with any currently marketed products. Thus, to minimize delays to the
5064   timeline, the approach of the aluminum phosphate diluent was employed. The starting point for
5065   formulation development associated with a lyophilized formulation, much like the liquid development
5066   efforts, used a platform formulation.

5067

5068   Once the initial matrix of sucrose, histidine, and polysorbate 80 was determined, two techniques to help
5069   shape the initial lyophilization cycle were used to better characterize the formulation. The first was the
5070   use of modulated differential scanning calorimetry to determine the glass transition temperature (Tg')
5071   for the formulation of choice. Second, the collapse temperature (Tc) was measured. These biophysical
5072   techniques resulted in a Tg' value of ~ -33ºC and a Tc value of ~ -30ºC. Both are well in line with past
5073   knowledge associated with sucrose-containing formulations.

5074

5075   Using Manometric Temperature Measurements (MTM), the initial lyophilization development was
5076   expedited. MTM is one of many lyophilization development technologies that has truly benefited early
5077   stage development and lyophilization robustness. Using the Tg' value, along with the formulation
5078   composition (glassy or amorphous), fill volumes, and the vial configuration, the identification of primary
5079   drying conditions can be obtained in a few runs rather than multiple interactions of development. The
5080   lyophilization cycle based on MTM was defined for early stage development (Table 7-5).

5081

5082   After establishment of the initial primary drying conditions using MTM, lab-scale runs utilized
5083   temperature probe data to monitor cycle progress. This was completed to ensure that throughout the
5084   development, the product temperature was staying below the Tg' and collapse temperature during
5085   primary drying. As development of the cycle for ramp rates, pressure, and secondary drying continued
5086   and was optimized, the cycle shifted from use of temperature probes to a time-/pressure-driven cycle.

5087

5088 **Table 7-5: Preliminary Lyophilization Cycle for A-VAX Vaccine**

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-25ºC
Primary Drying Time	1,500 minutes
Ramp to Secondary Drying	0.5ºC/minute
Secondary Drying Temperature	20ºC
Secondary Drying Time	420 minutes
Final Stage Postsecondary Drying	4ºC
Stoppering Pressure	800 mBarr
Stoppering Gas	Nitrogen
All conditions during lyophilization used 130 µbar pressure, based on past experience.	

5089

5090   ### 7.3.3. Adjuvant Development

5091 Early preclinical development needed to determine whether an adjuvant would be required for the
5092 vaccine. Based on experience from other conjugate vaccines on the market and in our portfolio, it was
5093 expected that an adjuvant would be required to ensure robust immunogenicity in the patient
5094 population.
5095
5096 As a starting point for choosing the adjuvant, the team assessed aluminum phosphate and aluminum
5097 hydroxide adjuvants. A main consideration in choosing the adjuvant was the robustness of adsorption as
5098 well as ensuring that the stability of the Ps-VLP conjugates was preserved post-reconstitution.
5099
5100 Results from the early work indicated that both adjuvants showed robust adsorption kinetics; however,
5101 the stability of the Ps-VLP conjugates was better with the aluminum phosphate adjuvant. The aluminum
5102 phosphate adjuvant significantly increased the anti-capsular Ps antibody levels, and the adjuvant
5103 mitigated ligand exchange between the Ps and the aluminum hydroxide. This ligand exchange impaired
5104 the immune response in animal models.
5105
5106 A correlation between the preclinical results and the clinical studies was observed, and the aluminum
5107 phosphate adjuvant dose level was selected during the Phase II clinical studies.
5108
5109 Aluminum phosphate is supplied from a commercial manufacturer and then pooled and sterilized prior
5110 to use as a diluent for the lyophilized drug product. Other vaccines in the pipeline have used aluminum-
5111 containing diluents with a standard formulation and filling process. As a start for development, the team
5112 decided to use the standard image (Table 7-6).
5113
5114 **Table 7-6: Adjuvant Formulation**

Component	Concentration

Aluminum phosphate	600 µg/mL
NaCl	150 mM
pH	5.0–8.0

5115

7.4.   Initial Risk Assessment: Cause and Effect

5116

5117   To prioritize the design space work early in program development, a preliminary risk assessment was
5118   performed. It utilized cause-and-effect matrices to examine the different process steps that could
5119   impact the critical quality attributes of the product (Table 7-8).
5120
5121   Utilizing knowledge gained from other Ps-VLP vaccines with formulation compositions similar to A-VAX,
5122   each of the specific process steps was examined. The scoring for the overall cause-and-effect matrices is
5123   outlined in Table 7-7.
5124

5125   **Table 7-7: Scoring Approach for Initial Risk Assessment**

Scoring of Process Parameters	
Impact Score	Ranking Criteria
10	Strong relationship known based on available data and experience
7	Strong relationship is expected
5	Not-so-strong relationship expected or unknown
1	Known to not have a relationship

5126
5127   Utilizing the approach from Table 7-7, the manufacturing unit operations associated with the product
5128   were scored to determine the risk/level of potential interaction (Table 7-8). The individual scores were
5129   assigned based on prior knowledge from other vaccine programs in the company's product line and on
5130   literature review.
5131
5132   After scoring for individual interactions, a total score was determined for each unit operation and
5133   quality attribute. These total scores were determined by summing the respective individual scores
5134   horizontally against the specific unit operation and vertically for individual quality attributes. The total
5135   scores were then used to assess relative risk for individual quality attributes and to prioritize
5136   development work for specific unit operations.
5137
5138   Using this scoring, the highest-risk unit operations of formulation (including drug substance (DS)/buffer
5139   quality) and lyophilization were selected for further investigation during initial development efforts
5140   using design of experiment (DOE) studies. Scoring vertically allowed the team to better understand
5141   which parameters would appear to have the most significant impact on the product moving forward
5142   (i.e., potency, moisture, sterility, adsorption).

Table 7-8: Cause-and-Effect Matrix

Cause-and-Effect Matrix

Process Parameters	Potency	Purity	Identity	Dose	pH	Moisture	Appearance (Lyo)	Appearance (Recon)	Recon Time	Endotoxin /LAL	Sterility	General Safety	Sub-Visible Particulates	Adsorption	Formulation Composition	Score
Raw Material (DS)	10	10	10	5	5	1	1	5	1	5	5	5	5	10	5	83
Raw Material (Buffer)	1	5	1	1	10	7	7	7	5	5	5	5	1	7	10	77
Raw Material (Vial/Stopper)	1	1	1	1	1	10	5	5	1	5	10	1	5	1	1	49
DS Thaw/ Handling	5	5	1	1	1	1	1	1	1	1	1	1	5	5	1	31
Formulation Compounding & Mixing	10	1	1	10	5	5	5	1	5	5	5	1	5	7	7	73
Filtration	5	5	1	5	1	1	1	1	1	1	10	1	7	5	1	46
Filling	7	1	1	10	1	1	5	1	5	5	5	1	7	5	1	56
Lyophilization	1	5	1	1	1	10	10	5	10	5	5	1	5	1	1	62
Capping	1	1	1	1	1	5	1	1	1	1	7	1	1	1	1	25
Visual Inspection	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
	42	35	19	36	27	42	37	28	31	34	54	18	42	43	29	

1   Since lyophilization of the drug product is required to ensure the TPP profile is achieved for the
2   vaccine, the aluminum adjuvant is provided in a separate diluent and has its own manufacturing
3   process and COA. Because the aluminum adjuvant has its own process and quality attributes, an
4   additional cause-and-effect matrix was generated (Table 7-9).
5
6   Prior knowledge learned through other vaccines similar to A-VAX and use of an aluminum
7   diluent aided in the initial risk assessment. Aluminum adjuvants' characteristics were studied in
8   several publications. S.L. Hem and collaborators have widely published on aluminum hydroxide
9   and aluminum phosphate adjuvants. The effect of autoclaving on aluminum phosphate adjuvant
10   was addressed in an article[2]; its impacts on pH (decreased by deprotonation and dehydration),
11   point of zero charge (decreased), and protein adsorption capacity (lyzozyme model, decreased)
12   were demonstrated. The amorphous structure was not affected by 30 or 60 minutes of
13   autoclaving. History with other sterilizable-in-place equipment shows that sterility will be
14   guaranteed if Fo of a minimum 20 minutes is reached during SIP operations.
15
16   **Table 7-9: Cause-and-Effect Matrix for Aluminum Adjuvant**

Quality Attribute Parameter	Sterility (Fo)	Homogeneity	Alum. Size	Alum. PZC	Alum. Adsorption Capacity	Score
Heating profile	5	1	5	5	5	21
Sterilization temperature	10	1	10	10	10	41
Sterilization duration	10	1	10	10	10	41
Cooling profile	5	1	5	5	5	21
Mixing speed	5	10	5	3	3	26
Impeller configuration and vessel geometry	1	5	5	1	1	13
	36	19	40	34	34	

17
18   Similar to Table 7-8, scores were added vertically (quality attributes) and horizontally (process
19   parameters) to determine which should be examined during design space development. Higher
20   scores were associated with the sterilization temperature and duration, which can be extended
21   to the heating/cooling profile, and with the mixing speed. These parameters will be evaluated
22   during design of experiment studies described for aluminum in the next section.
23

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[2] Burrel, Lindblad, White, Hem, Stability of aluminum-containing adjuvants to autoclaving, Vaccine 17,
2599–2603, 1999

7.5.   Design Space Development

To complete the initial risk assessment tool, the steps to be further studied during developmental work included the formulation compounding step and examination of the levels of excipients and pH associated with the product. Thawing and handling of the DS will not be further studied as a result of information learned from the downstream DS team, as well as experience with thawing and handling of the DS for related vaccines in the pipeline.

Moving into the lyophilization process for the product, the parameters to be investigated include: primary and secondary drying, ramp rate, and chamber pressure during the lyophilization run. Although the freezing process may impact the product's quality attributes, knowledge gained from past lyophilized vaccines shows the risks associated with freezing are minimal, and they will not be extensively examined early in product development. However, if issues arise during scale-up to commercial scale, additional development efforts will focus on examining the freezing process associated with the A-VAX vaccine.

Although in the example outlined in this document freezing was not investigated as part of the lyophilization process, it should be noted that freezing would be part of the process to examine. It is known that different methods of freezing can significantly impact the overall crystal structure (i.e., faster freezing (LN2 blast freezing) and can lead to smaller ice crystal structure vs. shelf freezing or controlled freezing with larger ice crystal structure. These differences in ice structure can impact the overall drying properties of the drug product and should be examined. It should not be assumed that freezing would not impact the lyophilization process, and it should be examined during routine development.

For aluminum adjuvants, two major factors will be further investigated during early development. These factors are the impact of mixing shear and the impact of thermal treatment on the aluminum particle characteristics.

7.5.1.   Leveraging Prior Knowledge: Parameters That Will Not Be Studied

Based on the C&E matrix and prior knowledge, the following parameters will not be explored further in the case study. The reason is either the risk is low to the final drug product or prior knowledge has been gained through literature reviews or experience with other vaccines similar to A-VAX.

7.5.1.1. Hold Time Studies

Once the formulation for the lyophilization is determined, hold studies will be conducted to determine acceptable hold times in the selected formulation. The data will demonstrate that this process intermediate can be held at 2–8°C and 25°C for seven days without significant degradation or impact to product quality. The parameters for assessment will be based on the following criteria: pH, appearance, total protein, antigenicity, and other characterization assays such as DLS.

7.5.1.2. Drug Substance Preparation and Handling

An assumption has been made that all the Ps-VLPs are maintained frozen. Each of the drug substances will be thawed using standard procedures and will be discussed in this document.

67   **7.5.1.3. Sterile Filtration**

68   An assumption has been made that the appropriate filter membrane, size, and membrane
69   housing have been chosen based on experimental data, which will be discussed.

70   **7.5.1.4. Vial Filling**

71   Vial filling is a standard platform process, with the respective vial and stopper configuration.
72   Required filling tolerances have been previously demonstrated for similar formulation
73   compositions.
74
75   ## 7.5.2. Formulation Experiments and DOE

76   Following the completion of the initial risk assessment tool, it was identified that a better
77   understanding of the formulation composition was necessary to ensure that a robust process
78   and product were achieved. To accomplish these activities, the formulation development
79   studies will be conducted in two phases utilizing design of experiments.

80   • Determine the optimal final formulation matrix following the reconstitution process with
81   the aluminum phosphate adjuvant. This will be determined based on liquid studies showing
82   the conditions of the formulation necessary to ensure robust stability for a short period of
83   time and rapid adsorption to the aluminum phosphate adjuvant.

84   • Identify the lyophilization matrix, and complete design space studies on the actives and
85   excipients associated with the formulation. The overall adjuvant formulation matrix would
86   be defined based on the overall formulation matrix required to support lyophilization.
87
88   **Formulation Optimization DOE**
89
90   **1.5.2.1 Design Space for Formulation Matrix Following Reconstitution**
91

92   To initiate formulation development, it was necessary to identify a formulation that would
93   ensure that all five serotypes would bind to the adjuvant in a timely manner and consistently
94   absorb to aluminum so that the immunogenicity of the vaccine was maintained.
95

96   In the first set of formulation DOE studies, the formulation excipient levels along with pH and
97   aluminum adjuvant concentrations were varied to determine an optimal formulation for the
98   pentavalent Ps-VLP vaccine. The optimal binding for all five serotypes will be determined for the
99   product along with the respective design space. Factors investigated included the concentration
100   of sucrose, salt, and adjuvant along with a pH range from 5.0–8.0 (Table 7-10).
101

102   **Table 7-10: Factors Determining Binding of Antigens to Aluminum**

Excipients	Range
Sucrose	4%–10%
NaCl	0–150 mM
pH	5.0–8.0
Aluminum phosphate	0.4–0.6 mg/mL as aluminum
Antigens constant factor	Ps 1-VLP to PS 4-VLP at 100 µg/mL and Ps 5-VLP at 10 µg/mL

103
104   Using the ranges listed in Table 7-10, the first DOE study was determined to investigate four
105   factors.
106
107   **Table 7-11: Study Design for DOE to Determine the Drug Product Matrix**

Run	Aluminum Concentration (mg/mL)	pH	Sucrose (%)	NaCl (mM)
1	0.4	5.0	4.0	150.0
2	0.8	8.0	10.0	0.0
3	0.4	5.0	10.0	150.0
4	0.4	5.0	10.0	0.0
5	0.4	5.0	4.0	0.0
6	0.8	8.0	4.0	0.0
7	0.8	5.0	4.0	150.0
8	0.4	8.0	4.0	150.0
9	0.8	5.0	10.0	150.0
10	0.8	8.0	4.0	150.0
11	0.8	5.0	4.0	0.0
12	0.4	8.0	10.0	150.0
13	0.4	8.0	4.0	0.0
14	0.6	6.5	7.0	75.0
15	0.6	6.5	7.0	75.0
16	0.8	5.0	10.0	0.0
17	0.4	8.0	10.0	0.0
18	0.8	8.0	10.0	150.0
19	0.6	6.5	7.0	75.0
Response: Polysaccharide binding to aluminum phosphate based on immunoassay such as ELISA or nephelometry.				

108

109   One of the important aspects of formulation will be quantitation of the individual antigens.
110   Given that an immunoassay will be utilized for quantitation, that may lead to variability
111   associated with the analytical methods following formulations.

112

113   An immunoassay specific for each serotype was used to measure the overall binding of the
114   serotype-specific antigen to the aluminum fraction. The result from the DOE for percent of
115   binding for each serotype was captured and presented. As expected, the binding of conjugates
116   to aluminum did vary depending on the formulation investigated (Table 7-12).

117

118   **Table 7-12: Example % of Binding of Ps-VLP Conjugates**

Run					
	Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
1	80	85	98	98	98
2	30	30	55	49	50
3	82	78	80	78	97
4	83	79	97	45	96
5	82	78	55	42	99
6	20	20	30	30	95
7	98	98	95	95	98
8	30	30	70	70	82
9	85	85	99	99	95
10	40	40	80	80	86
11	82	78	80	49	50
12	20	20	50	50	50
13	20	20	50	50	50
14	59	56	95	95	95
15	57	57	100	100	100
16	85	85	50	50	89
17	20	20	30	30	90
18	30	30	60	60	90
19	57	55	93	93	93
Data in table shows the impact of salt, aluminum concentration, and pH on the binding.					

119

120   Through the DOE work completed examining the impact of pH and aluminum concentration on
121   binding, as expected, there appeared to be a strong correlation between pH and adsorption.

122   Additionally, it has been observed the overall concentration of aluminum did not have a
123   significant impact on binding. The Ps 1-VLP conjugate appeared to show the best example for
124   the impact of pH on binding (Figure 7-1). As a result, utilizing Ps 1-VLP to optimize the pH range
125   would ensure a robust formulation is achieved.
126
127   **Figure 7-1: Ps 1-VLP Binding as a Function of pH and Aluminum Concentration**
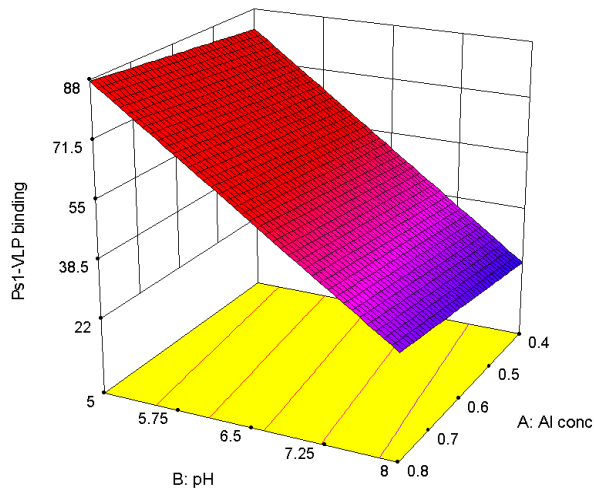
128
129   In addition to examining the impact of pH and aluminum concentration on Ps-VLP binding to
130   aluminum, the DOE also examined the impact of salt and sucrose concentrations on adjuvant
131   binding. Four of the serotypes (1, 2, 3, and 5) indicated that there was no significant impact to
132   binding when varying the concentrations of the excipients. However, for serotype 4, there was a
133   strong correlation observed related to the concentration of sucrose and salt (Figure 7-2). As with
134   the impact of pH, future formulation development will center on ensuring that serotype 4 would
135   adsorb to aluminum and meet the required TPP.
136

137   **Figure 7-2: Ps 4-VLP Binding as a Function of NaCl and Sucrose**
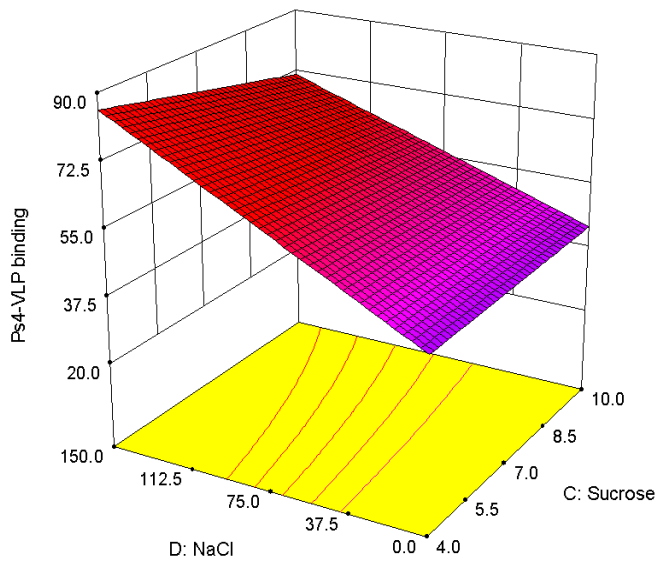
138
139

140   **7.5.2.1. Determination of the Lyophilized Matrix**

141

142   Once the matrix for the final formulation is determined, the antigen formulation and the
143   adjuvant formulation will be separately evaluated. The next phase is to lyophilize the antigens in
144   the matrix and determine that the binding is maintained following lyophilization.

145

146   The parameters would mainly be the limits of adsorption to aluminum for the five Ps serotypes
147   within the wider design space of the lyo matrix. The factors for design space and the DOE for
148   these optimizations are shown in Table 7-13.

149

150   **Table 7-13: Factors Determining Edges of Formulation to Ensure Binding Is Maintained**

Excipients	Range
Sucrose	3–7%
Histidine	5–15 mM
Polysorbate 80	0%–0.03%
pH	6.0
Antigens constant factor	Ps 1-VLP to 4 at 100 µg/mL and Ps 5-VLP at 10 µg/mL
Response: Cake cosmetics, moisture, and binding to aluminum phosphate diluent on reconstitution as determined as optimal in the first study.	

151

152   For simplicity, the second DOE evaluating the lyophilization matrix is not discussed here.
153   However, it was observed that under the optimal conditions, there is consistency of binding and
154   the necessary stability profile was achieved.
155
156   Using the ranges from Table 7-13, a DOE experiment was initiated to vary the different factors
157   to understand if there are any issues related to the sucrose, histidine, and PS 80 concentration
158   on cake appearance, moisture, and overall binding to aluminum following reconstitution.
159
160   **Table 7-14: Optimization of Formulation for the Lyophilized Matrix**

Run	Histidine (mM)	PS 80 (%)	Sucrose (%)
1	15.0	0.30	7.0
2	15.0	0.00	3.0
3	5.0	0.30	3.0
4	5.0	0.30	7.0
5	10.0	0.15	5.0
6	10.0	0.15	5.0
7	15.0	0.30	3.0
8	5.0	0.00	3.0
9	15.0	0.00	7.0
10	10.0	0.15	5.0
11	5.0	0.00	7.0

161
162   The data in Table 7-15 is an example showing that the drug product is robust based on the key
163   parameters of the lyophilized cake, moisture, reconstitution time, and binding of each of the Ps-
164   VLPs within the limits of the excipients, which are histidine, PS 80, and sucrose.
165
166   **Table 7-15: Binding Analysis within the Drug Product**

Run	Moisture %	Recon time sec	% Binding of Ps-VLP conjugates				
			Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
1	1	10	55	50	90	90	90
2	1.2	125	56	55	92	92	92
3	1.4	24	59	56	95	95	95
4	0.9	16	46	49	98	98	98
5	1.1	18	59	69	100	100	100
6	1.4	17	57	57	100	100	100
7	1.1	20	57	55	93	93	93

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Run	Moisture %	Recon time sec	% Binding of Ps-VLP conjugates				
			Ps 1-VLP	Ps 2-VLP	Ps 3-VLP	Ps 4-VLP	Ps 5-VLP
8	0.8	20	55	56	91	91	91
9	1.0	22	54	59	99	99	99
10	1.1	25	59	46	97	97	97
11	0.9	29	61	59	98	98	98

7.5.2.2. Plcaeholder for text

The samples that were prepared were placed on stability and monitored under accelerated conditions to ensure alignment with the TPP and VVM14 requirements. The conditions investigated included one month of 50ºC thermal stress as well as both refrigerated and room temperature stability through 24 months to support shelf life.

Following the completion of the development, the lead formulation was identified based on both the adsorption and stability results associated with the design of experiments. The lead formulation was XX.

7.6.   Dosage Administration and Stability

This section focuses on the dosage administration instructions at the clinic for delivering the vaccine. It is assumed that the vaccine will be administered by medical personnel. The vaccine will be supplied as two components: the lyophilized vaccine, packaged with an aluminum phosphate adjuvant for reconstitution.

To reconstitute the vaccine, personnel first will withdraw the aluminum phosphate with a syringe and inject it into the lyophilized vial. They will mix the vial well, and the instructions will be based on data generated by monitoring the uniformity of the vaccine as measured by product uniformity. Although ideally the vaccine should be given soon after reconstitution, it may need to be held for a time. To support the period of use following reconstitution of the vaccine, stability data will be used. An example of the experimental design is shown in Table 7-16, used to measure the quality attributes associated with the reconstituted vaccine.

Table 7-16: Stability of Vaccine Following Reconstitution

	Time in hours							
	0	2	4	6	12	24	48	72
Appearance								
pH								
Subvisible particles								
Total protein								

Saccharide concentration								
Protein adsorption to aluminum								
Saccharide adsorption to al								

192

193   The data will support the in-use period. However, because the vaccine is a preservative-free
194   product, the time that the vaccine is held post-reconstitution should be limited.

195

196   ## 7.7.   Lyophilization Process Development and DOE

197   **Lyophilization process development**
198

199   Based on prior knowledge of the lyophilization process development and scale-up, primary
200   drying is one of the most critical process steps in terms of scale dependency and product quality.
201   Many models are available in the literature to calculate product temperature, sublimation
202   kinetics, and sublimation time once the heat and mass transfer for a given equipment and a
203   given product are known (*Pikal. J. Parentr. Drug Assoc.. 1985, 39 (No. 3), 115–138; Mascarenhas*
204   *et al. Comput. Methods. Appl. Mech. Eng. 1997, 148, 105–124*). Models are also available to
205   calculate all shelf temperature and chamber pressure combinations that would ensure that the
206   product temperature remains below the collapse temperature throughout primary drying
207   (*Chang et al. 1995. Pharm. Res. 12:831–837; Nail et al. 2008. Biopharm Int. 21:44–52; Giordano*
208   *et al J. Pham. Sci. 2011.100(1),311–24*).

209

210   More recently, more advanced approaches have been published. They propose to take into
211   account intrinsic batch heterogeneity and transfer parameters' uncertainty (*Fissore et al.*
212   *Advanced approach to build the design space for the primary drying of a pharmaceutical freeze-*
213   *drying process. J. Pharm. Sci. Vol. 100 (11), pgs 4922–4933*). For the lyophilization cycle
214   development of the A-VAX vaccine, a standard approach of experimental measurement of heat
215   (Kv) and mass (Rp) transfer coefficients coupled with a monodimensional model (for example,
216   *Giordano et al J. Pham. Sci. 2011.100 (1), 311–24*) was used to define optimal freeze-drying
217   conditions during primary drying.

218

219   *Experimental determination of heat transfer coefficient Kv at lab scale*

220

221   A gravimetric method was used to determine the heat transfer coefficient Kv values throughout
222   the shelf for vials directly loaded in the shelf, as described in *Brülls M, Rasmuson A. Int. J. Pharm.*
223   *2002; 246(1-2):1–16.* Other averaging spectroscopic methods can be used (*Kuu et al. 2009. J.*
224   *Pharm. Sci. 98:1136–1154*); however, they do not provide information about the heat transfer
225   heterogeneity resulting from radiation phenomena at the edges of the shelf. Figure 7-3 below
226   details the different five locations of vials on the heating shelf of the freeze dryer.

227

228   **Figure 7-3: Various Zones of the Heating Shelf in Terms of Heat Transfer**

229   Named from (a) to (e). Half a shelf is represented, and vials are in direct contact. (a) type vials
230   represent 1.3% of the total number of vials, (b) type vials represent 9.6%, (c) represent 5.6%, (d)
231   represent 15.3%, and (e) represent 68.2%.
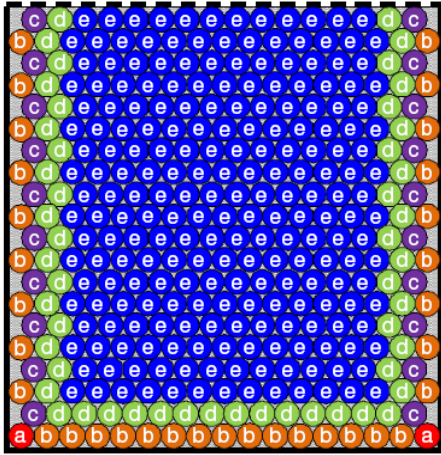
232
233

234   The table below gives an example of overall heat transfer values measured at 100 µbar as a
235   function of vial locations:

236

Vial location	Kv, W.m-2.K-1
(a)	35.2±3.4
(b)	24.5±2.0
(c)	16.3±0.9
(d)	11.8±1.0
(e)	9.3±0.7

237
238   *Kv* is pressure dependent and was therefore calculated at different pressure for each vial
239   location identified above in the different zones of the heating shelves.
240

241   **Figure 7-4: Kv Values As a Function of Pressure for Different Vial Locations in the Lab-Scale**
242   **Freeze Dryer**

243   Diamonds for (b) vials, squares for (c) vials, triangles for (d) vials, and circles for (e) vials
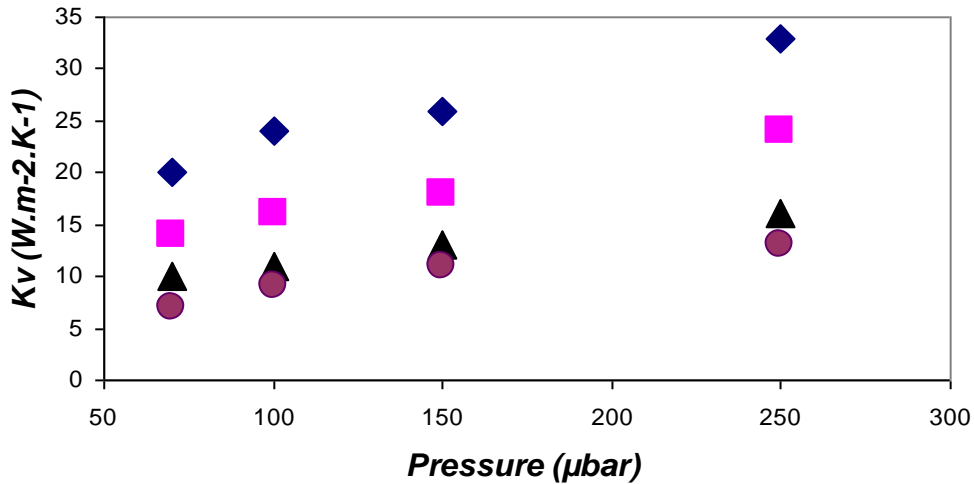
244
245   *Experimental determination of resistance to mass transfer coefficient Rp of the product*
246
247   A pressure rise method was used to determine Rp values as a function of the dry layer thickness
248   (L_{dried}) in the freeze-dried cake (*Milton et al. 1997. PDA J. Pharm. Sci. Technol. 5:7–16*). The
249   measurement was repeated three times to evaluate an average and the variability associated
250   with the Rp values.
251
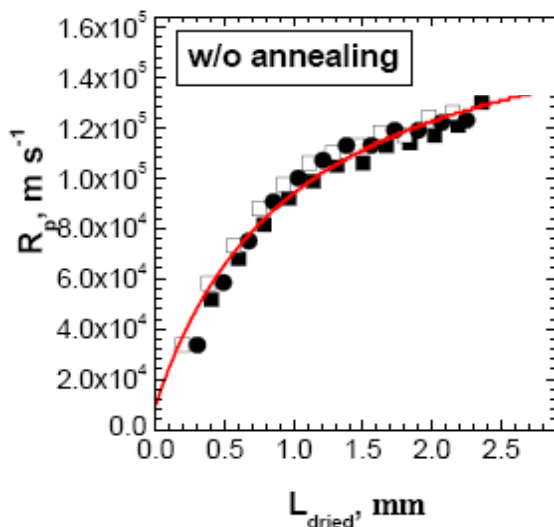
252
253
254   *Definition of the design space for primary drying using mathematical modeling*
255
256   For the A-VAX vaccine, the modeling and the design space representation described in (*Fissore*
257   *et al. Advanced approach to build the design space for the primary drying of a pharmaceutical*

258   *freeze-drying process. J. Pharm. Sci. Vol. 100 (11), pgs 4922–4933*) was chosen. An example of
259   this representation is given in the figure below:
260
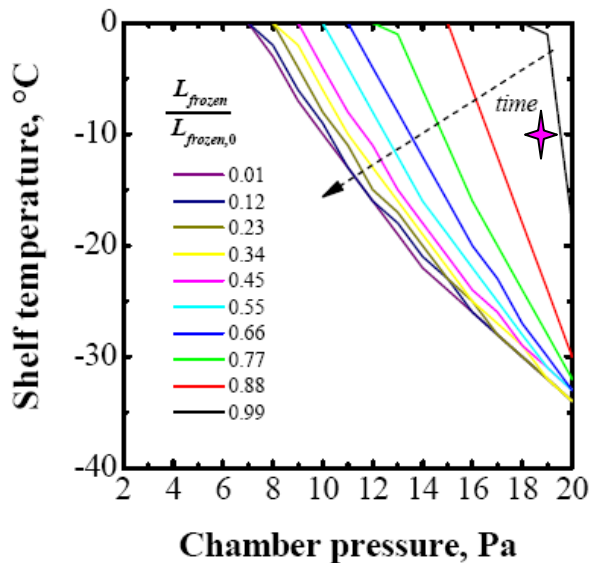
261
262
263   In this design space representation, chamber pressure is represented in the abscise axis and
264   shelf temperature is represented in the ordinate axis. A quasi-steady state monodimensional
265   model was used to calculate at a given value of *Rp* (i.e., at a given value of the dry layer
266   thickness) all combinations of shelf temperature and chamber pressure values that would set
267   the product temperature at the sublimation interface equal to the collapse temperature. These
268   combinations are represented by the color solid lines in the graph, each color corresponding to
269   a given dry layer thickness.
270
271   For example, for a frozen layer thickness equal to 88% of its initial value (prior to sublimation),
272   the combinations of T_{shelf} and P_c are represented by the red solid line. For a frozen layer
273   thickness equal to 1% of its initial value, they are represented by the purple solid line.
274
275   Above this solid line, the product temperature at the sublimation interface is above the collapse
276   temperature. Below this line, the product temperature at the sublimation interface is below the
277   collapse temperature; and the area below this line represents the design space for primary
278   drying conditions at a given *Rp* value.
279
280   The figure below represents the calculation of the primary drying design space of our A-VAX
281   vaccine, for different values of the frozen layer thickness compared to the initial thickness (77%,
282   45%, and 1%) and the (b) type locations. The (b) type locations were selected to define T_{shelf} and
283   P_c values because they have the higher Kv values after (a) type locations, whose number was
284   considered negligible, and therefore are the vial location at risk to exceed collapse temperature
285   during primary drying.
286
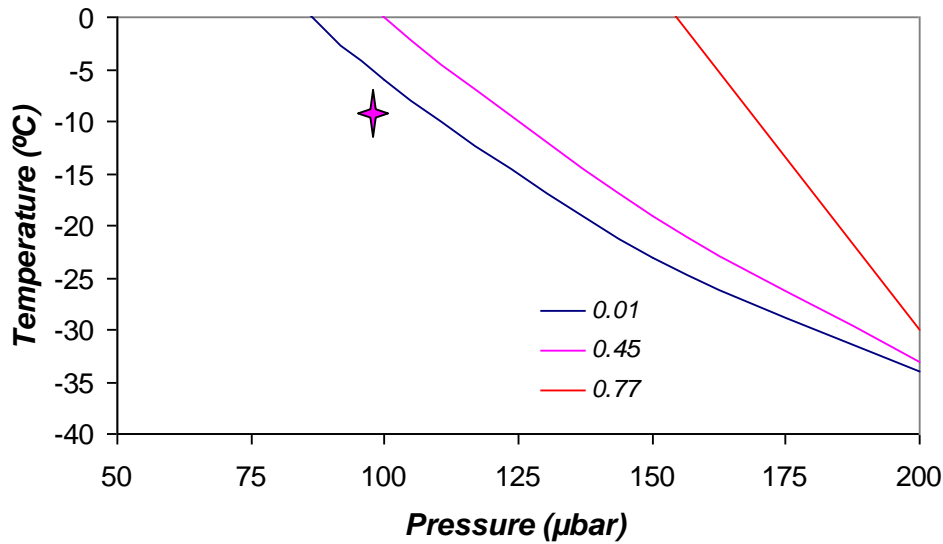
287
288   The selected conditions are represented by the purple star on the graph. T_{shelf} = -10C and Pc=100
289   μbar will ensure product temperature below the collapse temperature for all vial locations
290   (except (a) location) and throughout the duration of primary drying.
291
292   Primary drying time was selected by calculating its value in these conditions for (e) type
293   locations, which have lower Kv value and therefore the longer sublimation time. The calculated
294   sublimation time is 620 minutes. The selected sublimation time is 720 minutes, to include a 100-
295   minute safety margin. The maximum calculated sublimation rate during primary drying in these
296   conditions is 0.34 kg h^{-1}m^{-2}.
297
298   *Experimental validation of the model at lab scale*
299
300   The table below gives, for important process parameters, the maximum difference (*Δmax*)
301   between calculated and measured values at the pilot scale throughout the duration of the
302   primary drying. The measurement system is specified in the table:
303

Process parameter	Δmax during primary drying
Product temperature	*Average of 5 t-type Thermocouple: ΔTmax=0.7°C* *Pressure rise test measurement: ΔTmax=0.9°C*
End of sublimation time	*MKS/Pirani gauge ratio: Δtsublimation=30min*
Maximum sublimation flow rate	*TDLAS: Δ(max sublimation rate)= 0.05 kg h-1m-2*
Rejection rate based on cake appearance	*Δ(rejection rate)=0.2%*

304
305   The good agreement between calculated and measured important process parameters validated
306   the use of this model and this design space approach to define primary drying process
307   conditions.
308
309

310   *Definition of freezing and secondary drying conditions*
311
312   Based on prior knowledge, it was demonstrated that the freezing rate, within the capabilities of
313   an industrial freeze dryer, did not have any impact on product quality. As mentioned earlier, it
314   would be important for the team to investigate the impact of freezing early in development to
315   determine the impact of freezing. In this example, only a few different parameters were
316   examined, but freezing should be examined routinely since it can impact the overall
317   lyophilization process substantially.
318
319   Shelf temperature ramp rate was then set to 0.3°C/min. Similarly, previous data demonstrated
320   that up to 40°C was an acceptable product temperature for secondary drying for all serogroups.
321   The setpoint was then set at 30°C for 10 hours to achieve moisture levels lower than 2%.
322
323   **Table 7-17: Pilot Scale Optimized Lyophilization Cycle for A-VAX Vaccine**

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-10ºC
Primary Drying Time	720 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	4ºC
All conditions during lyophilization utilized 100 µbar pressure.	

324
325   **Lab-scale lyophilization DOE**
326
327   Based on initial risk assessment, the main areas to examine include freezing, primary and
328   secondary drying, pressure control, and ramp rate.
329
330   Applying the output of the initial cause-and-effect risk assessment, a series of development
331   activities were executed to further understand the sensitivity of product quality attributes to
332   process parameters. The first of these activities was a screening DOE, whereby the potential
333   impact of high-risk process parameters could be further assessed.
334
335   The screening study was designed as a one-quarter fractional two-level DOE on the parameters
336   outlined in Table 7-18. This design was selected to allow direct evaluation of main effects while
337   screening for the potential presence of two-way interactions. Lyophilization-related quality
338   attributes of potency, moisture, appearance, and reconstitution time were evaluated across all
339   runs. The ranges explored for each parameter were selected to be >3X NOR for expected
340   performance in similar commercial equipment.

341

342   **Table 7-18: Design of Experiment to Screen Lyophilization Parameters**

Factor	Low	Set point	High
Sucrose	-15%	0%	+15%
Chamber Pressure	50 µbar	100 µbar	150 µbar
1° Drying Shelf Temperature	-15°C	-10°C	-5°C
Shelf Temperature Ramp Rate	0.1°C/min	0.3°C/min	1.0°C/min
2° Drying Shelf Temperature	25°C	30°C	35°C
2° Drying Duration	8 hr	10 hr	12 hr

343

344   The results of this DOE suggest the following:

345

346   •   The process parameters explored did not have a statistically significant response (95% CI) on
347   cake appearance or potency.

348

349
350
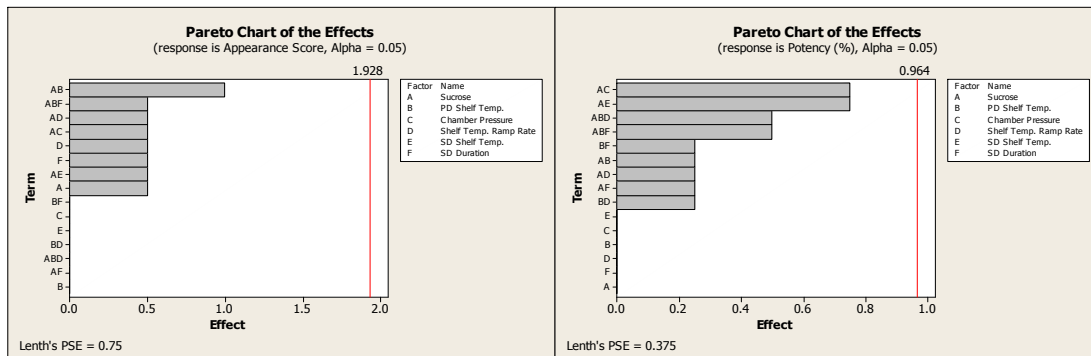
351   •   Statistically significant effects were observed for product moisture, specifically sucrose, shelf
352   temperature ramp rate, secondary drying temperature, and secondary drying duration.
353   Additional work should be performed to understand specific risks for this quality attribute
354   and the parameters indicated.

355

Main Effects Plot for Moisture (%) / Pareto Chart of the Effects (response is Moisture (%), Alpha = 0.05)
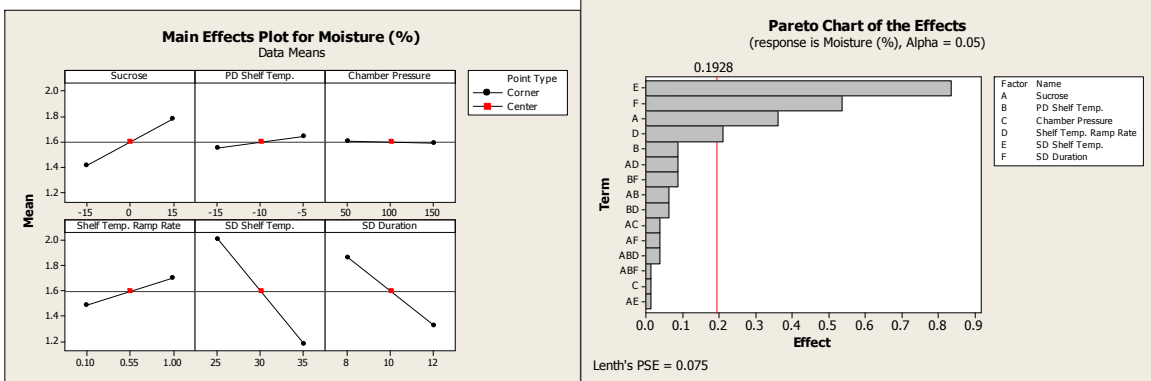
- Statistically significant effects were also observed for reconstitution time, specifically secondary drying temperature and secondary drying duration. It is important to note that while the effects were statistically significant, the range of values observed (2–20 seconds) is well below the maximum specification of 120 seconds.

Main Effects Plot for Reconstitution Time (seconds) / Pareto Chart of the Effects (response is Reconstitution Time (seconds), Alpha = 0.05)
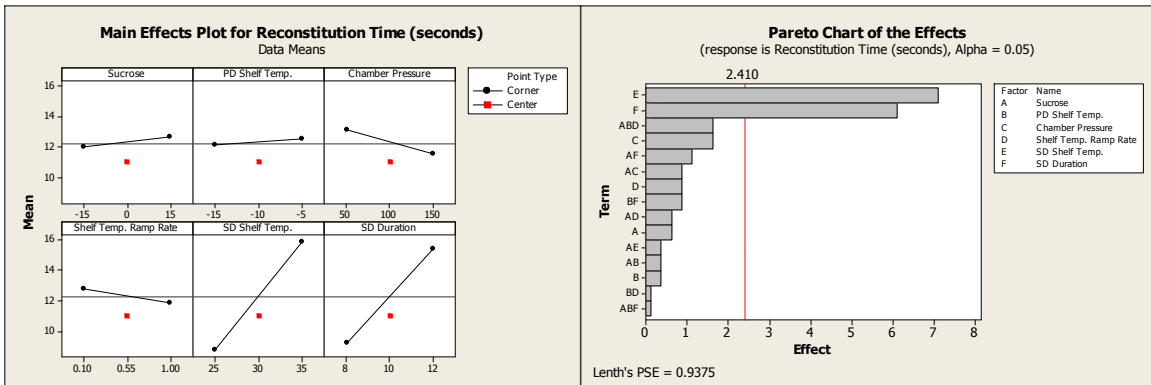
- Two-way interactions were not observed for the conditions explored in this DOE for any of the lyophilization-related critical quality attributes.

- Over the range of conditions evaluated, primary drying shelf temperature and chamber pressure did not have a statistically significant response on any of the quality attributes. While this would suggest that these parameters are not important to the process, it is well documented that these parameters are important to maintaining appropriate product temperature during sublimation and successful removal of ice from the product prior to removal of bound water in secondary drying. If controls can be implemented to ensure that primary drying is completed at the commercial scale and product temperature is monitored during scale-up, this data may be used to justify a reduced criticality for these parameters.

With statistically significant, but not functionally meaningful, effects on reconstitution time and no statistically significant effects on potency and appearance, future development efforts during scale-up should focus on product moisture (and associated parameters) and implementation of a control strategy to ensure successful primary drying completion and product temperature.

381   ### 7.7.1. Adjuvant Sterilization Process Development

382
383   Based on the initial risk assessment, the main focus of development efforts will be on the
384   sterilization process of the aluminum suspension.

385
386   The sterilization step must guarantee product sterility, while delivering a homogeneous
387   aluminum suspension of consistent characteristics. The sterilization step is performed in a
388   jacketed vessel under mixing. (FIGURE XX to show process investigated.)

389
390   ### 7.7.2.   Aluminum Sterilization DOE

391
392   Since the sterilization process will be examined, a flow diagram (Figure 7-5) indicates the overall
393   process associated with sterilization.

394
395   **Figure 7-5: Aluminum Sterilization Process**
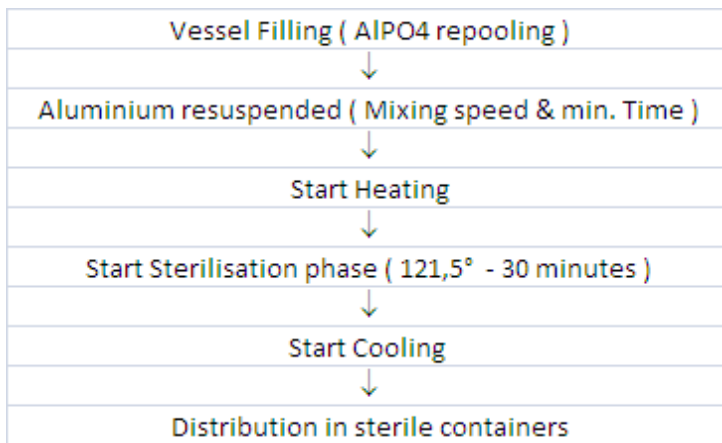
396
397
398   With knowledge of the process and the cause-and-effect matrix, the parameters of sterilization
399   temperature, mixing speed, and cycle duration (Table 7-19) were examined using a design of
400   experiment.

401
402   **Table 7-19: Adjuvant Sterilization DOE**

Parameter Investigated	Range
Sterilization Temperature	119.5 ºC–123.5ºC (target 121.5°)
Sterilization Duration	30 minutes
Cycle Duration	100–250 min (target 160 min)
Mixing Speed	104–310 rpm (target 210 rpm)
Quality Attributes Evaluated	pH, Zeta Potential, PZC, Particle Size, Adsorption Capacity, Viscosity, Settling Velocity, Turbidity, Free Phosphate

403

CMC-VWG

404 Because aluminum phosphate production is already in place for other vaccines, a lot of historical
405 information and prior knowledge is already available. The system is well understood, critical
406 process parameters are identified, and the design of experiment will focus on the
407 demonstration of the process robustness of the sterilization step performed in a new stainless
408 steel vessel, associated with scale-up considerations.
409
410 Two factors will be evaluated, split in three quantitative factors studied through a full two-level
411 factorial design with three additional central points (reference conditions):
412
413 • The impact of mixing speed: A boundary condition is the minimal speed defined to
414   guarantee aluminum suspension homogeneity. The maximal speed is defined from the
415   scaling-up studies and will cover the worst-case conditions identified for larger-scale vessels
416   that will be used in the future. Scale-up considerations for mixing are described in chapter
417   7.4, Adjuvant Scale-up Considerations.
418 • The temperature profile: It is the combination of the sterilization temperature and the
419   kinetics of heating/cooling. Sterilization temperature is fixed at 121.5°C, and the variation
420   range around the target value is fixed to 2°C, taking into account the overall precision of the
421   temperature probes and the process control system. Sterilization step duration is fixed to 30
422   minutes. Sterilization duration starts automatically (PID) when sterilization temperature is
423   reached and ends automatically after 30 minutes (PLC-controlled).
424
425 Overall process duration is composed of the heating, sterilization, and cooling steps. Extremes'
426 profiles will be evaluated (short to long heating/cooling kinetics). The short temperature profile
427 is associated with the lowest temperature (119.5°C) and must guarantee a minimal Fo value for
428 sterility assurance. The long temperature profile is associated with the highest temperature and
429 is the worst case for temperature impact on aluminum properties.
430
431 The main output of the DOE will be the PZC, the particle size, and the adsorption capacity.
432 Adsorption capacity can be measured with a model protein (allow to make the link with
433 previous aluminum phosphate-based vaccine development), and in the case of the A-VAX
434 vaccine development, the impact on the binding of the worst-case serotype will also be
435 evaluated.
436
437 The DOE is based on an equivalence approach, with a target of robustness demonstration.
438
439 Objective of the DOE is demonstration that evaluated changes do not impact aluminum quality.
440

441   **Table 7-20: DOE for Aluminum Sterilization and Responses for Measured Quality Attributes**

Mixing Speed	Temperature	Duration	PZC	Adsorpt. Capacity mg Lyz / mg Al	Size by SLS µm (D [v, 0,5])	Remarks
210	121.5	160	5.3	0.99	2.9	Central Point
210	121.5	160	5.4	0.87	3.6	Central Point
210	121.5	160	5.2	0.91	3.1	Central Point
104	119.5	100	5.5	1.06	3	
104	119.5	100	5.5	0.97	3.5	
104	123.5	100	5.3	0.96	4	
310	123.5	100	5.4	0.88	3.3	
104	119.5	250	5.1	0.86	3.8	
310	119.5	250	5.4	1.01	3.5	
104	123.5	250	5.1	0.82	3.2	
310	123.5	250	5.1	0.79	3.9	

442
443   Based on manufacturing experience with aluminum phosphate and on characteristics of
444   aluminum adjuvant used in A-VAX preclinical and clinical development, acceptance ranges are
445   defined for PZC, particle size, and adsorption/binding:

446
447   5,0 ≤ PZC ≤ 5,6
448   0,7 ≤ Ads. Capacity Lyz. ≤ 5,6
449   2,5 ≤ Size by SLS ≤ 5,0

450
451   Particle size by Static Light Scattering (SLS) is not affected by mixing speed, sterilization
452   temperature, or temperature profile:
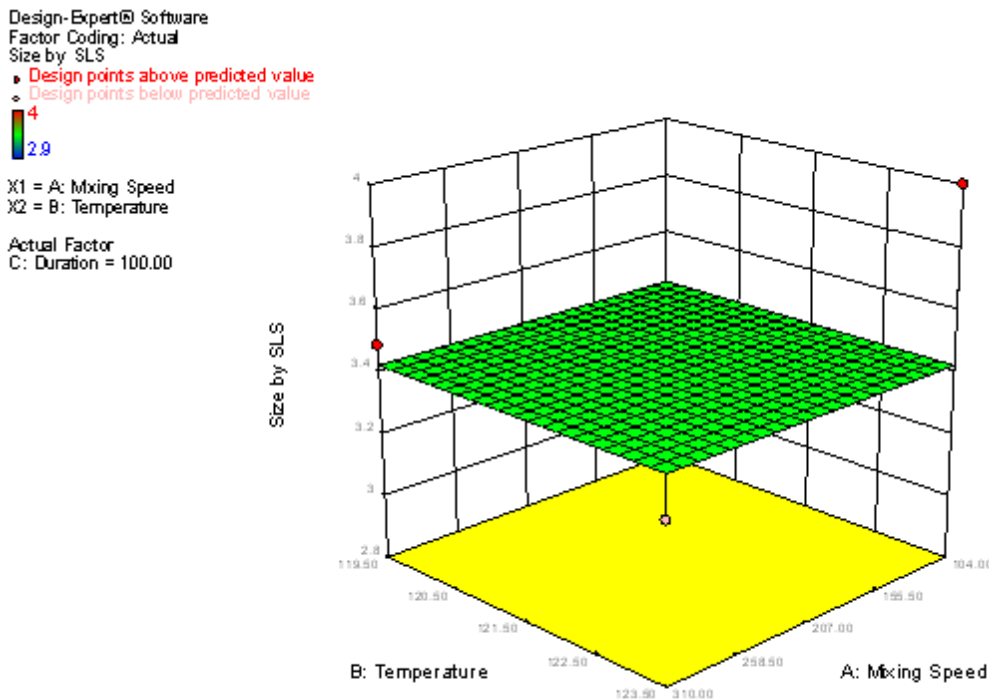
453

454   Experimental data are all in the range of acceptance criteria. However, DOE analysis allows us to
455   understand the impact of some parameters on aluminum characteristics:
456
457   PZC is impacted by the temperature and the duration of the complete sterilization cycle
458   (p-value < 5%, ANOVA analysis).
459

460
461
462   Adsorption capacity is also affected by the temperature and the cycle duration (p-value < 5%,
463   ANOVA analysis).
464

Design-Expert® Software
Factor Coding: Actual
Adsorpt. Capacity
- Design points above predicted value
- Design points below predicted value
1.06
0.79

X1 = A: Temperature
X2 = B: Duration
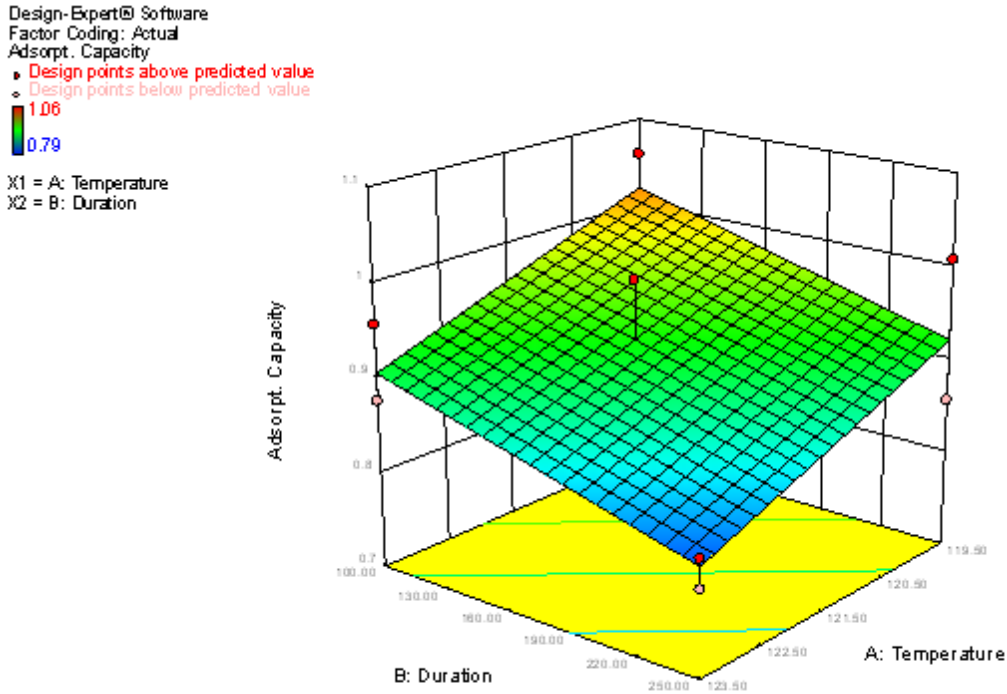
465
466
467 Two-way interactions were not observed for the conditions explored in this DOE.

468
469 This kind of experimental plan does not allow us to model curvature effects. However, it is
470 possible to check with the central points that the model is not affected by the absence of
471 curvature modeling.

472
473 A lack of fit parameter is calculated by comparing the measured values and the predicted values.

474
475 The lack of fit is found not to be significant, and it is not necessary to add experimental points to
476 take into account curvature in the modeling.

477
478 From the prediction model build from the DOE, it is possible to determine potential risk of
479 failures.

480
481 At reference (target) conditions or for the most critical parameter combinations, predicted
482 responses and associated 95% confidence intervals are inside acceptance criteria:
483

484   **Table 7-21: Impact of Process Parameters on Quality Attributes: Predicted Response Based on**
485   **Model from DOE**

Response	Mix. speed	Temp.	Duration	Low spec	High spec.	Prediction	95%CI low	95%CI high
PZC	*	121.5	160	5	5.6	5.3	5.3	5.4
PZC		119.5	100			5.5	5.4	5.6
PZC		123.5	250			5.2	5.1	5.3
Adsorpt. Capacity	*	121.5	160	0.7	1.2	0.9	0.9	1.0
Adsorpt. Capacity		119.5	100			1.0	0.9	1.1
Adsorpt. Capacity		123.5	250			0.8	0.8	0.9
Size by SLS	*	*	*	2.5	5	3.4	3.2	3.7

*: Not significant factor, predictions valid for the whole studied range

486
487
488   Conclusion of the DOE is demonstration of process robustness, but attention must be paid to
489   the heat treatment conditions (duration and temperature).
490
491   **Figure 7-6: Aluminum Sterilization**
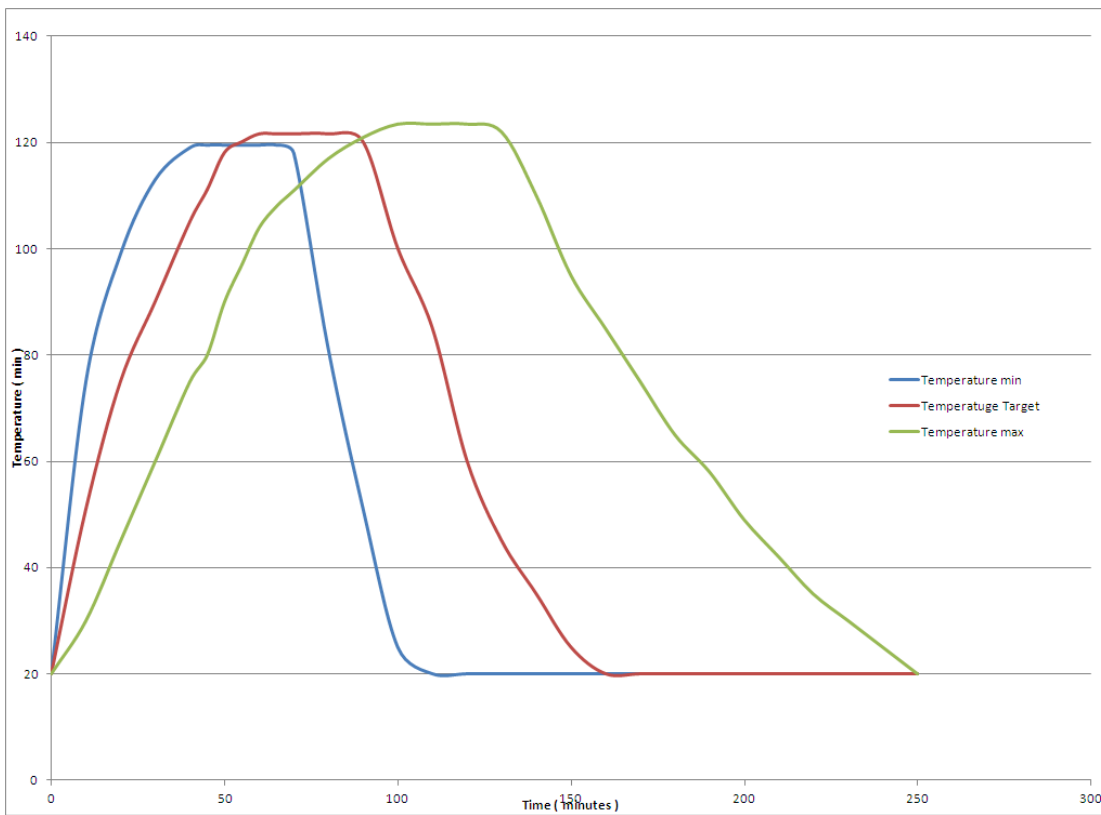
492
493

494   ## 7.8.   Scale-up Risk Assessment: FMEA Analysis

495   Moving into scale-up, additional learnings obtained from design space activities were applied
496   along with known scale-up risks to perform a higher-rigor-level risk assessment (FMEA).
497
498   ### 7.8.1.   Failure Modes and Effect Analysis

499   Following the completion of initial lab-scale DOE work and continued development into the
500   commercialization space, a second-stage risk assessment was conducted based on failure modes
501   and effect analysis (FMEA). The analysis leverages process understanding and the known risks
502   associated with different unit operations during the formulation and filling processes. The FMEA
503   aids in the determination of potential failures that could occur within the process and helps to
504   identify critical process parameters (CPPs). Once the CPPs are identified, adequate control
505   strategies can be utilized to ensure a robust process is achieved. For each unit operation, scores
506   of severity (S), occurrence (O), and detectability (D) are assigned. For the A-VAX study, the
507   scoring system is listed below (2).
508
509   **Table 7-22: Scoring System for FMEA**

Score	Severity	Occurrence	Detection
9 "HIGH risk"	Process failure potentially impacting one or more critical product quality attribute(s) leading to product rejection.	> 20% (very frequent)	No way to detect excursion. Not tracked and not alarmed.
7	Potential impact on product quality or consistency (e.g. product-related substances). Investigation needed prior to product release.	~ 5-20% (frequent)	Difficult to detect excursion, and not until after it has impacted the process.
5	No impact on product quality, but deviation from manufacturing procedures that requires justification. Likely deterioration in process performance attributes (e.g. yield) or ease of process operability.	~ 1-5% (occasional)	Excursion can be detected, but not until after it has impacted the process.
3	No impact on product quality. Potential for minor deterioration in process performance attributes (e.g. yield) or ease of process operability.	< 1% (rare)	Excursion is usually detected and corrected prior to impacting the process.
1 "LOW risk"	No impact to process performance attributes or product quality.	0% (never observed)	Excursion is obvious and always detected prior to impacting process.

510

Process Step	Key Process Inputs	Ranges Evaluated	Potential Failure Mode	Potential Failure Effects	SEVERITY	OCCURRENCE	DETECTION	Current RPN
What is the process step	What are the Key Process Inputs? (KPIV's)	What are the ranges expected to experienced during normal operations?	In what ways can Key Inputs go wrong? (Process fail to meet requirements)	What is the impact on the Key Output Variables (customer requirements) or internal requirements?	How Severe is effect ? (9,7,5,3,1)	How frequent is cause likely to Occur? 9, 7,5,3,1	How probable is Detection of cause? 9, 7, 5, 3, 1	Risk Priority # to rank order concerns
Raw Materials (DS)	Sucrose concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	pH	Set point: 6.5 Range: 5.5 - 7.5	Improper formulation of Buffer, or incomplete buffer transfer, pH probe calibration or pH instrument issues	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	NaCl Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Histidine Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Polysorbate Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, adsorption, recon time, potency	3	3	3	27
	Antigen Concentration		Problem with dilution, analytical testing issue, aggregation / degradation of DS	Potency, concentration, stability,	9	3	5	135
	Bag Volume	+/- 15%	Under filled bag	Potency, concentration, stability,	9	5	1	45
	Storage Temperature	Set point: 4ºC Range: 0 - 10ºC	Wrong with cold chain, shipping deviation, cold storage equipment deviation, wrong TOR reporting	Stability, potency	1	3	3	9
Formulation / Mixing	Mixing Time	Set point: 15 minutes Range: 10 - 20 minutes	Don't mix product, not mixed long enough, too long mixing time	Stability, potency, concentration	5	3	1	15
	Mixing Speed	Set at Point: 200rpm Range: 150rpm - 250rpm	Too fast or too slow, no stirring at all	Stability, potency, concentration, Free Ps,	5	3	1	15
	Mixing temperature	Set point: 4ºC Range: 0 - 10ºC	Too warm, too cold, loss of temperature control	Stability, potency, concentration	3	5	1	15
	Dilution Buffer Volume Added	+/- 15%	Weighing error, under filled bags, overfilled due to error, Calculation error for dilutions, Line losses	Concentration, potency, stability	7	3	5	105
	DS Volume added	+/- 15%	Weighing error, under filled bags, overfilled due to error, Calculation error for dilutions, Line losses	Concentration, potency, stability	7	3	5	105
	Dilution Buffer Sucrose concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer pH	Set point: 6.5 Range: 5.5 - 7.5	Improper formulation of Buffer, or incomplete buffer transfer, pH probe calibration or pH instrument issues	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Bufffer NaCl Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer Histidine Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Dilution Buffer Polysorbate Concentration	+/- 15%	Improper formulation of Buffer, or incomplete buffer transfer,	Improper formulation of DP, could impact product stability, moisture, cake appearance, adsorption, recon time, potency	3	3	1	9
	Loading temperature	Set point: -50ºC Range: -45 - -55ºC	loss of temperature control of cabinet, improper recipe	Stability and Potency	1	3	1	3
	Freezing time (Duration)	Set Point: 60 minutes Range: 50 - 70 minutes	too short	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	1	21
	Initial Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high	Cake Appearance, Moisture, Stability, Potency, Recon Time	1	3	3	9
	Ramp rate to 1º Drying	Set Point: 1ºC / minute Range: 0.5 - 1.5ºC / minute	too fast or slow ramp	None Identified	1	3	3	9
	Primary Drying temperature	Set Point: -10ºC Range: -15ºC - -5ºC	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Primary Drying duration	Set Point: 720 minutes Range: 660 - 780 minutes	too short	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Primary Drying Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	9	3	5	135
	Ramp rate to Secondary Drying	Set Point: 0.3ºC / minute Range: 0.1 - 0.5ºC / minute	too fast or slow ramp	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	5	105
	Secondary Temperature	Set Point: 30ºC Range: 25 - 35ºC	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	7	3	5	105
	Secondary Duration	Set Point: 600minutes Range: 540 - 660 minutes	too short or long	Cake Appearance, Moisture, Stability, Potency, Recon Time	5	3	5	75
	Secondary Pressure	Set Point: 100µBarr Range: 75µBarr - 125µBarr	too high or low	Cake Appearance, Moisture, Stability, Potency, Recon Time	3	3	5	45
	Stoppering Temperature	Set Point: 5ºC Range: 0 - 10ºC	too high or low	Sterility, Stability, Moisture	1	3	5	15
	Stoppering Pressure	Set Point: 800mBarr Range: 750 - 850mBarr	too high or low	None Identified	1	3	5	15
	Stoppering Gas	Nitrogen	wrong gas utilized, leak in gas line	Stability, Moisture	9	1	5	45
	Stoppering Force		too high or low	Sterility, Stability, Moisture	9	3	3	81
Aluminum	**Sterilization**							
	Temperature	Set-Point : 121.5 ºC Range : 119.5 - 123.5 ºC	too high (impact on Alum) or low (Impact on Sterility)	Sterility, Adsorption Capacity, PZC	9	3	1	27
	Duration	Set-Point : 30 min.	too short or long	Sterility, Adsorption Capacity, PZC	9	3	1	27
	pressure	Set-Point : 0.5 bar	too high (bursting disc) or low (risk of sterility failure)	Sterility	9	3	1	27
	Mixing time	Continuous mixing during heat./ster./cool.	too high (risk of shearing) or none (temperature non-homogeneity)	Sterility, Particle Size	9	3	1	27
	Mixing Speed	Scale dependent param. DOE-20L scale Set-Point : 210 rpm Range : 104 - 310 rpm	too high (risk of shearing) or low or none (temperature non-homogeneity)	Sterility, Particle Size	9	3	1	27
	Heating & Cooling time	Range : 100 - 250 min.	too long or short	Adsorption Capacity, PZC	3	3	1	9
	Distribution of Product							
	Mixing speed	Scale dependent param. DOE-20L scale Set-Point : 210 rpm	not enough speed to stop settling	Non-Homogeneity	7	3	1	21
	Mixing time	Min. 15 minutes	not enough time to ensure homogenous	Non-Homogeneity	7	1	1	7
	Speed of filling		Settling can occur if filling speed is too low	Non-Homogeneity	7	3	1	21

511

512   ## 7.9.   Scale-up Considerations and Site Transfer Activities

513   ### 7.9.1.   Formulation Scale-up Considerations

514   During early development activities, the formulation was shown to be highly robust as regards
515   the serotypes binding to the aluminum adjuvant. Two factors shown to have an impact on
516   binding (pH and sucrose concentrations) can be readily controlled during scale-up and
517   commercialization. As a result, scale-specific considerations are not expected to be high risk.
518
519   ### 7.9.2.   Freeze-drying Scale-up Considerations

520
521   A successful scale-up and transfer of a freeze-drying cycle imply that their performances are
522   equivalent between lab or pilot scale and industrial scale (i.e., that product temperature: time
523   profiles are identical). By performance, one should consider cycle robustness, cycle time, and
524   product quality (potency, residual moisture, dissolution time).
525
526   To ensure successful scale-up, several aspects need to be taken into account during cycle
527   development and process transfer. The ones listed below are a subset of the aspects that would
528   be examined during scale-up and process transfer:
529   •   Industrial process configuration: trays configuration, heat transfer map, sublimation rates
530   within the design space, door placement, temperature jacketed units, gas injection (single or
531   multi port, continuous), sensor type (Pirani vs. mks), and condenser location
532   •   Industrial equipment performance: choke flow, shelf temperature homogeneity, radiative
533   effects, and condenser capacity
534
535   Details of these considerations are available in the study guide appendix.

536   **7.9.2.1. Industrial Equipment Configuration Vs. Pilot:**
537   The basic rule is to ensure that all components in the process that influence the heat and/or
538   mass transfer characteristics, and therefore the sublimation rate during primary drying, are
539   identical between the pilot and the industrial scale. In other words, identify and implement
540   what can be identical between the pilot and the industrial scale. These components include:
541
542   •   **Freeze-drying trays (if any)**: Figure 7-7 below represents the level of heterogeneity in
543   overall vial heat transfer coefficient Kv, as a function of its location on an aluminum tray.
544   Vials on the edges of the tray can receive up to 60% more energy than vials located in the
545   middle of the tray. The level of heterogeneity can vary as a function of the tray's material
546   (aluminum, stainless steel, plastic) and its configuration (bottomless vs. standard).
547

548   **Figure 7-7: Heat Transfer Profile in Commercial Lyophilization Unit**
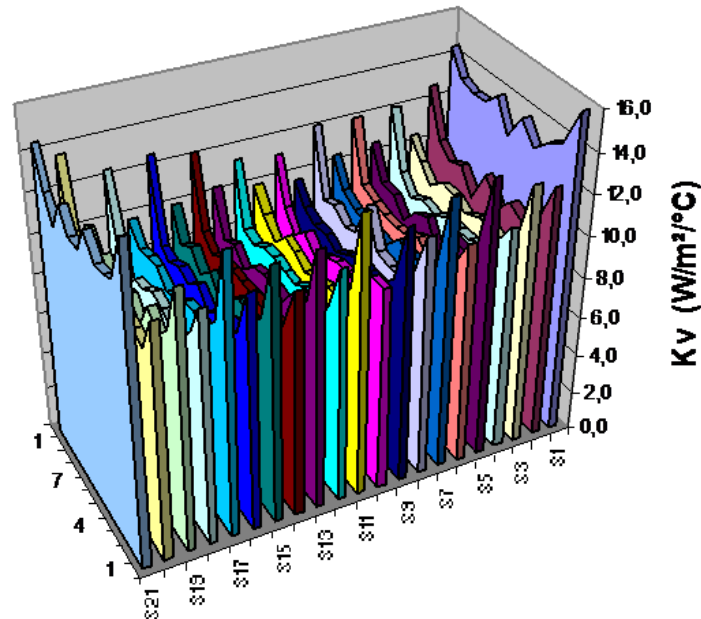
549

550   •   **Componentry:** As with trays, it is important to use identical vials during freeze-drying cycle
551   development because they can have very different overall heat transfer coefficient values
552   Kv, as demonstrated by Pikal et al. For example, important differences have been
553   demonstrated in the literature. (*Pikal M.J., Roy M.L., Shah S., 1984: Mass and heat transfer*
554   *in the freeze-drying of pharmaceuticals: Role of the vial. Journal of Pharmaceutical Sciences,*
555   *73, 1224–1237.)*

556   •   Similarly, stoppers used for development should be the same, and moisture uptake studies
557   at the lab scale should be done with residual moisture levels in the stopper equivalent to
558   what the industrial process delivers.

559   •   Design of the condenser: The position of the condenser (i.e., inside the freeze-drying
560   chamber vs. external condenser with a separating valve) can significantly impact mass-
561   transfer characteristics, and similar design should be used during development when
562   possible.

563   •   Two types of pressure gauge are commonly used for pressure control during primary and
564   secondary drying: Pirani type (heated wire sensor; reading is impacted by the gas
565   composition) and MKS type (capacitance sensor; reading independent of the gas
566   composition). Using the same type of sensor is critical because there is a ~1.6 ratio between
567   the two when atmosphere in the chamber is saturated with water vapor, which is the case
568   during primary drying.

569

570   **7.9.2.2. Industrial Equipment Performance Vs. Pilot:**

571

572   Equipment-imposed boundaries and intrinsic heat and mass transfer differences must be
573   identified, measured, and taken into account during cycle development. In other words, identify
574   and control what cannot be identical between the two scales. These parameters include:

575

576   •   Shelf temperature homogeneity is critical and is verified during commissioning of the
577   equipment. But it has also been shown that the difference between the shelf temperature
578   setpoint and the actual surface temperature of the shelves during primary drying can be
579   important and significantly different, as a function of scale and sometimes equipment itself.
580   Reported values in the literature are in the 2°C to 7°C range, as a function of sublimation
581   rates.

582
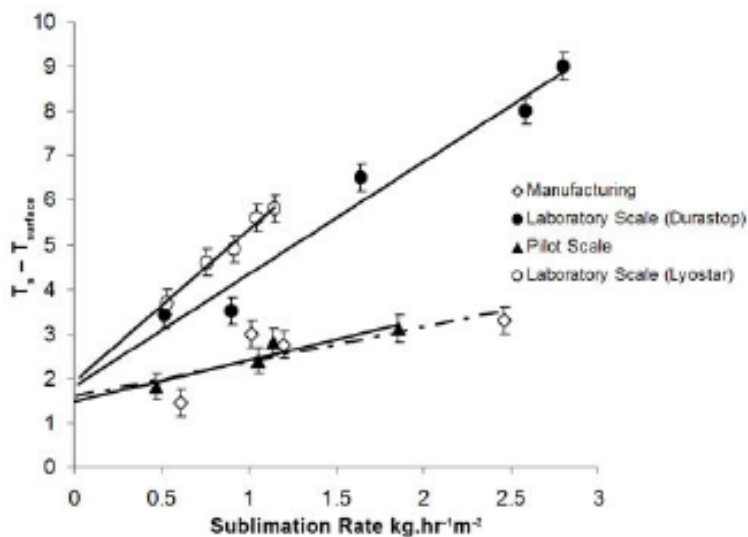
Figure 1. Difference between shelf temperature (T_s, fluid inlet) and shelf surface temperature ($T_{surface}$) obtained as a function of sublimation rate for different lyophilizers. Error bars represent estimated errors ($\pm 0.2°C$) in thermocouple measurements.

583

584   *AAPS PharmSciTech* 2006; 7 (2) Article 39 (http://www.aapspharmscitech.org).
585

586   •   Radiative contribution to overall sublimation heat transfer coefficient often depends on
587   equipment scale and design. This "edge effect" is mainly related to differences in chamber
588   parts emissivity values and potentially chamber wall temperature difference as a function of
589   equipment size and cooling technology used after sterilization. In most cases, radiative
590   contribution is higher in pilot scale equipment, leading to shorter primary drying times for
591   the identical freeze-drying recipe.
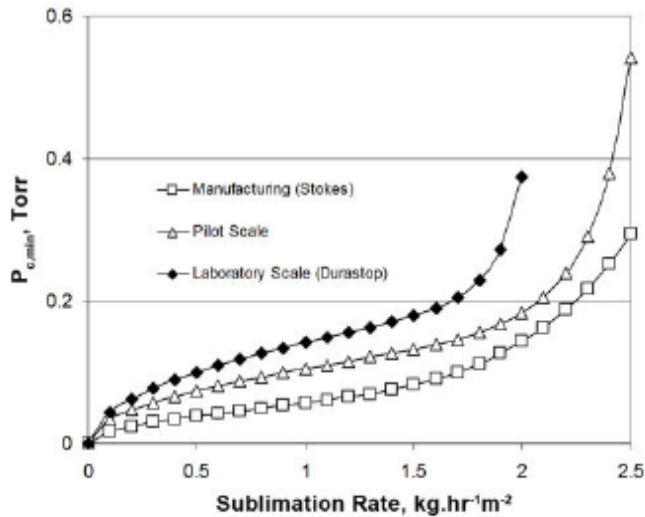
Figure 4. Comparison of minimum chamber pressure ($P_{c,min}$) as a function of sublimation rate.

592

594

595   Primary drying time should be carefully monitored during scale-up, and adequate safety margin
596   should be applied to primary drying time to compensate for this edge effect.
597

598   •   The choke flow corresponds to the maximum water vapor flow rate that can pass through
599   the spool toward the condenser. This value is a function of equipment design and pressure
600   and should be measured to ensure that for any cycle scaled-up and transfered to industrial
601   equipment, instantaneous sublimation rate is always lower than choke flow value at the
602   corresponding pressure, to avoid loss of pressure control in the drying chamber.

603   •   Similarly, maximum condenser capacity (expressed in $g.min^{-1}$) should be measured to secure
604   primary drying and avoid loss of temperature control of the condenser.

605   •   Freeze-drying cycle design should be compatible with heating and cooling performance of
606   the industrial equipment at full load.
607

608   The freeze-drying cycle should be robust enough to absorb all these intrinsic differences,
609   keeping the product temperature always below its collapse temperature throughout primary
610   and secondary drying.
611

612   7.9.3.   Lyophilization Process Scale-up and Transfer from Pilot Scale to Industrial Scale

613   *Industrial freeze-dryer characteristics vs. pilot scale*
614

615   Table 7-23 below compares the main characteristics of the industrial freeze-dryer in which the
616   product is transferred with the ones of the pilot scale equipment in which the lyo cycle was
617   developed.
618

619   **Table 7-23 Industrial Freeze-dryer Characteristics vs. Pilot Scale**

Characteristics	Pilot scale equipment	Industrial scale equipment
Shelf area	*1m2*	*40m2*
Batch size	*4,000 vials*	*160,000 vials*
Condenser	*External*	*External*
Trays	*Bottomless trays*	*No trays-direct contact- Automatic Loading System (ALS)*
Pressure gauge	Capacitance	Capacitance

620

621   During the cycle development, bottomless trays were used to mimic direct loading on the
622   shelves, and the same pressure gauge, same vials, and same stoppers were selected.
623
624   *Choke flow measurement in the industrial unit at 100 µbar*
625
626   Methodologies to accurately measure the choke flow in a freeze dryer are described in the
627   literature. As an example, a simple protocol is described by *Patel et al., Chemical Engineering*
628   *Science, Volume 65, Issue 21, 1 November 2010, pages 5716–5727*.
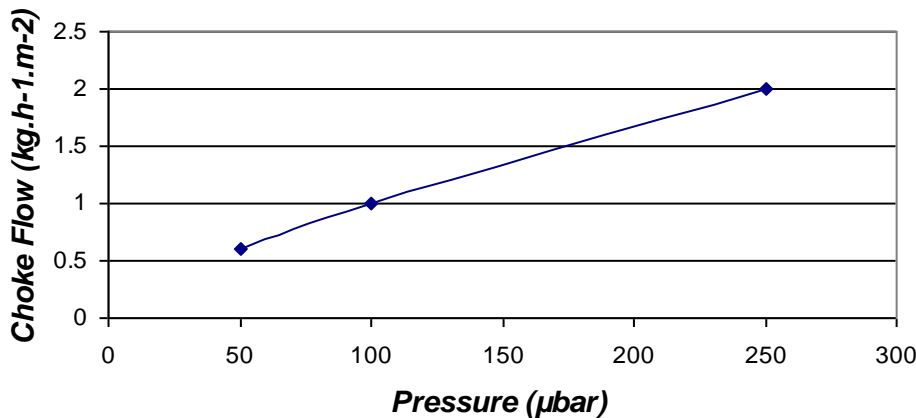
629
630
631   The choke flow for the industrial unit was measured at approximately 1 kg.h^{-1}.m^{-2} at 100 µbar,
632   the operating pressure of our freeze-drying cycle. This value is way above the 0.34 kg h^{-1}m^{-2}
633   calculated by the model at pilot scale and, therefore, choke flow was not considered as a
634   concern for our vaccine in this unit.
635
636   *Heat transfer measurement in the industrial unit at 100 µbar*
637
638   The table below gives the overall heat transfer values measured at 100 µbar as a function of vial
639   locations in the industrial unit; as a reminder, the Kv values obtained in the pilot unit are
640   reported in the right column:
641

Vial location	Kv, W.m-2.K-1 Industrial scale	Kv, W.m-2.K-1 Pilot scale
(a)	40.2±3.4	35.2±3.4
(b)	29.5±2.0	24.5±2.0
(c)	21.3±0.9	16.3±0.9
(d)	10.8±1.0	11.8±1.0
(e)	8.1±0.7	9.3±0.7

642
643   Moreover, the proportion of each vial location is changed in the industrial unit compared to the
644   pilot unit. It was evaluated that (a) location vials represent 0.05% of the total number of vials,
645   (b) location vials represent 5.2%, (c) represent 2.1%, (d) represent 9.4%, and (e) represent
646   68.2%.
647
648   *Freeze-drying cycle parameters adjustment for scale-up*
649
650   The design space was redefined for the industrial-scale process, taking into account these
651   difference in Kv values for different vial locations, and is represented in the figure below.
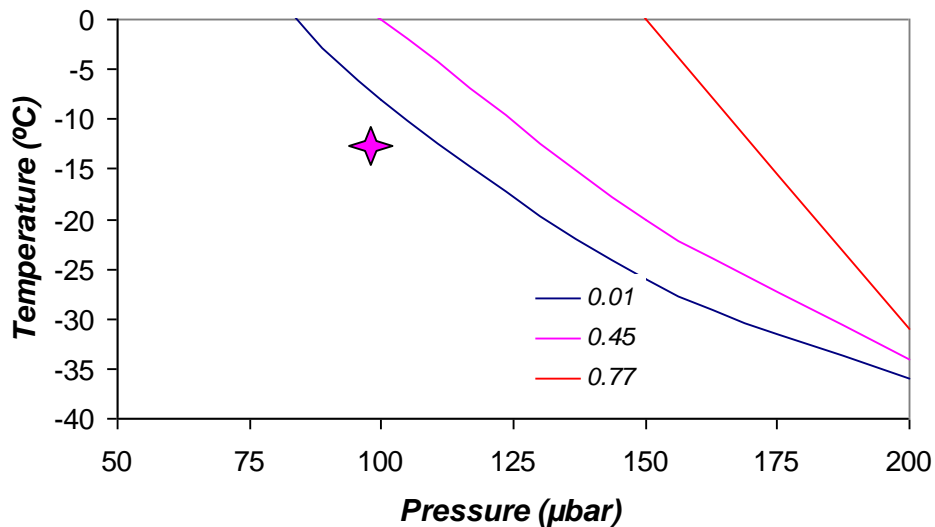
652
653   The shelf temperature was set 3°C lower compared with the pilot-scale conditions because of
654   higher Kv value of (b) location vials. The primary drying time was therefore increased to 960
655   minutes to compensate for the lower (e) location vials' Kv value, the decreased shelf
656   temperature value, and to include a 120-minute calculated safety margin. In these conditions,
657   the calculated maximum flow rate during primary drying is equal to 0.32 kg.h^{-1}.m^{-2} and remains
658   far below the choke flow of the industrial equipment. Choke flow is therefore not a concern for
659   this process.
660

661   The selected cycle for the industrial-scale process is given in Table 7-24 below:

662   **Table 7-24: Industrial-Scale Lyophilization Cycle for A-VAX Vaccine**

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-Load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-13ºC
Primary Drying Time	960 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	4ºC
All conditions during lyophilization utilized 100 µbar.	

663

664   *Freeze-drying process scale-up*

665

666   Engineering runs at full scale were performed prior to process qualification and validation to
667   check for cycle suitability at industrial scale. In some cases, the active ingredient is not available
668   and a proper placebo has to be identified. This is the case for our cooties vaccine; a placebo
669   formulation containing (everything but active ingredient) was characterized and demonstrated
670   similar freeze-drying characteristics as the actual drug product: glass transition at maximum
671   cryoconcentration Tg', collapse temperature Tc, and resistance to mass transfer Rp as a function
672   of dry-layer thickness during primary drying.

673

674   The following attributes were measured during these runs; additional attributes may be
675   measured as well during transfer (i.e., product temperature, pressure):

676   •   Actual primary drying duration vs. setpoint for primary drying duration $\delta t_{sublimation}$: It was
677   determined considering completion when the Pirani value meets and equals the capacitance
678   value. An example is described in the figure below:
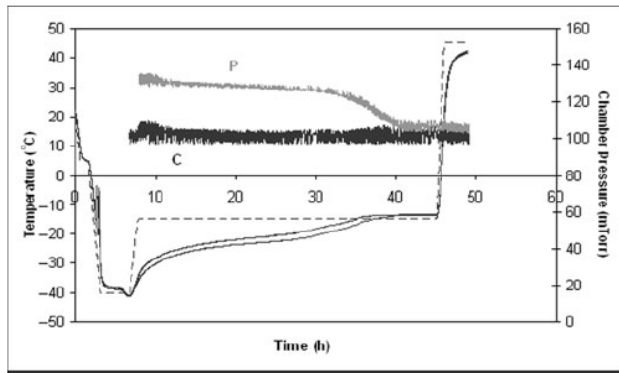
Figure 1. Temperature and pressure profiles of a freeze-drying cycle. The broken line represents the shelf setpoint temperature, the smooth lines represent thermocouple product temperatures, and line P and line C represent the chamber pressure measured by Pirani gauge and capacitance manometer, respectively.

679
680 Controlled nucleation in freeze drying: effect of pore size in the dried product layer, mass
681 transfer resistance, and primary drying rate. Konstantinidis et al. 2011. J. Pharm. Sci. Apr 4.

682 • Residual moisture: samples were taken at corners and center of each shelf

683 • Cake appearance and associated rejection rate

684

685 Results are gathered in Table 7-25 below:

686

687 **Table 7-25: Scale-up Results**

	δtsublimation (min)	Residual moisture (%±σ)	Rejection rate %
Engineering run 1	45	0.7±0.4	0.8
Engineering run 2	60	0.8±0.3	0.4

688
689 Engineering runs were successful and confirmed the cycle adjustment performed with the
690 model. Primary drying time was about one hour longer than calculated, but the remaining safety
691 margin was considered acceptable to proceed with process validation.
692

693   ## 7.9.4. Adjuvant Scale-up Considerations

694
695   Sterilization will occur through jacketed vessel (no direct steam injection to avoid product
696   dilution). Design of the vessel must guarantee efficiency of SIP process.
697
698   Characterization of aluminum particles' rheological properties (mass per unit volume, apparent
699   viscosity, settling velocity) have allowed appropriate impeller configuration selection to
700   guarantee homogeneity through mixing. It also helps to build scale-up models regarding
701   agitation. Particularly, it will allow defining per vessel size a minimal mixing speed for
702   homogeneity. The scale model for the sterilization vessel is discussed below, and aided in
703   defining a scale-independent process.
704
705   The vessels at intermediate scale (DOE scale) (20 L) and commercial scale (500 L) are in
706   geometric similitude. That means that they have the same shape, one being a uniform scaling
707   (enlarging or shrinking) of the other; in other words, the ratio of all corresponding dimensions is
708   equal. Main characteristics of the vessels are:

709   • Torispherical bottom
710   • 1 axial flow impeller
711   • No baffles
712   • H/D = 1
713   • d/D = 0.4
714   • Y/D = 0.2
715

Geometry parameters
- Vessel diameter : D
- Liquid height : H
- Impeller diameter : d
- Impeller clearance : Y
- Impeller speed : N

Scale-Up/Scale-Down parameters
- Liquid height : $\frac{H}{D}$
- Impeller diameter : $\frac{d}{D}$
- Impeller clearance : $\frac{Y}{D}$

716   **Scale-up of agitation**
717   **speed:**
718   The scale-up is performed
719   at constant volumetric
720   power dissipated in the
721   vessel (P/V). It allows to
722   reproduce at both scales the particle attrition and breakage rate resulting from fluid stress and
723   mechanical impacts of the particles (mainly particle-impeller collisions). In turbulent regime, the
724   power dissipated by the mixing in the liquid is given by:
725

726
$$P = \rho\ Np\ N^3\ d^5$$

727
728   Where:
729   • P is the dissipated power (W)
730   • ρ is the density (kg/m3)
731   • Np is the power number (-), Np = 0.32 for our axial impeller
732   • N is the agitation speed (rps)
733   • d is the impeller diameter (m)
734
735
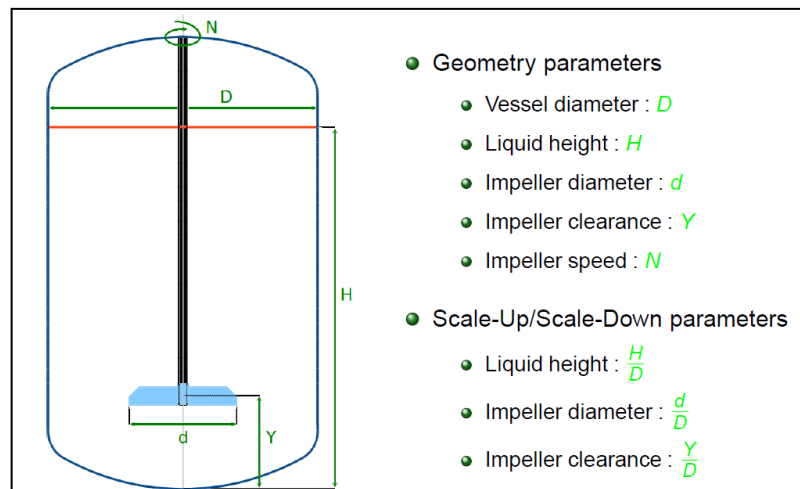
736 **Calculation of minimal agitation speed:**
737
738 For the DOE and for the process operated at large scale, the suspension must remain
739 homogeneous during sterilization. The minimal speed required for homogeneous suspension is
740 measured at small scale; the extrapolation to larger scale uses the Grenville law (one level of
741 impeller).

$$N_{min} * cst = N_{js} = \frac{x'}{N_P^{\frac{1}{3}} D^{\frac{2}{3}}} \left(\frac{g \Delta \rho}{\rho}\right)^{0.5} X_v^{0.141} d^{0.166} \left(\frac{Y}{D}\right)^{0.243}$$

742
743
744 Where:
745 • Njs is the minimal speed to get just suspended particles; nonhomogeneous (rps)
746 • x' is a constant depending of the impeller type (-)
747 • Np is the power number (-)
748 • D is the vessel diameter (m)
749 • d is the impeller diameter (m)
750 • Xv is the solid fraction (-)
751 • Y is the distance of the impeller from the bottom (m)
752
753 For equipment in geometric similarity, this law can be simplified to:
754
755 Nmin ~ D $^{-0.5}$.
756
757

758   Experimental studies confirmed this dependency on scale-up; the experimental curve is shown
759   below.
760
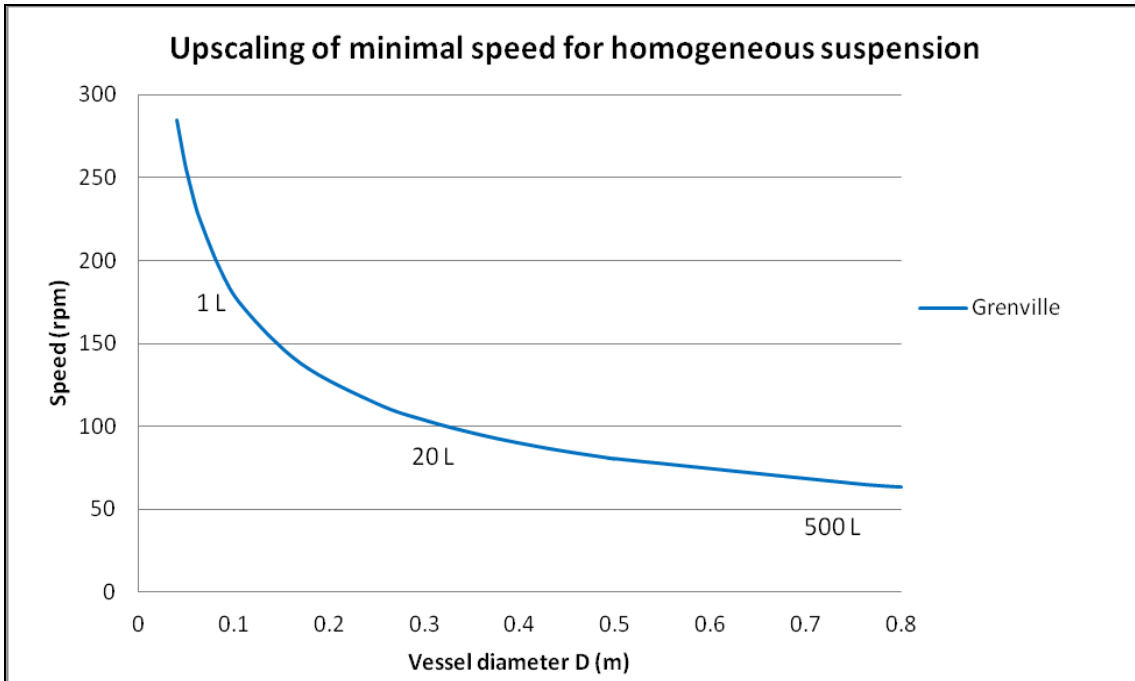
761
762
763   **Thermal transfer feasibility check**
764   The thermal transfer is scaled up maintaining a constant volumetric heat transfer rate (Q/V).
765   $Q = U\,A\,\Delta T$
766   • Q   = heat transfer rate (W)
767   • ΔT = temperature difference (K)
768   • A   = heat transfer area (m2)
769   • U   = overall heat transfer coefficient (W/m2K)
770   The overall heat transfer coefficient takes into account the convective resistance of the jacket,
771   the resistance of the vessel wall, the fouling of the jacket and vessel surface, and the convective
772   resistance of the process. In most applications, the heat transfer rate from the process side is
773   the limiting step (convection in the vessel).
774

775   Then :
776          $Q = h_{process}\,A\,\Delta T$
777   where $h_{process}$ is the heat transfer coefficient on the process side.
778
779   It can be shown that for a stirred vessel, with double jacket:
780          $h_{process} \sim Re^{2/3}/D$
781   Re being the Reynolds number
782
783   => $Q/V \sim (Re^{2/3}\,A\,\Delta T)/(D\,V)$
784

785 For equipment in geometric similarity, with a same fluid, the expression is simplified to:
786      $Q/V \sim (N/D)^{2/3}$
787
788 It was shown at 20 L scale that an agitation speed of 10 rpm was sufficient to assure a
789 nonlimiting heat transfer during sterilization.
790
791 ### 7.9.5. DOE Range

792 • The minimal value is fixed to assure that the aluminum suspension is homogeneous in the
793   vessel.
794 • The maximal value is calculated to reproduce the maximal shear produced at large scale.
795 a. **Minimal speed of DOE:**
796   The minimal speed required to get homogeneous suspension was measured at 1 L scale
797   (D= 0.11 m) and is 170 rpm.
798   => the extrapolation (see law above)

799   o   to 20 L scale (D = 0.30 m): Nmin = 104 rpm
800   o   to 500 L scale (D = 0.88 m): Nmin = 81 rpm

801
802 b. **Maximal speed of DOE:**

803
804   The maximal speed is calculated to cover the maximal particles' damages encountered
805   at commercial scale; this is a function of P/V.
806
807   Commercial scale:
808   The existing 500 L vessel has a maximal speed of 150 rpm.
809
810   =>   Pmax = ρ Np N^3 d^5 = 27 W
811   Pmax/V = 54 W/m3

812
813   20 L scale: P/V = 54 W/m3 => P = 1.1 W
814

$$N = \left[\frac{P}{Np * \rho * d^5} \right]^{1/3} * 60$$

815   =>   = 310 rpm
816
817 c. **DOE range:**

818   104 < N < 310 rpm
819
820   Reference (target) mixing speed for the DOE is placed at the middle of the range
821   (210 rpm).
822

7.9.6. Extrapolation of the Optimal Speed Determined by DOE

The extrapolation of the optimal speed determined at 20 L scale to the commercial scale is performed at constant P/V using the formula:

$$P = \rho \, Np \, N^3 \, d^5$$

Example:

- If the optimal speed in the DOE is 210 rpm

    =>   P = 0.3 W
              P/V = 17 W/m3

    At 500 L scale: P/V = 17 W/m3

    =>   P = 8.5 W

$$N = \left[\frac{P}{Np * \rho * d^5} \right]^{1/3} * 60 = 103 \text{ rpm}$$

- Homogeneity check:
  This speed is superior of the minimum speed required to maintain the suspension as homogeneous (81 rpm). The setpoint can then be fixed at 100 rpm.

- Thermal transfer check:
  $Q/V \sim (N/D)^{2/3}$

  It was shown at 20 L scale that an agitation speed of 10 rpm was sufficient to assure a nonlimiting heat transfer during sterilization. Extrapolation to 500 L scale at constant Q/V:
  $N_{500L} = N_{20L} * D_{500L}/D_{20L}$

  $_{=>} N_{500L} = 10*088/0.3 = 30$ rpm

  The setpoint of 100 rpm is superior to this lower limit.

7.9.7. Adjuvant Scale-up Transfer:

Confirmation runs were performed with same steps' duration and mixing speed defined by the scale-up model.

Homogeneity was checked by temperature profiles in different points of the vessel.
Homogeneity is also checked by Alum sampling and Al content measurement (+ turbidity as IPC).

862   ## 7.10. Control Strategy

863   ### 7.10.1. Parameter Criticality Assessment

864   Critical parameters were identified using a Pareto-type analysis of the FMEA results. The RPN
865   cut-off for criticality was selected at an RPN of 60, above which parameters would be considered
866   critical. In addition, all parameters with RPN <60 and a severity score of 9 were considered well-
867   controlled CPPs. The outcome of this assessment is shown below:

868

869
870

871   **Table 7-26: Operating Ranges for CPPs**

Parameter	Classification	Control Limits	Proven Acceptance Ranges	Control Strategy
Lyophilization Primary Drying Duration	CPPs	> 960 minutes	960 minutes	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Primary Drying Pressure		75–125 µBarr	50–150 µBarr	
Lyophilization Primary Drying Temperature		-11ºC to -15ºC	-5ºC to -15ºC	
Raw Material DS Ag Concentration		1.35–1.65 mg/mL	1–2 mg/mL	CoA, downstream process controls
Formulation Mixing/ Dilution Buffer Added		+/- 5%	NA	Batch record, gravimetric checks
Formulation Mixing/DS Added		+/- 5%	NA	
Lyophilization Ramp Rate to Secondary Drying		< 0.5ºC/min	0.1–1.0ºC/min	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Secondary Drying Temperature		28ºC–32ºC	25ºC–35ºC	
Lyophilization Stoppering Force		> 1,000 psi	NA	Equipment setup and routine preventative maintenance and qualification
Lyophilization Secondary Drying Duration		> 600 minutes	480–720 minutes	Lyo cycle automation and recipe selection, alarms, in-process monitoring
Lyophilization Stoppering Gas	WC-CPPs	Nitrogen	Nitrogen	Equipment setup, facility design
Raw Material DS Bag Volume		+/- 5%	NA	Batch-record calculations, CoA, container labels, gravimetric checks, downstream dispensing controls
Aluminum Sterilization Duration		30 minutes	NA	Batch-record procedures/eqt PID
Aluminum Sterilization Mix Speed		100 rpm	80–150rpm	Batch-record procedures
Aluminum Sterilization Mix Time		Defined by PID	NA	Eqt PID
Aluminum Sterilization Pressure				
Aluminum Sterilization Temperature		121.5ºC	199.5ºC–123.5ºC	Batch-record procedures/eqt PID

872

873   ## 7.11. Comparability Protocols for DP Lyophilization Site Change

874   ### 7.11.1. Introduction

875   It is anticipated that during the post-file life cycle of A-VAX, the site of drug product
876   manufacturing will be changed. The purpose of this comparability protocol is to describe the
877   process demonstrations that will be required to support such a change, specifically for the
878   lyophilization process. Other process changes or quality system reviews potentially associated
879   with a change in lyophilization site or equipment are out of scope. The purpose of this protocol
880   is to describe the scientific justification for the change, not necessarily the regulatory mechanics
881   to support the change. In actual execution, this approach could be supported through multiple
882   protocols.
883
884   ### 7.11.2. Description of a Planned Change

885   The definition of a site change will range from the addition of similar lyophilization units in the
886   current facility to transfer of the product to a new or existing facility in the same or different
887   location with either comparable or noncomparable lyophilization units, which may or may not
888   include process changes to maintain comparable product quality. In the case of a new facility,
889   the reporting categories suggested may not apply because of the need for quality system
890   reviews.
891
892   Site changes can be executed for a range of reasons, including:
893   •   Enable manufacturing flexibility in multiple units
894   •   Increase manufacturing capacity
895   •   Support local manufacturing in emerging markets
896   •   Distribute capacity to balance facility utilization across manufacturing network
897   •   Increase reliability/uptime
898   •   Improve/maintain existing equipment
899

900   **Table 7-27: Lyophilization Cycle Description**

Lyophilization Stage	Initial Cycle
Loading/Freezing Temperature	-50ºC
Freeze Time Post-load	60 minutes
Ramp to Primary Drying	1ºC/minute
Primary Drying Temperature	-10ºC
Primary Drying Time	960 minutes
Ramp to Secondary Drying	0.3ºC/minute
Secondary Drying Temperature	30ºC
Secondary Drying Time	600 minutes
Final Stage Post-secondary Drying	5ºC
All conditions during lyophilization utilized 100 µbar.	

901
902   The potential impact of the lyophilization process on critical quality attributes is described in the
903   attached risk assessment (see cause-and-effect matrix). A statistically designed experiment was
904   executed based on this risk assessment, and it concluded that the primary impacts of the
905   lyophilization cycle were on moisture and reconstitution time quality attributes.
906
907   7.11.3. Justification of Equivalency

908   The information required to support equivalency for the site changes described above will be
909   determined based on lyophilization and equipment performance comparability, with the
910   information required increasing with decreasing comparability, as shown in Figure 7-8. In the
911   scenarios outlined in Figure 7-8, the rationale for completing only one process validation lot is
912   based on knowing that during routine manufacturing, additional data would be captured and
913   utilized to monitor performance. This would be part of the continuous verification process.
914

915   **Figure 7-8: Equivalency Demonstration Decision Tree**
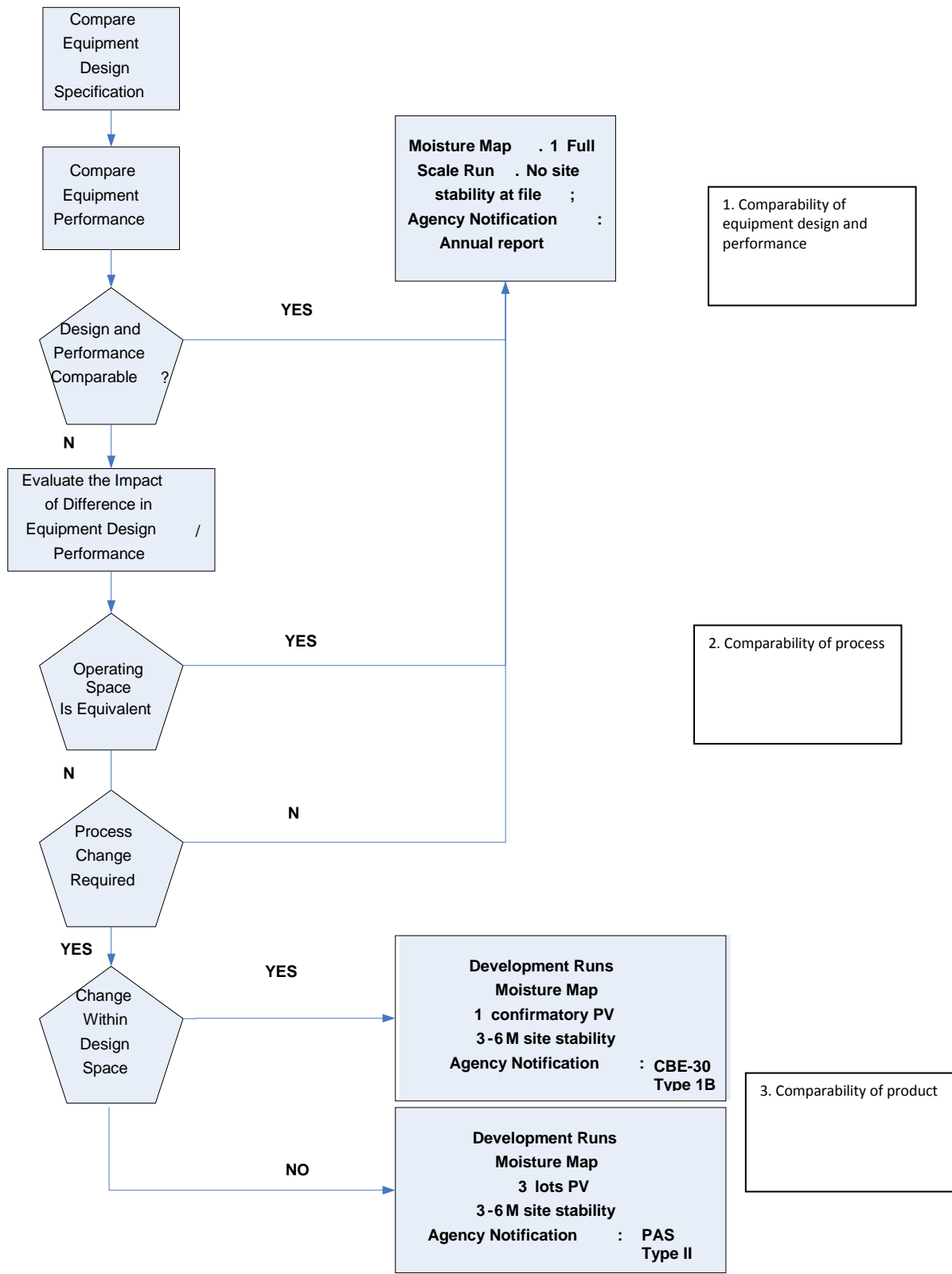
916
917

918 **Equipment Design Specification Comparability**
919 The equipment design specification comparability will be determined by a detailed evaluation of
920 various equipment elements that are known to impact lyophilization performance. This may
921 include the following:

922 • Manufacturer

923 • Automation system and system architecture

924   – SCADA sampling

925 • Chamber design

926   – Material of construction of internal shell, similar finish

927   – Dimensions/volume

928   – Door placement (mechanical, loading slot, etc.)

929   – Post-SIP cooling mechanism (jacketed or not jacketed)

930   o Insulative controls

931   o Consistency/limits

932 • Pressure control mechanism

933   – Gas injection (single or multiple point, continuous, location)

934   – Capacitance manometer vs. Pirani gauge, location

935 • Shelf design

936   – Number of shelves

937   – Use/type of trays

938   – Surface finish (similar)

939   – Number of trays/product vials per shelf

940   – Loading sequence (by row or tray/shelf)

941   – Shelf construction material

942   – Spacing between shelves

943   – Distance between silicone oil in shelves and vial (shelf thickness)

944   – Flow pattern/rate of flow of silicone oil in shelves, flow meter/control

945   – Working shelf area

946   – Shelf-by-shelf cooling capability

947   – Counter plate at top of chamber

948 • Shelf temperature control

949   – Heat transfer fluid used for shelf temperature control

950   – Location of probe for shelf temperature control

951   – Temperature control mechanism, algorithm, design

952 • Condenser configuration

953   – Above, below, beside

954   – Isolation valve (diameter, length, type)

955   – Deflector design

956   – Spool piece design (diameter, length)

957   – Construction type (coil, plate, internal, external)

958   – Maximum ice capacity (kg/)

959   – Ratio of usable shelf surface to ice capacity

960   – Number of compressors

961   – Refrigeration system type

962   – Cooling mechanism, compressor type

963   – Number of coils

964   – Backup system

965   – Number of vacuum pumps

966   – Number of vacuum boosters

967

968   There is a very broad range of potential equipment designs and possible differences in
969   specifications. Because of this range, the specification evaluation will need to be risk based and
970   dependent on the magnitude of the difference observed and the potential impact to process
971   parameter control and product quality. If significant differences in the equipment design are
972   identified, the design specifications will be deemed to be noncomparable and an equipment
973   performance evaluation will be conducted.

974

975   **Equipment performance evaluation**

976

977   As described above, if the equipment design is deemed to be noncomparable, a more detailed
978   comparison of the equipment performance must be performed. This shall include statistical
979   comparability of the following:

980

Performance Comparison	Acceptance Criteria
Pressure Control	+/- 10 ubar
Temperature Control	+/- 1°C
Chamber Leak Rate	<25 ubar-L/sec
Condenser Ice Capacity	> Reference cabinet
Shelf Temperature Uniformity	+/-1°C between and across all shelves
Heat Flux Studies	Range of target facility inside range of current facility

981

982   Again the assessment of comparability for equipment performance should be risk based,
983   including an assessment of the magnitude of difference and potential impact on process
984   parameters and product quality. This will include an assessment of the impact on CQAs and
985   determination of the necessity for a process change. If significant differences in these elements
986   are observed, the equipment performance will be deemed to be noncomparable and a process
987   change evaluation will be conducted.

988

989   **Process change evaluation**
990
991   If equipment performance is deemed to be noncomparable, an assessment will be performed to
992   determine whether a process change is required to accommodate the change in performance.
993   The approach to evaluate process changes will depend on the difference observed.
994
995   If the change in performance is observed in temperature control/uniformity, pressure control,
996   or heat flux, the change will be evaluated using the first principles mathematical model
997   described in the tech transfer section of the document. Using this approach, product
998   temperature and moisture responses can be predicted based on the observed differences in
999   temperature, pressure, or heat transfer.
1000
1001 If the change in equipment performance compared with the existing facility is small enough that
1002 it will not have a significant impact on the ability of the process to deliver product within defined
1003 the specifications and design space identified, no changes will be made to the process. A
1004 development run will be performed to confirm acceptable product performance, followed by a
1005 single process validation lot to demonstrate process/product comparability; this will include full
1006 CQA testing per release, extended characterization protocols, and three months of stability
1007 data. Additional data will be collected as manufacturing lots are completed. The data will be
1008 utilized for continuous verification that the process and site-to-site changes are acceptable.
1009
1010 If the change in equipment performance compared with the existing facility is large enough to
1011 suggest a process change outside the design space based on scale-down model predictions,
1012 development runs will be performed. The runs will support the new process prior to execution
1013 of a full series of three process validation lots to demonstrate process/product comparability,
1014 again including full CQA testing per release, extended characterization protocols, and three
1015 months of stability data.
1016
1017 7.11.4. Proposed Regulatory Reporting Categories
1018
1019 The present example of lyophilization DP site change can be submitted as part of an initial
1020 marketing authorization application (as Post-approval change management plan/protocol) or
1021 submitted after licensure as a change management/comparability protocol. The current change
1022 is an example that can be managed via a comparability protocol, which has been written to be
1023 independent of the manufacturing location; in this way, subsequent sites can be introduced
1024 with reference to the same comparability protocol using lower reporting categories.
1025
1026 With the application of QbD, the expanded process and product understanding serve to support
1027 the sponsor's ability to assess the change according to the decision tree and apply a risk-based
1028 approach as described above (process change evaluation).
1029 (Under the paradigm for post-approval change, the introduction of a new facility for a previously
1030 approved product requires regulatory review and approval.)
1031
1032 This type of change generally poses little risk of impact on product quality when the
1033 manufacturing site is a multi-product facility with established quality systems and a successful
1034 inspection history. In the European Union, a new secondary manufacturing site can be
1035 introduced without a specific product-related preapproval inspection. This is the case provided
1036 that the site is authorized for the type of pharmaceutical form and a manufacturing

1036   authorization and/or GMP certificate is provided with the application. A possibility to waive the
1037   Pre-Approval Inspection (PAI) is based on successful inspection history or recent PAI for a similar
1038   type of product.
1039
1040   Therefore, provided the inspection status is compliant and a comparability protocol has been
1041   approved, it could be expected that for the introduction of each new site, the following
1042   reporting categories might be proposed:
1043   1. In the case the comparability assessment confirms the equipment design or performance is
1044   comparable, the change falls within the initial design space. Therefore, the change could be
1045   reported as a minor notification (EU-Type IB; US-Annual report). This will be the case if the
1046   equipment performance difference is small enough that it will not require a significant
1047   change to deliver product within defined specifications and therefore no process changes
1048   will be necessary. For intra-site changes, if the site has already been approved for the EMA,
1049   no notification would be necessary and changing equipment within a site would be possible.
1050   2. In the case of a process change, but where the process is comparable and remains within
1051   the design space, the change could be reported as a minor notification with agency review
1052   (EU-Type 1B; US-CBE-30).
1053   3. In the case of a significant process change moving outside the design space, the change
1054   would be submitted as a regular variation (EU–Type II; US–PAS) and a modification to the
1055   design space/protocol should be considered.

7.11.5. Long-term Protocol Maintenance

1057   •   Update and/or withdraw this comparability protocol should the protocol become obsolete
1058   as a result of changes in the regulatory environment, identification of a new safety or
1059   scientific issue, and/or changes in technology.
1060

8.   Regulatory Section

1061

1062   The regulatory environment for incorporating design space into regulatory filings for vaccines is
1063   expected to evolve in coming years as regulators and vaccine companies gain more experience.
1064   This section of the case study explores the application of Quality by Design (QbD) concepts to
1065   the content of regulatory filings. These examples were developed in the absence of significant
1066   precedents; the applications will continue to evolve as experience is gained. The regulatory
1067   section concludes with a section on future challenges.
1068
1069   The section was created to introduce topics where there is tremendous potential value from
1070   applying the principles. However, there are also enough unanswered questions that it is
1071   important to emphasize the fluid and exploratory nature of the discussion. The additional
1072   product knowledge gained through the application of QbD concepts is expected to: 1) provide
1073   more strength to the data set supporting operational ranges and control strategy elements
1074   described for the product; and 2) justify management of change in a manner that increases the
1075   assurance of maintaining product quality while ensuring appropriate assessment across the
1076   spectrum, from gaining full prior-approval board of health review to empowering companies'
1077   quality systems to oversee that change.
1078
1079   To utilize product and/or process knowledge captured in the design space, the design space
1080   must be captured in the regulatory filings and approved. Given the limited experience to date in
1081   managing change in the context of a design space, to accomplish this in the EU and US filings
1082   today, a change management plan could be submitted to clarify the anticipated treatment of
1083   changes envisioned for the product life cycle. Examples are provided.
1084
1085   The case study is a scientific document addressing the application of Quality by Design to
1086   vaccine development and product life cycle management. It is intended to serve as an example
1087   of potential ways that scientific principles and tools described under ICH documents Q8, Q9,
1088   Q10, and Q11 could be applied seamlessly during vaccine development and through post-
1089   approval life cycle management. The examples have been created as a teaching tool and as an
1090   opportunity to encourage stakeholder discussions on the application of these concepts.
1091
1092   These examples are not presented as a mock submission, nor is there any expectation that the
1093   combination of illustrative examples would represent a realistic filing. The scientific principles
1094   are discussed and data are provided to demonstrate how the assignment of quality attributes,
1095   conduct of risk assessments, performance of experiments, and development of design space
1096   and control strategy could be utilized in regulatory filings to enhance the depth of product
1097   knowledge, increase the robustness of process control, and facilitate continuous improvement.
1098   We have indicated what data could be presented to support the analysis, where summary
1099   information is appropriate, and how the data would be analyzed in each of the process sections.
1100
1101   The focus of discussion in this document is on US and EU approaches. There are potential
1102   applications in multiple other regions; however, they are not addressed given the regional
1103   regulations.

1104

1105   This section will address the following regulatory aspects:

1106   • Incorporating prior knowledge and design space information into initial regulatory filings.

1107   • Applying the scientific principles behind the FDA PV guidance throughout the product life
1108   cycle. Proposals for change management are based on existing precedents and exploration
1109   of emerging opportunities.

1110

1111   8.1.   Assessing Change Within the Context of the Life Cycle of a Vaccine

1112   Throughout the development and commercial phases of a vaccine's life cycle, changes in the
1113   starting materials, manufacturing process, process control strategy, and analytical control
1114   strategy are inevitable. Drivers for these changes may include external influences, such as
1115   availability of material supplies and new technologies, and internal influences such as a need to
1116   improve productivity, decrease variability, or respond to changes in a company's supply
1117   network.

1118

1119   The spectrum of changes and the reasons for them are similar throughout all of the
1120   pharmaceutical and biotech industry, across small molecules, biotherapeutics, and vaccines.
1121   However, the implications of such changes and the tools employed to manage and assess the
1122   impact of these changes vary significantly between these product classes. Boards of health have
1123   generated specific guidance (or detailed sections within guidance) pertaining to these subclasses
1124   individually.

1125

1126   Generally speaking, the requirements for managing and assessing changes for vaccines have
1127   been among the most restrictive or conservative. The reasons for this conservative stance
1128   include the diversity of products in the class, the complexities of their manufacturing processes,
1129   the challenges of analytical characterization of the drug substances and products, limited
1130   specific knowledge of mechanisms of action, and a high demand for safety given that vaccines
1131   are typically given to healthy individuals and often to infants.

1132

1133   The expected contribution from this case study to the field of vaccine development is to
1134   illustrate how application of product and/or process knowledge as captured in the enhanced
1135   process understanding, design space, and control strategy can enhance continuous
1136   improvement, change management, and the assurance of product quality.

1137

1138   A robust process development program will study the effects of variation in material inputs,
1139   independent process parameters, and upstream quality attributes. These variables will have
1140   been assessed on the basis of their effect on the downstream process parameters, intermediate
1141   quality attributes, and critical quality attributes (CQAs) of the drug substance and drug product.
1142   This development program will drive the definition of design space, process control strategies,
1143   and analytical control strategies. The availability of the enhanced data set provides the
1144   underpinning for improved life cycle management.

1145

1146   Among the most significant contributions and benefits of QbD are decreasing the potential for
1147   unanticipated impact on CQAs and more objectively (less subjectively) defining the ranges for
1148   critical process parameters (CPPs) and non-CPPs.

1149

1150 ### 8.1.1. Changes During the Development Phase

1151 Throughout vaccine development, there will be changes made to the manufacturing process,
1152 including the modification of processing steps, scale-up of unit operations, and revisions to
1153 formulation and container-closure systems. While these must be handled on a case-by-case
1154 basis, data gathered at a smaller scale make a significant contribution to the design of protocols
1155 to demonstrate product comparability. For those operations where product and/or process
1156 understanding has sufficient depth, it may be possible to build arguments for utilizing analytical
1157 and nonclinical bridging data in lieu of collecting clinical bridging data.

1158

1159 During development, companies describe a manufacturing process and control strategy in an
1160 investigational filing (IND, IMPD, or equivalent document in other countries as required) and,
1161 depending on the significance of a change, report changes as development continues if required
1162 by the boards of health. As these changes would be followed up with additional testing in
1163 clinical trials, where safety and immune response, or even efficacy, are subsequently evaluated,
1164 generally the burden of proving comparability before and after a change at this stage is
1165 relatively low. Indeed, companies' concerns about observing clinical results inconsistent with
1166 earlier preclinical and clinical findings or confounded with the main objectives of the clinical
1167 study discourage companies from making large changes during this phase of the life cycle. The
1168 concerns drive companies to lock down major product and process design decisions relatively
1169 early in the development of vaccines compared with other product classes.

1170

1171 The case study provides examples of changes that could be justified largely through design
1172 qualification, process evaluation, and product characterization. In some situations, the
1173 subsequent clinical data are robust with respect to yielding acceptable clinical response even
1174 after moderate process changes and variability in the CQAs that are used to characterize the
1175 vaccine product. In such cases, the robustness to process change begins to illustrate that the
1176 historical paradigm for vaccine development that "… the product is the process …" can, in fact,
1177 within at least some ranges and for some moderate changes, be shown to be overly
1178 conservative.

1179

1180 ### 8.1.2. Post-approval Changes

1181 Companies are responsible for assessing, prior to distribution of a product, the effect of any
1182 post-approval chemistry, manufacturing, and controls (CMC) changes on the identity, strength,
1183 quality, purity, and potency of the product as they may relate to the product's safety or efficacy.
1184 Such an assessment generally includes data that demonstrate that the pre- and post-change
1185 products (i.e., the products manufactured prior to and subsequent to a manufacturing change)
1186 are comparable. In a QbD environment, the analysis is facilitated because of the available
1187 enhanced process and product knowledge. The company must report significant post-approval
1188 CMC changes to regulatory agencies, in one of the reporting categories described by each
1189 regulatory body.

1190

8.2.   Regulatory Applications Would Contain a Hybrid of Traditional and QbD Filing Content

1191
1192

1193   • Industry will generally implement QbD for vaccines in certain process steps ("Targeted QbD
1194   Implementation" for vaccines), and filings with the enhanced approach applied to targeted
1195   steps will be standard.
1196   • QbD implementation for vaccines may be limited to those areas that would benefit most
1197   from QbD and where the strength of the product characterization capability and process
1198   equipment understanding is consistent with the enhanced approach. Most likely areas for
1199   application are those that require changes post-licensure (e.g., equipment changes, process
1200   changes, process optimization, site changes).
1201   • Comparability protocols (post-approval change management protocols/expanded change
1202   protocols) provide a flexible mechanism to implement QbD across the product life cycle
1203   (e.g., by including comparability protocols in initial marketing authorization or submitting
1204   these post approval).

1205

1206   Today a company can apply both traditional and enhanced development approaches, based on
1207   QbD principles, to different aspects of the production process in developing a drug substance
1208   and drug product, as the approaches are not mutually exclusive. Both approaches may generally
1209   be used in a single vaccine submission, giving rise to a hybrid submission.

1210

1211   The focus areas/process steps chosen for QbD study are driven by the individual project
1212   expectations. In the first instance, implementation may be limited to those areas that would
1213   benefit most from QbD, most likely areas that require most of the changes post licensure, such
1214   as equipment changes, process changes, process optimization, and site changes. It is highly likely
1215   that the extent of application of QbD will vary among process steps. Steps are chosen for
1216   evaluation based on impact on the QTPP, prior knowledge, reproducibility, yield, and expected
1217   process changes such as site/scale. The outcome of these choices for a filing is a submission
1218   where a complete arsenal of QbD principles is applied to a subset of the process steps and an
1219   approach that is primarily traditional is applied for the remainder of the process. In summary,
1220   industry will most likely implement QbD for vaccines in certain process steps; hence, "Targeted
1221   QbD Implementation" for vaccines will result in filings with a combination of enhanced and
1222   traditional elements.

1223

8.3.   Guidance on Dossier Content for QbD Regulatory Submissions

1224

1225   • ICH Q11 lists expectations in terms of dossier content (S.2 Drug Substance) for the
1226   traditional and enhanced approaches. Points to Consider for ICH Q8/9/10 implementation
1227   provide considerations for development of the control strategy and its life cycle.

1228

1229   ICH Q11 lists expectations in terms of dossier content (S.2 Drug Substance) for the traditional
1230   and enhanced approach. The key elements for QbD files are: the linkage between material
1231   attributes and process parameters and the CQAs, and also the control strategy, which can
1232   include a proposal for a design space. The quality target product profile (QTPP) and potential
1233   CQAs of a drug product are discussed in ICH Q8R.

1234

1235   Points to Consider for ICH Q8/9/10 implementation provide considerations for development of
1236   the control strategy and its life cycle. They also provide guidance regarding the level of
1237   information that is expected in an enhanced regulatory filing. Not all studies performed/data
1238   generated during product development needs to be submitted; however, sufficient information
1239   should be provided to address the following:

1240   •   The scientific justification of the proposed control strategy

1241   •   The scientific rationale for the DOE studies conducted

1242   •   A concise description of methodologies used to conduct these studies and to analyze the
1243   generated data

1244   •   The summary of results and conclusions drawn from these studies

1245

1246   The sections of the case study lay out appropriate packages to summarize the analysis
1247   performed and enable appropriate review in line with the proposals in the Points to Consider.

1248

1249   As highlighted in Q11, the minimal requirements for manufacturing process development in the
1250   traditional approach are as follows:

1251

1252   •   Identifying potential CQAs associated with the drug substance so that those characteristics
1253   having an impact on product quality can be studied and controlled

1254   •   Defining an appropriate manufacturing process

1255   •   Defining a control strategy to ensure process performance and drug substance quality

1256

1257   An enhanced approach to manufacturing process development would additionally include the
1258   following elements:

1259   •   Identifying, through prior knowledge, experimentation, and risk assessment, the material
1260   attributes and process parameters that can have an effect on drug substance CQAs

1261   •   Determining the functional relationships that link material attributes and process
1262   parameters to CQAs

1263   •   Developing an appropriate control strategy using the enhanced approach in combination
1264   with QRM (quality risk management); for example, the strategy can include a proposal for a
1265   design space(s) and/or real-time release testing (RTRT) or potentially reduced end-product
1266   testing

1267

1268   In either the traditional or enhanced approach, there is an expectation that CQAs will be
1269   identified. This remains a particular challenge in vaccine development. Examples of the range of
1270   options for different polysaccharides are provided.

1271

1272   Understanding the appropriate level of documentation for enhanced regulatory submissions is
1273   evolving as submissions are made. The level of detail in a QbD filing should be sufficient for a
1274   regulatory reviewer to understand how conclusions were derived. Cited studies should be
1275   summarized with detail that is sufficient to convey an understanding of the purpose of the
1276   study, the data collected, how it was analyzed, the conclusions reached, and the impact of the
1277   study on the manufacturing process. The risk assessment tools and study results, on which a
1278   design space is based, should be adequately described. However, it is important to note that not

1279 all the studies performed and/or data generated during product development are expected in
1280 the submission.
1281
1282 This case study includes examples of ways to present in the dossier risk assessments, results of
1283 DOEs, and design spaces to facilitate understanding of the conclusions drawn and enable health
1284 authority reviews. A related analysis is also applied to the treatment of prior knowledge.
1285
1286 For initial filings or post-approval QbD submissions, guidance suggests the dossier contains a
1287 statement by the applicant describing the proposed regulatory outcome and expectations. For
1288 post-approval changes this can be presented in the form of a post-approval change
1289 management plan.
1290

1291 8.3.1. Use of Prior Knowledge

1292 Prior knowledge is information gained from experience and may come from production of
1293 previous products, literature searches, and/or experiments on related products. Prior
1294 knowledge is a key component in making appropriate risk assessments of critical quality
1295 attributes (CQAs), process parameters, and process inputs and outputs (as per the ICH guidance
1296 Q11).
1297
1298 Prior knowledge can be applied for multiple purposes such analyzing potential risks of a process
1299 step, doing design of experiments based on historical understanding of the strengths and
1300 limitations of a process step, and ensuring that the design of process steps is based on a
1301 contemporary understanding of the technology.
1302
1303 The application of prior knowledge is clearest when dealing with platform processes, as has
1304 been seen with the development of monoclonal antibodies where a number of unit operations
1305 can be covered by the platform. However, there are numerous applications in vaccine
1306 development that can utilize this springboard concept. Platform processes in vaccines can cover
1307 single unit operations such as conjugation and lyophilization; there are also wider applications
1308 such as polysaccharide production, the development of new drug delivery systems, introduction
1309 of formulation excipients, inclusion of adjuvants, and the manufacture of a drug substance
1310 without further process optimization. Such platform processes will be based on extensive prior
1311 knowledge with other vaccines and other large molecules. The extent of the use of prior
1312 knowledge is limited by the scientific strength and presentation of the platform and the options
1313 to demonstrate the relevance of the cited scientific data.
1314
1315 Companies may choose not to cross-reference data between products. There are real challenges
1316 to be addressed to facilitate incorporating information from another filing, although doing so
1317 can have significant payoff and should be considered. Deciding how to incorporate prior
1318 knowledge into an application is not at all trivial because 1) it may include an extremely large
1319 data set if referring to production data, 2) both CMC and clinical data may be required to
1320 support relevance, and 3) relevance of historical data must be justified.
1321
1322 The key point to consider with regard to prior knowledge is the ability to adequately document
1323 the information and relate it with good rationale to the contemporary situation. Prior
1324 knowledge can be applied extensively as long as the arguments made based on the data are

1325   scientifically sound, clear relationships exist between the scaled-down models and commercial
1326   scale, and appropriate supporting information can be provided for reference.
1327
1328   ### 8.3.2. Design Space

1329   Establishing a design space can be done by linking the process inputs and variables to the CQAs
1330   through design of experiments (DOEs), failure modes and effects analysis (FMEA), and life cycle
1331   knowledge. A design space can be determined operationally through a combination of proven
1332   acceptable ranges derived from multivariant experiments and/or through modeling. The
1333   rationale for the inclusion of these parameters in the design space should be provided in the
1334   dossier, and in some cases it is helpful to provide a rationale as to why some parameters were
1335   excluded.
1336
1337   In the QbD paradigm, movement within an approved design space is not viewed as a change and
1338   will not require review or approval, but will be managed in the company quality system. As
1339   manufacturing experience grows and opportunities for process improvement are identified, the
1340   operating parameters could be revised within the design space without the need for post-
1341   approval submission. The same is true for design spaces built with mathematical models. In all
1342   cases, continuous process verification can help to verify performance within the design space.
1343
1344   Presentation in the dossier can include a description of the design space in tabular format,
1345   including the variables (material attributes and process parameters, as appropriate) and their
1346   proposed ranges. Examples of how to present the design space in a QbD submission can be
1347   found in the Annex 2c of ICH Q8. The present case study also includes examples of ways to
1348   present design spaces/modeling in a regulatory submission.
1349

1350   ### 8.3.3. Control Strategy

1351   The control strategy can include a number of interacting elements that assure full control of the
1352   product to be marketed. In the dossier the control strategy should be summarized in Module 3,
1353   Section P.5.6 with a scientific justification provided for the strategy. Additional information can
1354   be presented in other sections of the dossier (refer to Q8, Q11). Consideration should be given
1355   to the identification of potential residual risk that might remain after the implementation of the
1356   proposed control strategy and proposals for managing these residual risks.
1357
1358   Continual improvement of the control strategy through such methods as continuous process
1359   verification might be introduced into an application using a post-approval change management
1360   plan, which would set out the applicant's proposed regulatory outcome and expectations.
1361

1362   ### 8.3.4. Process Validation

1363   •   Traditionally, process validation has been used to prove that the manufacturing process can
1364   consistently produce the product meeting specifications. The process validation exercise has
1365   traditionally encompassed production of three consecutive lots of product that met the
1366   specifications. In the context of Quality by Design, the same objectives of process validation
1367   may be established through a life cycle approach leveraging process development and data
1368   from studies at commercial scale along with continuous process monitoring. This section
1369   describes a potential application of this approach along with its impact on the regulatory

1370   submission. The validation discussion is an important element of the case study because of
1371   the potential to utilize small-scale data.

1372

1373 Managing variability is one of the key ideas for managing a process. A QbD development effort
1374 will define the interrelatedness of process variables. FDA's Process Validation guidance is
1375 evaluated here because the paradigm presented for process validation is based on Quality by
1376 Design and the application of multiple guidance documents that have been developed and
1377 authored in the last decade. These guidance documents include Q8, Q9, Q10, Q11, and the
1378 associated Q&A for the first three ICH guidelines. Further guidance is expected as the QbD
1379 concept matures.

1380

1381 There are two guidance documents that discuss the impact of the enhanced approach on
1382 process validation. Question 2 (under "For General Clarification") in the ICH "Q8, Q9, and Q10
1383 Question and Answer" document states the following regarding the process validation
1384 methodology using the enhanced approach:

1385

1386 The objectives of process validation are unchanged when using ICH Q8, Q9, and Q10. The main
1387 objective of process validation remains that a process design yields a product meeting its
1388 predefined quality criteria. ICH Q8, Q9, and Q10 provide a structured way to define product
1389 critical quality attributes, design space, the manufacturing process, and the control strategy.
1390 This information can be used to identify the type and focus of studies to be performed prior to
1391 and on initial commercial production batches.

1392

1393 The answer to the next question from the same document (Question 3 under "For General
1394 Clarification") recognizes that "process validation also has a lifecycle (process design, process
1395 qualification, and ongoing process verification)." This approach describing these same stages of
1396 the process validation life cycle is further elucidated in the second guidance document, FDA's
1397 "Guidance for Industry: Process Validation: General Principles and Practices." This document
1398 was written as an application of the subject ICH documents. In the absence of similar guidance
1399 from other industry groups or health authorities, the terms and concepts from the latter
1400 document are utilized in the remainder of the section.

1401

1402 The life cycle approach to process validation described in the guidance should be utilized for unit
1403 operations where QbD concepts have been applied in development. This section will not repeat
1404 the concepts outlined in the guidance, but will give additional suggestions toward application of
1405 the concepts contained therein.

1406

1407 Those unit operations where development has occurred through a traditional approach would
1408 be expected to have process validation conducted in the traditional fashion and filed as such.
1409 CTD Section 3.2.S.2.5 should clearly delineate different validation approaches for different unit
1410 operations based on the differences in process development approaches to facilitate the
1411 understanding of this section by the reviewer. Only process validation through an enhanced
1412 approach is discussed throughout the remainder of this section.

1413

1414 A graphical description of the suggested approach is captured below in Figure 8-1. Note that the
1415 figure does not include all outputs from each stage of the process validation, but focuses on
1416 those pertinent to the process validation approach described hereafter.

1417

1418 **Figure 8-1: PV Strategy for Unit Operations Developed through an Enhanced Approach**
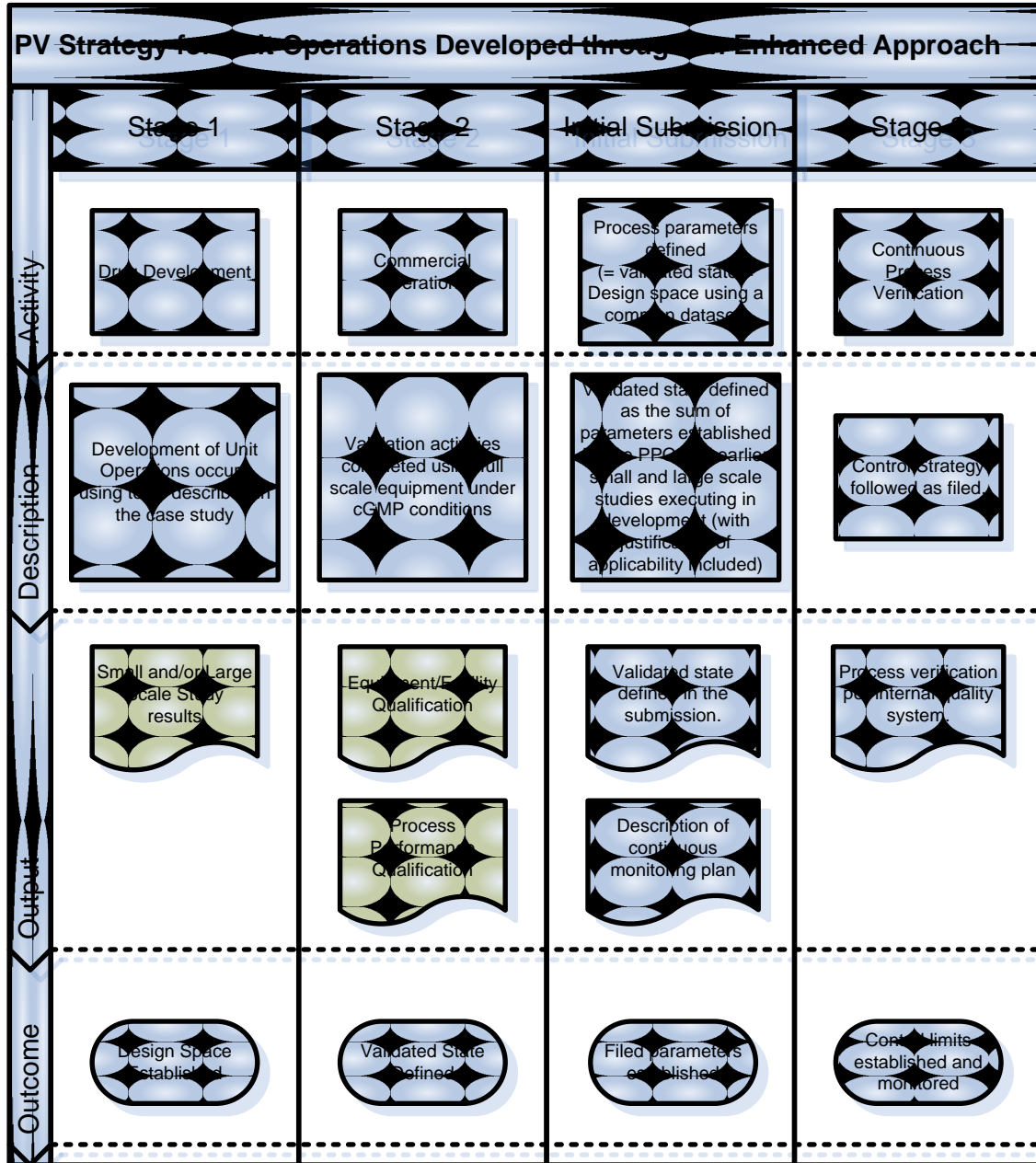
1419
1420

1421   *Stage 1: Process Design*
1422
1423   With the new guidance, the process validation life cycle begins in process design. Identification
1424   and quantification of process parameters critical to product quality need to be discussed in the
1425   submission. The guidance allows for limits of quantification to be established at either small-
1426   scale or full-scale development lots or during Stage 2 process qualification.
1427
1428   In the traditional approach to process validation, all parameters were frequently challenged
1429   during the process validation study itself; therefore, this data was often provided in 3.2.S.2.5
1430   Process Validation and/or Evaluation. With the new guidance, much of the data developed
1431   during process design will ultimately define the validated state, and as such may be described in
1432   other sections based upon early data collection efforts with robustness studies using models
1433   when scientifically justified. Discussion of Stage 1 in 3.2.S.2.5 should establish the design space
1434   as the basis for the validated process referencing ranges based on earlier data collection efforts.
1435   Portions of the process that must be validated during Stage 2 should also be highlighted.
1436
1437   *Stage 2: Process Performance Qualification*
1438
1439   Stage 2 has two elements: i) the facility and equipment design and the qualification of both to
1440   support the full-scale manufacturing process; and ii) the process performance qualification used
1441   to establish that a process is in a state of control and capable of reliably producing product with
1442   the desired specifications.
1443
1444   Per the guidance, the Process performance qualification (PPQ) "combines the actual facility,
1445   utilities, equipment (each now qualified), and the trained personnel with the commercial
1446   manufacturing process, control procedures, and components to produce commercial batches."
1447
1448   As most ranges are established during Stage 1, the PPQ would be expected to be run at set
1449   points within normal operating ranges. However, some operations and studies require
1450   execution under all conditions required to produce commercial batches (e.g., aseptic processing
1451   simulation) or concurrently with commercial manufacturing (e.g., column resin or TFF filter re-
1452   use studies). These studies are executed in the traditional approach and, as such, their
1453   description is not impacted by the QbD approach.
1454
1455   With this approach to process validation, the validated state may be described as the
1456   culmination of parameters established during both PPQ and process development. As the design
1457   space is created from the same data set, the design space submitted should be equivalent to the
1458   process description and to the validated parameters. This approach greatly simplifies evaluation
1459   of changes post approval.
1460
1461   *Stage 3: Continued Process Verification*
1462
1463   Although continuous verification is routinely part of GMP monitoring, Stage 3 represents a stage
1464   in the process validation life cycle not typically described in a product dossier developed solely
1465   with the traditional approach,. It should describe establishment of a continuous verification
1466   plan. It would not be expected to submit control limits because a statistically significant data set
1467   is not typically available at the time of submission. Additionally, control limits are expected to
1468   change as a result of continuous process improvement throughout the product life cycle. As a

1469   result, the dynamic nature of these values along with their periodic review during inspection
1470   negates the value in their submission.
1471
1472   A description of the continuous monitoring plan and potential for establishing control limits
1473   based on data collected over time should be discussed.
1474
1475   *Post-licensure evaluation of changes to the validated state of the process*
1476
1477   A potential work flow for evaluating changes to a unit operation validated under an enhanced
1478   approach is shown in Figure 8-2:
1479
1480   **Figure 8-2: Potential Work Flow for Evaluating Changes to a Unit Operation Validated Under**
1481   **an Enhanced Approach**
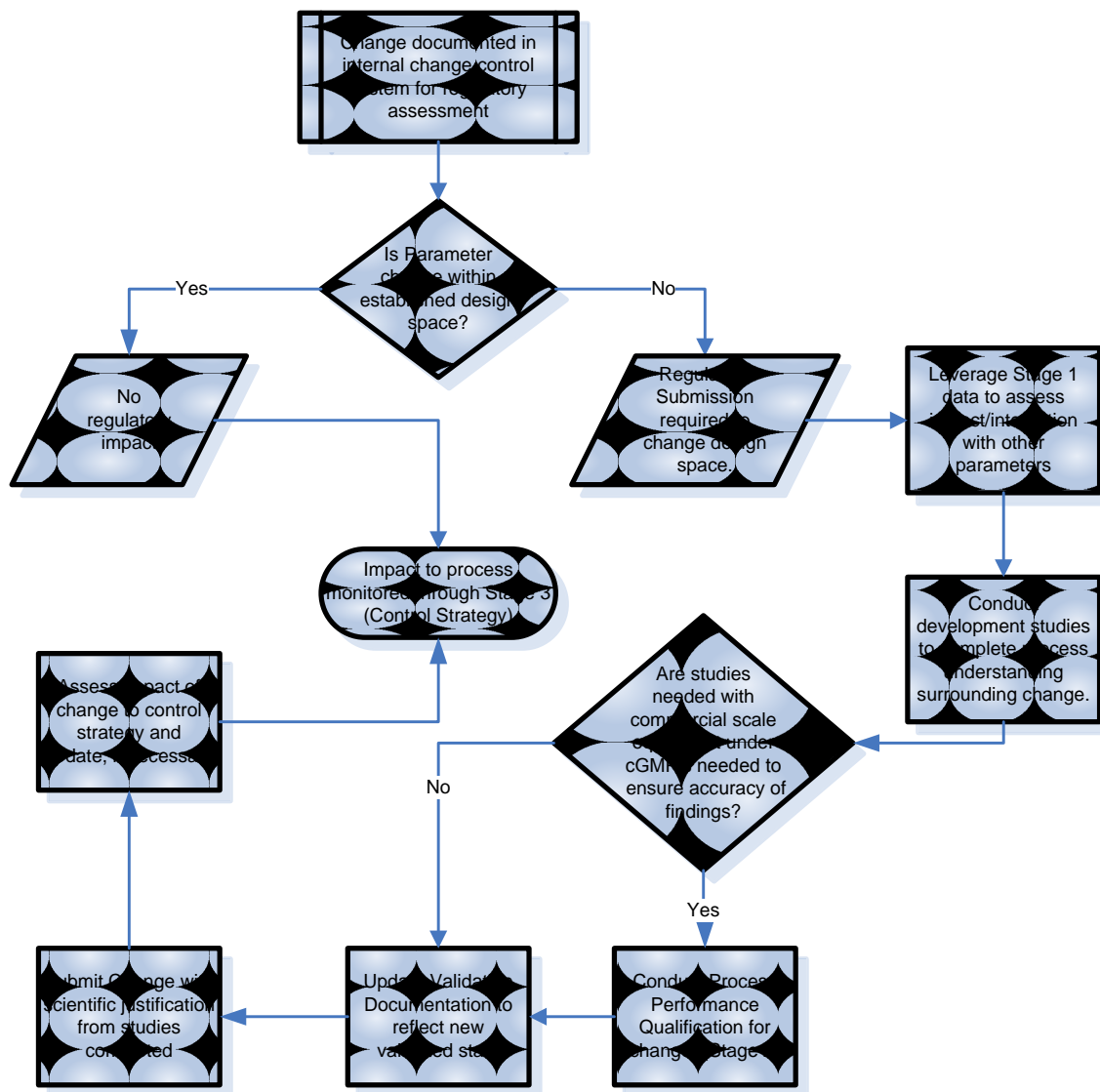
1482
1483

1484   It should be noted that although it is not explicitly stated, it is expected that cGMP and quality
1485   assessments of all changes are an intrinsic part of an internal change control system and would
1486   occur throughout the process listed above.
1487
1488   Evaluation of the first question is critical for the remainder of the work flow to be accurate. The
1489   established design space may be described for these purposes as the design space submitted to
1490   and approved by the health authority. The classic example would be to create a new parameter
1491   set point and allowable ranges outside the normal operating range, but within the design space
1492   previously filed and therefore validated parameters. As these changes affect neither the filing
1493   nor the validated state, there is no regulatory impact from a design space perspective. Internal
1494   quality systems ensure that cGMP concerns (e.g., documentation) are addressed. In addition,
1495   the established continuous monitoring program provides assurance post change that there has
1496   not been a negative impact to the process. However, note that movement within the design
1497   space may still constitute a regulatory impact based on cGMP and statutory considerations.
1498
1499   In the case where the parameter change is outside the design space, the process is more
1500   complex. Often, this would involve movement of a set point or ranges outside the ranges
1501   previously filed and validated. Other changes could involve use of a parameter not previously
1502   considered (e.g., introduction of PAT), expansion of a range based on new process
1503   understanding, or other change to the process outside what has been observed or reported as
1504   part of process development. All of these changes would be handled under a prior approval
1505   mechanism or at a lower reporting category if they have been appropriately downgraded based
1506   on approval of a change protocol. The appropriate data required to support these will leverage
1507   prior knowledge already generated.
1508
1509   The overall approach in the flow chart is to leverage previous process development data and
1510   supplement with additional studies to create new data in the design space using the same risk
1511   assessments and approaches as in the initial filing. The need to reexecute PPQ should be based
1512   on the same rationale included in the original process development and validation approach
1513   that determined whether the parameter needed to be demonstrated under commercial
1514   conditions or had been sufficiently demonstrated in stage 1 studies. In either case, the new
1515   validated state is defined, and the impact to the process is confirmed via continuous process
1516   monitoring.
1517
1518   The variation should clearly explain how previously filed data and risk assessments were
1519   leveraged with supplemental data and how the filed validation approach was applied to create
1520   the package needed. Additionally, the change to the process description and stage 1 and 2
1521   process validation should be clearly documented. A statement should also be included that
1522   justifies that the stage 3 continuous monitoring plan is sufficiently robust to capture any impact
1523   of the change on the process.
1524

8.4.   Appropriate Regulatory and Quality Oversight

- Understanding of CQAs and their linkage to critical process parameters and the design space allows clear identification of the parameters that may affect product safety or effectiveness and thus require the most stringent regulatory approval and oversight.

- Only a limited number of lots can be tested in clinical trials. One role of the case study is to illustrate examples where it is scientifically sound to establish criticality and process understanding beyond the information provided by clinical experience.

- Based on ICH Q8, working within a design space, which will have been approved in the initial license application, is *not considered a change* from a license perspective. Movement within the design space would not require regulatory notification because the space has already been assessed and approved. However, based on ICH Q10, *all changes* should be evaluated by a company's change management system.

Identification of critical quality attributes and linkages with process parameters provides a strong rationale for making risk-based decisions about the appropriate oversight. Those process parameters with a potentially significant impact on CQA(s) are expected to be subject to the most stringent levels of oversight. Design spaces are composed of acceptable ranges for the CPPs (critical process parameters) identified for each unit operation. The design space may also require regulatory control of critical raw materials. Other parameters not associated with CQAs are controlled and monitored in the quality system to ensure process and product consistency.

The case study cannot provide a definitive treatment with regard to the designation of regulatory commitments. As highlighted in the validation guidance, "all attributes and parameters should be evaluated in terms of their roles in the process and impact on the product or in-process material, and re-evaluated as new information becomes available. The degree of control over those attributes or parameters should be commensurate with their risk to the process and process output."

The case study is also important to demonstrate how the targeted experimentation guided by risk assessment and data collected through the DOE expands the available knowledge about process scale and reproducibility. Based on the knowledge generated, there is increased confidence in the understanding of the appropriate parameters to monitor and control throughout the process. There is also additional clarity about the appropriate point to execute testing to ensure the most critical attributes are appropriately controlled.

Movement within a design space does require an assessment of the risks associated with the particular move. This assessment would be performed within a company's quality system (as per ICH Q10), and a conclusion that the proposed change is supported by the existing product and process knowledge would be required.

8.5.   Procedural Framework for Enhanced/QbD Filings

- The provision of the data and information for the design space and control strategy can be submitted either at the time of the initial application or during post-approval submissions.

- ICH guidance introduces the concept of a change management plan in the *Q8/9/10-Implementation* paper *"Q8/9/10 Points to Consider,"* finalized in June 2011, where it is proposed that the plan can be incorporated into regulatory submissions as part of the manufacturing process description.

- The purpose of a change management plan is to facilitate more effective and proactive management of future changes resulting from business or technical reasons, and the plan would be part of continuous improvement of the manufacturing and control processes. For products already licensed, the EU Variation Regulation 1234/2008 was revised in January 2009 and introduced the option to file a design space as variation application. The Classification Guideline refers to the introduction of a new design space or an extension of an approved design space for the active substance or finished product. In the United States, a new design space will be introduced as a PAS.

- In addition to the introduction of design space, the concept of a Post-approval Change Management Protocol (CMP) was introduced through the revised EU Variation Regulation 1234/2008 that went into effect in January 2010. The CMP concept provides a flexible mechanism to implement enhanced/QbD principles across the life cycle of a product and occurs in two steps (Figure 8-3). The CMP concept can also be included in the initial marketing authorization application and then follow the variation procedures for the implementation step.

8.5.1.   How QbD Can Facilitate Stronger Control Strategies and Defined Pathways for Continuous Improvement

The enhanced (QbD) approach brings opportunities to include information on increased knowledge of the product and process that can be used to support the range of available regulatory approaches. The appropriate extent of regulatory oversight depends on how the design space and control strategy are defined and approved.

A robust process development program will study the effects of variation in material inputs, independent process parameters, and upstream quality attributes. These variables will have been assessed on the basis of their effect on the downstream process parameters, intermediate quality attributes, and CQAs of the drug substance and drug product. A number of potential scenarios are envisioned for life cycle management. Examples of each of these are described within the case study as noted below:

1. Parameters are noncritical, and therefore controls may be managed by a company's quality systems. This will be the most routine type of change.
2. Critical parameters are well-defined. Adjustment within licensed ranges may be made within firm's quality systems including confirmation of no adverse effect on CQAs.
3. Critical parameters are well-defined. Adjustment outside a licensed range is required to complete improvement. Confirmation of no adverse effect on CQAs and comparability can be shown, but must be managed through a regulatory reporting mechanism.

1607   4.   New knowledge regarding process parameters highlights potential for impact. New CPPs
1608   are defined. Confirmation of no adverse effect on CQAs and comparability can be shown,
1609   but must be managed through a regulatory reporting mechanism.

1610   Among the most significant contributions/benefits of QbD is a decrease in the potential for
1611   items of the fourth type and more objective (less subjective) definition of the boundaries of
1612   each of the first three scenarios .
1613
1614   In cases where strong characterization tools are available, FDA and EMA have both facilitated
1615   the application of a specific type of supplement as a tool that may diminish the reporting
1616   requirements after a company has demonstrated a lack of adverse effect. This tool of a
1617   comparability protocol has a very specific set of conditions that are prescribed for it to be
1618   applicable to a change. While a comparability protocol potentially diminishes the reporting
1619   requirements after a company has demonstrated a lack of adverse effect, the comparability
1620   protocol itself must be approved as a prior approval supplement. Furthermore, it significantly
1621   reduces the flexibility of the company in responding to unexpected observations during
1622   execution of the protocol.
1623
1624   In this case study, we have selected one or two examples of situations where a comparability
1625   protocol is likely to have potential value. Refer to examples in the Purification and DP sections
1626   (and expand detail as appropriate in next paragraphs). In these sections, we outline some of the
1627   reasons why a comparability protocol may be useful or valuable in these instances as well as
1628   acknowledging any additional risks or costs incurred by choosing to use the comparability
1629   protocol. In the analysis, we point to how changes to one or more aspects of the case study may
1630   change the risk-reward balance of this analysis from the company (and potentially for the
1631   reviewing BOH).
1632
1633   a.   A spectrum of risk has been articulated by health authorities, from examples cited as very
1634   acceptable: polysaccharide changes, cell bank location/process to examples highlighted as
1635   posing significant risk such as cross-linked conjugate. The examples in the case study are
1636   chosen to demonstrate where the additional data sets may reduce the perceived risk.
1637   b.   The range of application of animal models and/or clinical studies vs.
1638   physiochemical/analytical/process comparability is also demonstrated.

1639   We also provide details of what a comparability protocol should include in these particular
1640   vaccine examples. And we look at how a company may have to react to unexpected data that
1641   may be generated during execution of the protocol and how a comparability protocol filed with
1642   an original marketing authorization may be maintained to ensure it remains relevant at various
1643   stages of the product life cycle.
1644
1645   8.5.2. Scope for Regulatory Flexibility and the Post-approval Change Management Plan

1646   Once a design space has been established, movement within the approved design space can
1647   occur without further regulatory review; consequently, this is anticipated to reduce post-
1648   approval submissions.
1649
1650   The provision of the data and information for the design space and control strategy can be
1651   submitted either at the time of the initial application or during post-approval submissions.

1652   Alongside this information, future post-approval changes can be presented in a "post-approval
1653   change management plan." In addition, ICH guidance introduces this concept in the *Q8/9/10-*
1654   *Implementation* paper *"Q8/9/10 Points to Consider,"* finalized in June 2011, where it is proposed
1655   that it can be incorporated into regulatory submissions as part of the manufacturing process
1656   description.
1657
1658   The purpose of the change management plan is to facilitate more effective and proactive
1659   management of future changes resulting from business or technical reasons, and the plan would
1660   be part of continuous improvement of the manufacturing and control processes. This enhanced
1661   process knowledge and prospective thought about appropriate analysis and data sets to support
1662   process changes will also accelerate handling of reactively driven post-approval changes that are
1663   the consequence of deviations, OOS, or other findings such as CAPAs.
1664
1665   It is anticipated that the level of regulatory oversight with an enhanced QbD filing will be
1666   inversely proportional to the demonstrated product and process knowledge and application of
1667   risk management. Thus, even for changes outside the design space that require regulatory
1668   oversight, a greater scope for a reduced reporting category is anticipated.
1669
1670   Based on ICH Q8, a change within an approved design space is not considered a change from a
1671   license perspective. However, all changes should be evaluated by a company's quality control
1672   system, which provides the mechanism to ensure that the manufacturing process is maintained
1673   within the boundaries described by the design space. This assessment would examine the risks
1674   associated with the particular move. Following the assessment, if the conclusion is that the
1675   proposed change is supported by the existing product and process knowledge, it can be
1676   concluded the change is within the design space. Thus, this enables the management of some
1677   CMC changes based on clearly defined and agreed-upon risk-based criteria without additional
1678   regulatory filing. However, if this condition is not met, then the standard regulatory application
1679   appropriate for the given change would have to be submitted.
1680
1681   The enhanced QbD approach brings increased process understanding, which reduces the risk
1682   that process changes will adversely impact product quality. We therefore anticipate that, once
1683   industry and health authorities have experience with and confidence in the application of QbD
1684   to vaccines, the regulatory application requirements for process steps filed under the QbD
1685   approach could be different from those for process steps filed under the traditional approach.
1686
1687   Additional avenues for potential regulatory flexibility are discussed in the following sections;
1688   please refer to examples found in Section 8.7 for the European Union and Section 8.10 for the
1689   United States.
1690

1691   ## 8.6.   Regulatory Framework for Enhanced/QbD Filings in the European
1692   Union

1693   **For products already licensed, the EU Variation Regulation 1234/2008 was revised in January**
1694   **2009 and introduced the option to file a design space as variation application. The**
1695   **Classification Guideline refers to the introduction of a new design space or an extension of an**
1696   **approved design space for the active substance or finished product, items B.I.e.1 and B.II.g.1,**

1697   respectively. These changes are handled as Type II variations (the standard being a 60-day
1698   timetable).
1699
1700   **In addition to the introduction of design space, the concept of a Post-Approval Change**
1701   **Management Protocol (CMP) was introduced through the revised EU Variation Regulation**
1702   **1234/2008 that went into effect in January 2010.** The CMP concept provides a flexible
1703   mechanism to implement enhanced/QbD principles across the life cycle of a product and occurs
1704   in two steps (Figure 8-3). The CMP concept can also be included in the initial marketing
1705   authorization application and then follow the variation procedures for the implementation step.
1706
1707   *The first step* introduces the protocol to the license using a Type II variation as detailed in the
1708   Classification Guideline (**Introduction of a post-approval change management protocol** for the
1709   active substance and final product). In this step, the protocol presents a description of the
1710   proposed change; a risk assessment of the impact of the change on product quality, safety, and
1711   clinical performance; a description of the methods used to evaluate the effect of the change;
1712   the acceptance criteria for which the proposed change will be evaluated; and a commitment to
1713   update the protocol (if needed).
1714
1715   The protocol also includes how the changes will be reported to the regulatory authorities
1716   following approval of the protocol. This reporting is the second step or the implementation of
1717   the change.
1718   *The second step,* or the implementation variation, can be managed either via a Type IA_{IN} or a
1719   Type IB variation procedure as detailed in the Classification Guideline. For a
1720   biological/immunological medicinal product, reporting under the current guidance is restricted
1721   to a Type IB.
1722
1723   Following the introduction of the CMP into the Classification Guideline, the EMA issued a Q&A
1724   document (EMA/CHMP/CVMP/QWP/586330/2010) providing information regarding the
1725   expectations in terms of content of the CMP. For example, for multiple changes a CMP can be
1726   used; however, in the submission a justification to demonstrate that the changes are
1727   interrelated is required. The Q&A document also details how a change should be implemented
1728   and reported and provides timelines for approval, etc.; and it describes the classification for a
1729   change to an already approved protocol for biologicals as a Type IB variation, B.I.e.z (active
1730   substance), and B.II.g.z (finished product), respectively.
1731

Figure 8-3: Post-Approval Change Management Protocol EU Submission Procedure
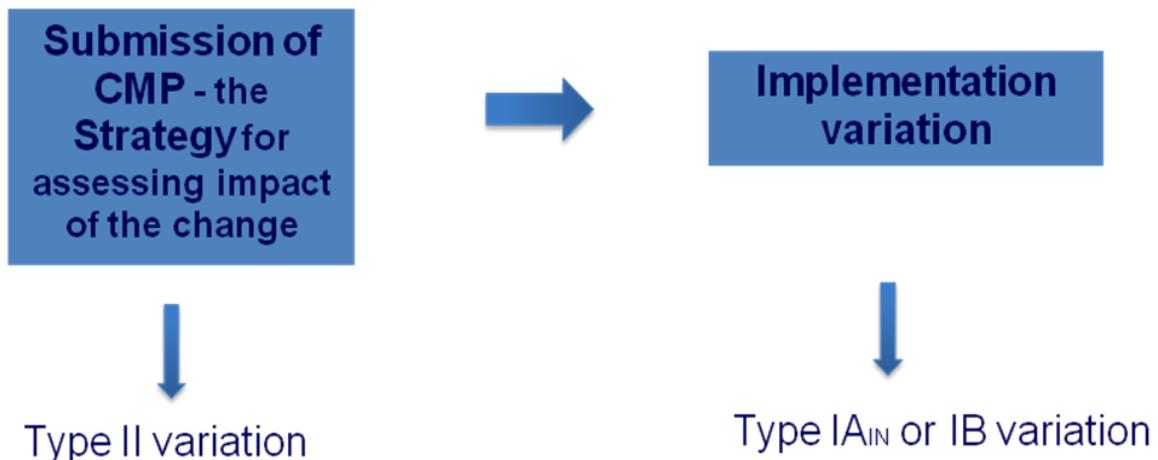
1733
1734

8.7.   Scope for Regulatory Flexibility in the European Union

1736   The current and future regulatory environment for enhanced/QbD applications and variations
1737   introducing or changing design space and the CMP is expected to evolve over coming years. As
1738   manufacturers gain experience in the use of these regulatory paths and the authorities increase
1739   their assessment of these applications, regulations and guidance will develop or existing ones
1740   will be further amended.
1741
1742   The appropriate degree of regulatory oversight is based on the level of relevant scientific
1743   knowledge that will be provided in the registration application or variation and existing
1744   guidance. As more experience is gained, more flexibility can be introduced. Thus, the following
1745   sections explore how flexibility can be introduced to new applications and post-market product
1746   life cycle management by the application of the principles of the enhanced (QbD) approach and
1747   how the existing regulatory guidance could evolve in the future.
1748

8.8.   Reduction in End-Product Testing

1750   **The control strategy focuses on performing the appropriate testing at the appropriate point in**
1751   **the process and eliminating testing as appropriate. A further reduction in end-product release**
1752   **testing and/or implementation of skip testing could be achieved using the principles of QbD.**
1753
1754   A traditional approach involves a discrete sample size that represents the minimal sampling
1755   expectations and will detect only major deviations in the manufacturing process. Use of an
1756   enhanced approach would make it possible to monitor relevant parameters that may involve
1757   assessing a CQA directly or indirectly using parameters associated with the CQA (e.g.,
1758   temperature, pressure, pH, speed, time, etc.) Because the testing is during the manufacturing
1759   process (in-line, on-line, at-line), it does not represent discrete sampling; therefore, the data
1760   generated lends itself more to statistical analysis and trending of these parameters. This type of
1761   testing can be described under the umbrella of "real-time release testing" (refer to draft
1762   guideline on Real Time Release testing EMA/CHMP/QWP/811210/2009; Rev 1 was published in
1763   March 2010 in the European Union). Real-time release testing currently is unlikely to replace

1764   end-product testing for a vaccine candidate; however, it can provide an opportunity for
1765   increased regulatory flexibility in the end-product testing. In addition, end-product testing will
1766   be required for other aspects of product quality such as in stability studies or OMCL release
1767   activities.
1768
1769   ### 8.8.1.   Flexibility in the Implementation of the CMP

1770   **Evolution in the existing legislation will facilitate the application of the CPM for biologics.**
1771   The existing legislation on CMP provides a broad guidance of the applicability and use of the
1772   CMP. The CMP can be used to manage proactively and strategically the manufacturing and
1773   control changes during the life cycle of a product, and it could become a critical tool for life cycle
1774   management of CMC. It is therefore envisaged that CPM legislation will evolve in coming years
1775   to further facilitate the application of CMPs for biologics, including vaccines.
1776
1777   The following subsections explore how the existing CMP guidance could evolve in the future to
1778   be more flexible in the implementation of this concept for biologics.

1779   **8.8.1.1. Reporting category of the implementation variation of a CMP**
1780   Under current guidance, the reporting category for biologicals is a Type IB. As many biological
1781   changes have a default categorization of a Type II, the CMP provides a mechanism to downgrade
1782   the reporting category for these changes. However, in the guidance the category for the
1783   implementation variation does not distinguish between active substance and final product. It
1784   could be envisaged that certain changes, provided they are implemented as approved, will carry
1785   little or no risk regarding an impact on the quality, safety, or efficacy of the product. Thus, the
1786   possibility to report the implementation variation as a Type IA or Type IA$_{IN}$ could potentially be
1787   supportable given the quality of the data package generated.

1788   **8.8.1.2. Cross-references within the dossier**
1789   A dossier can be structured well using smartly written text and cross-linking. This would lead to
1790   a reduction in post-approval changes. It is not related only to QbD but it is a general point to
1791   consider. There are specific opportunities available as companies begin to take advantage of
1792   filing a design space. Increased regulatory flexibility could be achieved by greater cross-
1793   reference within the dossier.
1794
1795   For example, methods for the determination of acceptance criteria for the CQAs or CPP could be
1796   cross-referred from the dossier section containing the description of the design space/CMP to
1797   the product dossier. When a minor method change is required, the dossier is updated via a
1798   regular variation, and the design space/CMP automatically reflects this change without
1799   additional regulatory action.

1800   **8.8.1.3. "Common/generic protocols" and combining work sharing and the CMP**
1801   The CMP protocol procedure is also interesting to manage in a strategic manner changes that
1802   are "common/generic" in nature. Changes that share common elements to demonstrating
1803   quality, safety, and efficacy might include secondary operations such as packaging or filling. The
1804   elements included in such a protocol would be equivalent, regardless of manufacturing site.
1805   Therefore, the use of common/generic protocols should be envisaged because this would
1806   greatly enhance the wider applicability of the CMP principle. The protocols are entirely

1807   consistent with the facility and equipment knowledge and the types of data packages generated
1808   as part of enhanced programs.
1809
1810   In a similar way, it could be envisaged that a CMP could be written for a change that may affect
1811   multiple products of the same vaccine family. Thus, this type of CMP could be written and
1812   submitted in a work-sharing procedure for the vaccine family. Subsequently, for each product,
1813   individual secondary implementation variations providing the data could be submitted. An
1814   example might be a bulk manufacturing site of a drug substance that is present in the drug
1815   product of a number of vaccines in a vaccine family. This concept is similar to the US expanded
1816   change protocol (ECP), which takes a more holistic approach; it offers the use of a protocol
1817   providing the approach and acceptance criteria that can be applied to multiple manufacturing
1818   process changes or a process change across multiple related product types or manufacturing
1819   process platforms. Again, the facility and product knowledge generated should facilitate.
1820
1821   The current Q&A document (EMA/CHMP/CVMP/QWP/586330/2010), which provides
1822   information regarding the expectations in terms of content of the CMP, does not preclude the
1823   possibility of writing a protocol that could be used for a number of products. The CMP is an
1824   integral part of Module 3, and thus it is possible to write a protocol that becomes specific via
1825   cross-references within the dossier. However, this does infer that the vaccine family dossier
1826   structure needs to be sufficiently similar for the products to enable correct cross-referencing to
1827   occur. (Refer to Drug Product section).
1828

1829   ## 8.9.   Regulatory Framework for QbD Filings in the United States

1830   • Limited FDA references exist to illustrate implementation of QbD principles into vaccine
1831   regulatory filings.
1832   • Design space information may be incorporated into regulatory filings as part of an original
1833   license application or as a supplement to an approved license. In addition, design space
1834   information may be an important addition to a comparability protocol [i.e., 21 CFR 601.12(e)
1835   filing] by supporting prospectively defined acceptance criteria captured in the filing.
1836   • The scope of regulatory flexibility will be defined by the ability of analytical methodologies
1837   to address two questions related to clinical significance of a change and robustness of the
1838   analytical methodology applied to assessing the change.
1839
1840   There are limited FDA references to the implementation of QbD principles beyond the adoption
1841   of principles contained in ICH documents Q8, Q9, and Q10. CDER issued a manual (dated Febr
1842   8th, 2011) outlining and clarifying how CMC reviewers should apply the recommendations in the
1843   ICH Q8(R2), Q9, and Q10 guidances to new drugs approved under the FD&C Act; however,
1844   vaccines are regulated under a separate set of regulations and a different statutory authority.
1845   For new drugs regulated by CDER, reviewers are directed to ensure that applications contain at
1846   least the minimum information on pharmaceutical development described by ICH Q8(R2) as: "At
1847   a minimum, those aspects of drug substances, excipients, container-closure systems, and
1848   manufacturing processes that are critical to product quality should be determined and control
1849   strategies justified."
1850

1851   The difference between vaccines and small molecule drugs in statutory authority and
1852   promulgated regulations in the United States adds a layer of complexity to the regulatory
1853   landscape. However, the concepts captured in the ICH guidance documents are consistent with
1854   the implementation of comparability protocols at CBER. This case study illustrates application of
1855   the principles of Q8, Q9, Q10, and Q11 to vaccine development and post-approval life cycle
1856   management through the enhanced process and project knowledge gained.
1857
1858   There are two means to incorporate a design space into a biologics license application (BLA):
1859   •   The incorporation of design space information into the original BLA or as part of a
1860   supplement to an approved BLA to support directions within the filed master batch record.
1861   •   The inclusion of design space to support acceptance criteria to be used under a regulatory
1862   comparability protocol (i.e., not to be confused with an assessment of product
1863   comparability performed by the license holder).
1864
1865   *License application.* Regarding incorporation of design space concepts into an original BLA, it is
1866   already fairly common for license applications to contain analogous types of data that provide a
1867   summary of product knowledge gained during the vaccine development process that supports
1868   operating parameters, specifications, and/or protocols. For example, stability protocols are
1869   often incorporated into the BLA to support extension of shelf life based on real-time
1870   commercial-scale stability experience; and these protocols prospectively define how change
1871   would be managed as additional data become available. The examples in this case study seek to
1872   illustrate additional means by which a more formalized QbD process can be used to enhance the
1873   control strategy and to establish a change management plan for review and approval by the
1874   FDA.
1875
1876   *Comparability protocol*. A comparability protocol (CP) is a well-defined, detailed, written plan for
1877   assessing the effect of specific CMC changes on the identity, strength, quality, purity, and
1878   potency of a specific drug product as they may relate to the safety and effectiveness of the
1879   product. A CP describes the changes that are covered under the protocol and the specific tests
1880   and validation studies and acceptable limits to be achieved to demonstrate the lack of adverse
1881   effect for specified types of changes on the safety or effectiveness of a product.
1882
1883   Upon approval of the CP, the FDA may determine that certain changes evaluated in accordance
1884   with the protocol may be reported at a reduced category. By providing an opportunity for FDA
1885   to review and approve the CP before it is used by the license holder to evaluate a change, FDA
1886   gains greater assurance that the change is being properly evaluated and, therefore, that there is
1887   less potential for the change to have an adverse effect on the safety or effectiveness of the
1888   product (*62 FR 39890*; 24 July 1997). Subsequent to implementation of the revised regulation,
1889   the FDA issued a number of guidance documents and conducted workshops to explore means to
1890   apply this regulatory approach to the reporting of changes in: manufacturing process; analytical
1891   procedures; manufacturing equipment; manufacturing facilities; container-closure systems; and
1892   process analytical technology (PAT).
1893
1894   In this spirit, this section seeks to extend the exploration of the CP approach as a means to apply
1895   the process knowledge and product understanding gained through application of the QbD
1896   approach to vaccine development and post-market product life cycle management.
1897

1898   License applicants and license holders are responsible for assessing, prior to distribution of a
1899   product, the effect of any post-approval CMC changes on the identity, strength, quality, purity,
1900   and potency of the product as they may relate to the safety or efficacy of the product. Such an
1901   assessment often includes data that demonstrate that the pre- and post-change products (i.e.,
1902   the products manufactured prior to and subsequent to a manufacturing change) are
1903   comparable. Vaccine manufacturers must report pos- approval CMC changes to the FDA in one
1904   of the reporting categories described by the FDA. As part of its review and approval of a CP to
1905   evaluate the effects of a change if supported by the submission, the FDA may determine that a
1906   CMC change made under the CP will fall into a less restrictive reporting category. In many cases,
1907   using a CP will facilitate the subsequent implementation and reporting of CMC changes, which
1908   could result in moving a product into distribution sooner than if a protocol was not submitted.
1909
1910   ### 8.9.1. Licensed/Marketed Products
1911   The license for approved vaccines may be modified through the use of a supplement, which is
1912   filed under the Changes to Be Reported regulation, *21 CFR 601.12*. A new design space would be
1913   introduced into the license either as part of a supplement or a CP, which would provide the
1914   overall context for how the new design space information informs the ability of regulators to
1915   assess the means being used to evaluate impact of a change to product safety and effectiveness.
1916   The initial design space filing would generally be reviewed as a Prior Approval Supplement (PAS)
1917   as described under 21 CFR 601.12(e). A review action is taken within four months under PDUFA
1918   IV managed review process timelines.
1919
1920   For a CP filing, there is a second step in completing the reporting requirement for
1921   implementation of the change that requires a second submission. The follow-up submission is
1922   often submitted as a Change-Being-Effected-in-30-Days Supplement (CBE30); however, the
1923   agency has allowed increased regulatory flexibility and permitted the change to be reported at
1924   even lower categories [Change-Being-Effected Immediately (CBE) or Annual Report]. For
1925   changes to the manufacturing process, the design space data are incorporated into the license
1926   or license supplement as part of the justification of acceptance criteria to be applied to
1927   evaluation of the change.
1928
1929   Incorporation of design space data may be useful for other types of change that are amenable
1930   to use of a CP approach, such as changes to analytical procedures, manufacturing equipment,
1931   manufacturing facilities, and container-closure systems. For example, the understanding of
1932   CQAs gained from design space data collection may inform criticality of defined user needs and
1933   the most efficient means of assessing equipment capability in delivering these performance
1934   characteristics.
1935
1936   Other types of protocols that may be used during product life cycle management may benefit
1937   from design space information, including shelf life extension protocols and container-closure
1938   component interchangeability assessments. These protocols may also be submitted as
1939   supplements to approved vaccine license files and should include: a description of the proposed
1940   change; a description of how impact on product quality, safety, and clinical performance will be
1941   assessed; a description of the methods used to evaluate the effect of the change; and the
1942   acceptance criteria to be applied in evaluating the change. The protocol should also include how
1943   the changes will be subsequently reported to the FDA following approval of the protocol.
1944

1945   ## 8.10. Options for Continuous Improvement in the United States

1946   **The options for continuous improvement will be defined by the ability of analytical**
1947   **methodologies to address two questions related to clinical significance of the change and**
1948   **robustness of the analytical methodology applied to assessing the change.** To effectively
1949   implement design space in a vaccine license file, we will need to focus on providing information
1950   that not only evaluates the analytical result(s) within the context of the CQAs, but also provides
1951   a linkage back to the clinical relevance of the data. As nonclinical means of assessing
1952   immunological performance of a vaccine are validated and gain regulatory acceptance, we can
1953   hope to further advance our ability to address this question.
1954
1955   A second focus of potential reviewer questions can be expected around our level of confidence
1956   that the product is comparable if no change is observed in analytical results. The question here
1957   is whether the methodology is sensitive enough and how can we assure ourselves and the FDA
1958   that, in fact, there is not a significant impact on product safety or quality that has crept into the
1959   product after implementing change. For those changes that are amenable to definition of a
1960   design space, we can anticipate the ability to conduct dialog with regulatory health authorities
1961   to seek their advice on applicability.
1962
1963   ### 8.10.1. Managing Repetitive Change

1964   Because of the nature of Changes to Be Reported requirements in the areas of manufacturing
1965   facilities and equipment, CPs have also been used to decrease the regulatory reporting burden
1966   for repetitive and recurring changes. For example, qualification of a new working seed can be
1967   performed under a CP, which has undergone prior review and approval by the FDA to ensure
1968   that the regulatory authorities have confidence that the change will be assessed appropriately
1969   and that potential impact on product safety and effectiveness can be managed under the quality
1970   system.
1971
1972   In addition, more generic CP approaches have been used in instances where equipment- or
1973   facility system-related changes are being made and apply to multiple products. For example,
1974   replacement of terminal HEPA filtration casings throughout a large manufacturing facility can be
1975   a significant undertaking with potential to impact a variety of controlled manufacturing
1976   environments. It is possible to utilize a CP to define how systems and manufacturing
1977   environments will be assessed after a change and to achieve a lowered Changes to Be Reported
1978   category.
1979
1980   ### 8.10.2. Reporting Category of the Implementation Supplement

1981   Under current guidance, the FDA maintains a degree of flexibility in defining the Changes to Be
1982   Reported category for the supplement that provides results of a post-change assessment made
1983   under a CP. In general, these were handled as CBE30 submissions. However, in those instances
1984   where the FDA has sufficient confidence in the robustness of the comparability assessment,
1985   regulators have permitted subsequent reporting as CBE supplements or as part of the annual
1986   report. In the broader context, the precedents for enhanced downgrade to the reporting
1987   category are more limited with vaccines because of the more limited strength of the product

1988   characterization capability and the more risk-averse nature of the patient population for
1989   vaccines.
1990
1991   ### 8.10.3. Updating/Modifying the Content of the CP

1992   Currently modification of the CP for vaccines would be a PAS filing. Updating the original
1993   protocol should be requested only when the original one becomes invalid because of <u>substantial</u>
1994   changes to the proposed test methods/acceptance criteria or new knowledge that becomes
1995   available.
1996
1997   ### 8.10.4. Reduction in Lot Release Testing

1998   Vaccines are subject to lot release testing on every lot of product intended for distribution to
1999   the US marketplace unless granted a waiver. The conditions for requesting a waiver from lot
2000   release testing include a demonstrated ability of the quality unit to release product lots over a
2001   period of time that meet specifications and confidence that release testing achieves a full
2002   assessment of all CQAs.
2003
2004   It may be possible to engage the FDA in a dialog to define the parameters that would be
2005   expected to request a waiver from lot release and to move a product to surveillance mode;
2006   however, because of the complexities of some vaccines (e.g., whole virus vaccine), the utility of
2007   this approach may be more readily acceptable for recombinant antigen vaccines with more well-
2008   characterized CQAs and a more robust strength of product characterization capability.
2009

2010   ## 8.11. Future Challenges in QbD Implementation for Vaccines

2011   As noted at the beginning of this section, the regulatory environment for incorporating design
2012   space into filings for vaccines is expected to evolve in coming years as regulators and vaccine
2013   companies gain more experience. As we look to the development of concepts in implementing
2014   QbD for small molecules, it is possible to identify some areas for further development of
2015   approaches for implementation that have not been discussed with regulatory health authorities
2016   for large molecules or vaccines. These include:
2017
2018   ### 8.11.1. Secondary or Adaptive Acceptance Criteria in a CMP

2019   In the development of a CMP, acceptance criteria for CQAs/CPP are required to build the control
2020   strategy. During manufacturing, it is possible that in testing of these criteria, a result may be at
2021   the limit of acceptance/failure. This could be handled as a deviation in the usual way and the
2022   CMP could be refiled, or more proactively it could be envisaged that secondary or adaptive
2023   criteria could be developed in advance.
2024
2025   Thus, using secondary or adaptive acceptance criteria, regulatory flexibility can be built into a
2026   CMP. The secondary acceptance criteria would be provided, along with details of the
2027   investigation and analysis that will be followed to determine acceptance and thus to justify the
2028   final conclusion that quality is maintained.
2029

2030   Following the triggering of the secondary acceptance, if it is assessed that this movement
2031   outside the design space is likely to re-occur, the design space should be reassessed and
2032   modified. The modification of the design space will then need to be submitted for regulatory
2033   review; please refer to Section 8.6.
2034

2035   **8.11.1.1.   Updating/modifying the content of the CMP**

2036   Currently modification of the CMP for biologicals is by default a Type IB variation under the EU
2037   legislation. Updating the original protocol should be requested only when the original one
2038   becomes invalid because of *substantial* changes to the proposed test methods/acceptance
2039   criteria or new knowledge that becomes available. However, minor noncritical deviations from
2040   the agreed protocol should be allowed via a Type IA and should not preclude submission of the
2041   minor deviation at the time of the implementation variation.
2042

2043   For instance, minor changes to the acceptance criteria can be justified in the variation submitted
2044   for the implementation of the change, to avoid having to delete the former CMP and submit a
2045   new type II variation with the updated CMP reflecting the adapted acceptance criteria. It could
2046   be envisaged in the future that minor changes could be notified or at the same time as the
2047   submission of the implementation variation as mentioned above in 1.1.3.3. Comparability
2048   protocols written in a more generic fashion.
2049

2050   The CMP protocol procedure is also interesting to manage in a strategic manner changes that
2051   are "common/generic" in nature. Changes that share elements common to demonstrating
2052   quality, safety, and efficacy might include secondary operations such as packaging or filling. The
2053   elements included in such a protocol would be equivalent, regardless of manufacturing site.
2054

2055   Therefore, the use of common/generic protocols should be envisaged because this would
2056   greatly enhance the wider applicability of the CMP principle. Also, they are entirely consistent
2057   with the facility and equipment knowledge and the types of data packages generated as part of
2058   enhanced programs.
2059
2060

2061   # 9.   Implementation Section

2062   ## 9.1.   Executive Summary

2063   This section covers the following key points:

2064   •   Multiple stakeholders (patients, manufactures, and regulators) benefit from the enhanced
2065   approach to vaccine process development. (See ICH Q8 and Q11 for concepts and
2066   definitions.)

2067   •   The key value of the enhanced approach is an improved ability to predict the value stream
2068   measures of safety and efficacy, availability, and cost effectiveness.

2069   •   A value stream approach can be used to successfully prioritize business and regulatory
2070   drivers, which support investment in the enhanced approach.

2071   •   Return-on-investment (ROI) analysis for the enhanced approach needs to be specific to the
2072   company, regulatory agency, and product because ROI factors drive the value stream and
2073   each situation may have unique considerations. In this case study, we provide an example
2074   framework that can be used to develop an individualized approach.

2075

2076   ## 9.2.   Implementation Section Overview

2077   The objectives of this case study were to exemplify the utility of Quality by Design (QbD) tools
2078   for vaccine development, demonstrating that, in many cases, stakeholders can achieve superior
2079   value through implementation of the principles of the enhanced approach to process
2080   development (as defined in ICH Q8 and Q11). For this case study, determination of the costs and
2081   benefits of the enhanced approach for vaccine development was made using a value stream
2082   measure of improved efficiency. This measure was defined in terms of the organization's ability
2083   to predict:

2084   •   Safety and efficacy

2085   •   Product availability (robustness)

2086   •   Cost effectiveness

2087   Superior value was achieved because the enhanced approach to vaccine process development
2088   provided an improvement in the organization's ability to predict metrics that directly impacted
2089   the three universal goals most vaccine stakeholders desire: safety and efficacy, product
2090   availability, and cost effectiveness. The value stream analysis demonstrated that
2091   implementation of the enhanced approach improved the efficiency in developing vaccines to
2092   meet patient needs, providing value over the life of the product to all stakeholders: patients,
2093   regulators, and manufacturers. Since all stakeholders receive value, the case for investment in
2094   the enhanced approach is justified.

2095

2096   The enhanced approach to process development offers great benefits but requires additional
2097   investment over more traditional process development methods. This additional investment is
2098   made primarily by the manufacturer during the development process when there is no

2099   guarantee a product will even be launched. Value is returned to the manufacturer only if a
2100   product is launched and has a successful life cycle, thereby recouping the initial investment and
2101   generating profits for continued operation and additional investment.
2102
2103   Value stream analysis, focused on the universal goals all vaccine stakeholders desire (safety and
2104   efficacy, product availability, and cost effectiveness), can identify the value-generating levers
2105   supporting the business case for this additional investment. An analysis of this type provides
2106   analytical tools that can open a dialog and improve decision making.
2107
2108   For the purpose of this case study, the terms "traditional" and "enhanced" are used to
2109   differentiate two possible approaches. In a traditional approach, setpoints and operating ranges
2110   for process parameters are defined and the drug substance control strategy is typically based on
2111   demonstration of process reproducibility and testing to meet established acceptance criteria. In
2112   an enhanced approach, risk management and more extensive scientific knowledge are used to
2113   select process parameters and unit operations that impact critical quality attributes (CQAs) for
2114   evaluation in further studies; these studies establish design space and control strategies
2115   applicable over the life cycle of the drug substance.

2116   As discussed in ICH Q8, for a drug product, a greater understanding of the drug substance and
2117   its manufacturing process can create the basis for more flexible regulatory approaches. The
2118   degree of regulatory flexibility is generally predicated on the level of relevant scientific
2119   knowledge provided in the application for marketing authorization (refer to ICH Q11).
2120   Traditional and enhanced approaches are not mutually exclusive. A company can use a
2121   traditional approach to drug substance/drug product development, an enhanced approach, or a
2122   combination of both in a hybrid filing.

2123   The traditional approach refers to the methods manufacturers and regulators currently use in
2124   vaccine development. The traditional approach produces a safe and effective vaccine for the
2125   patient. However, the traditional approach may not fully investigate all the interactions in
2126   process inputs (e.g., parameters, raw materials) during development to identify those
2127   interactions impacting manufacturing. The traditional approach also may not always allow
2128   efficient technology transfer because it is less integrated, as well as less complete in
2129   identification of risks.
2130
2131   The enhanced approach explores the data from experiments at the lab scale through clinical
2132   material manufacturing scale to derive specifications for post-licensure manufacturing. It can be
2133   used for better processing and determination of when changes interact to affect the process
2134   and, ultimately, the resulting vaccine product. The enhanced approach allows a risk-based
2135   assessment that takes advantage of prior knowledge from earlier experiments. This permits
2136   effective and more information-based decisions and easier technology transfer. The enhanced
2137   approach also produces a safe, efficacious vaccine for patients, while allowing more flexibility
2138   for manufacturers and regulators by generating processes that are more robust and understood
2139   (refer to ICH Q11).
2140
2141   One impact of applying the enhanced vs. traditional approach to vaccine process development
2142   was to reduce overall investment during the product life cycle and improve the probability of
2143   predicting success before manufacturers and regulators have made substantial investments.
2144

2145   This approach is applicable to the decision process organizations undergo related to vaccine
2146   process development activities. A value stream approach as outlined provided the rationale and
2147   expected benefit in specific cases where an enhanced approach to vaccine process development
2148   generated superior value over traditional methods. Decisions to augment traditional methods
2149   by implementing the enhanced approach for vaccine development activities or to perform
2150   additional development studies for an existing manufacturing process need to be evaluated and
2151   made by each manufacturer or regulator on an individual basis.
2152
2153   Risk analysis outcomes are fed into a comparative analysis as outlined in the diagram below
2154   (Figure 9-1). The risk mitigation projects are then further refined through a value stream analysis
2155   of important business case levers. Comparison of traditional vs. enhanced process development
2156   identified those areas, specific to vaccines, where an enhanced approach provided value for the
2157   development of product and process knowledge while also reducing cost, resources, and
2158   development time over the product life cycle.
2159
2160   The six steps outlined in this case study provide a value stream tool to highlight possible
2161   advantages for specific areas of a vaccine manufacturing process if the decision to implement
2162   the enhanced approach to process development is made.
2163
2164   **Figure 9-1: Value Stream Approach to Determining Implementation Costs and Benefits**

2165
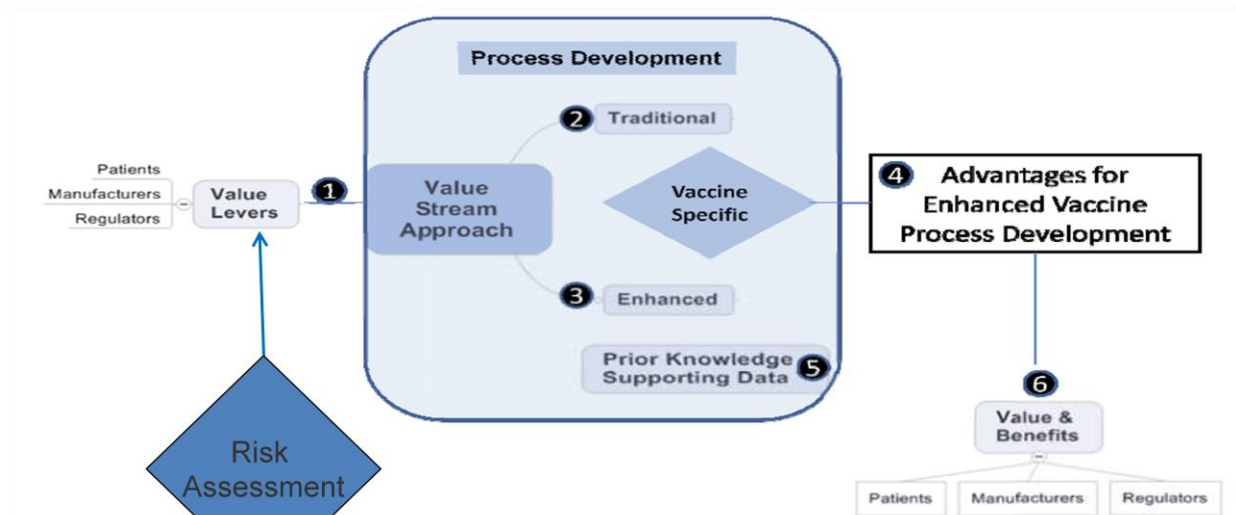
2166
2167

2168   The steps are as follows:
2169   1.   Identify all possible high-level business case value levers for manufacturers and regulators.
2170   Process and product risk areas, identified through a risk assessment tool or filter, generate
2171   high-risk items that can then be further prioritized through the value stream tool using
2172   these levers.
2173   2.   Qualitatively describe the traditional vaccine development process, and identify the
2174   disadvantage points and areas of opportunity based on previous risk analysis activities.
2175   3.   Qualitatively describe costs and benefits of using the enhanced approach in the areas that
2176   apply to vaccine process development.
2177   4.   Develop prioritization criteria, and select the business case levers where the enhanced
2178   approach is estimated to have potential beneficial impact.
2179   5.   Identify any prior knowledge or supporting data for the traditional or enhanced approach
2180   scenarios that is needed to support prioritized areas. Develop scoring ranges for
2181   implementation costs and benefits specific to vaccines.
2182   6.   Add detail supporting the scoring for the selected levers based on the key areas with greater
2183   estimated impact.

2184
2185   For this case study, examples from the process development chapters were selected. A few of
2186   the key business case levers/attributes were quantified in detail for each example. This exercise
2187   provides three examples of how to quantify and perform the six steps (Figure 9-1) for the
2188   determination of implementation costs and quantitative/qualitative benefits for the enhanced
2189   approach.
2190
2191   The benefits of implementing the enhanced approach must be large enough not only to cover
2192   implementation costs, but also to improve robustness and ultimately contribute to product
2193   availability, safety, and efficacy of the vaccine. For implementation of the enhanced approach,
2194   the key customers (patients, manufacturers, and regulators) must always be kept in mind.
2195

2196   ## 9.3.   Key Customers of the Enhanced Approach

2197   The key customers of the enhanced approach are the patients, manufacturers, and regulators.
2198   The ultimate customer of enhanced approach efforts is the patient. The value to the patients is
2199   accrued by increasing the associated value for manufacturers and regulators to provide a robust
2200   supply of safe and efficacious vaccines within the time frame they are needed. The enhanced
2201   approach increases value to the patients by identifying the critical attributes directly relevant to
2202   patient needs. In many cases, the current state of vaccine technology may limit the availability
2203   of product and process knowledge obtainable through the enhanced approach. In these
2204   situations, the most effective solution might be the traditional approach. Moreover, medical
2205   providers are also customers because they can provide better care to their patients if they have
2206   an adequate supply of the appropriate vaccine. The payers of vaccines benefit by the enhanced
2207   approach through better availability and lower costs resulting from efficient and robust vaccine
2208   processes.
2209
2210   Vaccine manufacturers are customers of the enhanced approach in multiple categories. Senior
2211   managers are interested in fewer interruptions in supply, robust manufacturing processes, and
2212   flexibility in increasing their supplies. The enhanced approach benefits the chemistry,

2213   manufacturing, and controls (CMC) process development customers since scale-up and
2214   technology transfer efforts can be more successful. A more complete CMC data package,
2215   developed using the enhanced approach, helps the regulatory groups compile high-quality
2216   submission documents. Site quality groups benefit from the enhanced approach with fewer
2217   nonconformances or regulatory actions. The additional product/process characterization
2218   associated with the enhanced approach also helps site quality groups expeditiously resolve
2219   manufacturing or testing issues that arise.
2220
2221   Regulators are key customers for the enhanced approach as well. They include the regulators in
2222   the review functions and in the inspectorate roles. Regulatory agencies, such as the FDA, EMA,
2223   and PDMA, can better assess the submissions due to the greater amount of process and product
2224   characterization information associated with the enhanced approach, as well as its focus on
2225   quality attributes.
2226

9.4.   Scope and Impact of the Enhanced Approach Implementation

2227

2228   The application of principles of the enhanced approach in the context of a new vaccine product
2229   candidate has the potential to impact and influence a vaccine's entire life cycle. To successfully
2230   apply the concepts as defined within ICH Q8, Q9, and Q10, some aspects of a manufacturer's
2231   pharmaceutical quality and associated systems will likely require "re-building/enhancement" to
2232   ensure application in a uniform and consistent manner. In that way, knowledge is conserved and
2233   the burden of repeating/verifying earlier work is streamlined or reduced. Execution of the
2234   enhanced approach to process development provides more knowledge of parameter design
2235   space. However, efforts to gain this knowledge are expected to increase compared with
2236   requirements of the traditional approach. In addition, start-up costs are associated with the
2237   enhanced approach, such as the cost of process and analytical equipment to execute design of
2238   experiment (DOE) development and associated cultural elements (e.g., the cost of training on
2239   such principles and retaining existing staff, development and maintenance of databases and
2240   knowledge bases, statistical services, and additional or contract staff for experiment execution
2241   and analysis).
2242
2243   Development costs likely increase based on enhanced development in comparison with
2244   traditional methods. Although there is an estimated cost increase, there are tangible gains, from
2245   both the manufacturer and regulatory perspectives, through knowledge management,
2246   information-based decisions, and operational flexibility linked to manufacturing processes.
2247   There is an expectation that the utilization of design space models results in gains for platform
2248   processes. These gains permit operational flexibility while maintaining a high degree of
2249   compliance through robust and reproducible operations. When platform process knowledge is
2250   supported by an enhanced process development approach, utilization of prior knowledge to
2251   support risk-based decisions is even more effective.
2252
2253   For an example illustrative vaccine, the estimated timeline for "break-even" ROI is about three
2254   years (Table 9-1). Within individual companies, the thrust is to integrate key concepts of the
2255   enhanced approach as fast as possible. This ultimately allows for timely market authorizations,
2256   such that the additional cost incurred with the enhanced approach models can be recovered
2257   even more quickly. There are also other tangible benefits linked to improved regulatory
2258   inspection performance. These benefits include shop-floor compliance drivers resulting from

2259 deviation management, product release, and the ease and effectiveness of introducing changes
2260 based on the established design space. Other qualitative indices include, but are not limited to,
2261 employee satisfaction, morale, and retention, including maintaining or improving the respective
2262 company's reputation.
2263
2264 **Table 9-1: Example Estimate for the Time to "Break-Even" ROI Analysis for Implementation of**
2265 **the Enhanced Approach**

Item	Enhanced Approach Example	Traditional Approach Example
Cost of Product Development	$550 milliona	$500 million (1)
Time for Development Completionb	5 years	7 years (1)
Break-Even Point (Full Market Penetration)c	3 years	N/A

2266 a Assumption: Introducing the enhanced approach principles to development and regulatory
2267 processes amounts to an increase in $50 million (excluding additional clinical studies) over the
2268 traditional approach and two years faster. Supplementary process development studies,
2269 required by the enhanced approach, are one of the factors for the increased cost of
2270 development in the enhanced vs. traditional approach.
2271 b Development completion time for the enhanced approach is estimated to be less than that for
2272 the traditional approach because of better data continuity and documentation, reduced reliance
2273 on full-scale demonstration runs, and less redundancy of process development efforts.
2274 c Assumption: Product sales for new vaccine candidate are $10 million at year one, $20 million
2275 at year two to a maximum of $50 million within three years of launch (1).
2276 N/A - Not applicable because no additional costs were incurred based on the traditional
2277 approach.
2278
2279 Reference:
2280 (1) Paragraph about waste is based on Better by Design, Sven Stegemann. World
2281 Pharmaceuticals Frontiers. 2010. Volume 1. pp 76 to 78
2282
2283 For the FDA's QbD pilot program for biologics, it was reported that "as of mid-2010 a total of
2284 five BLA and four post-approval supplements had been received." Within the same reference, it
2285 was noted that the FDA also extended its subscription period to its biologics QbD pilot program
2286 and the pilot results were not expected until 2015. (Quality by Design – Putting Theory into
2287 Practice, Siegfried Schmitt (Introduction, 2011)).
2288
2289 It is clear that the concept of QbD is still in its infancy. Although there are product candidates
2290 where this approach is being used, the full realization/gains are as yet unknown. The use of the
2291 enhanced approach for a new vaccine candidate has limitations from manufacturer as well as
2292 regulatory perspectives. Accordingly, if applied in its entirety, it requires a high degree of
2293 collaboration and upfront work from the sponsor (manufacturer) and the respective regulatory
2294 agencies. This concept requires an understanding of expectations and shared perspective from
2295 the manufacturer and the regulatory agencies, with the ultimate goal being the supply of a safe
2296 and efficacious product.
2297

2298   The enhanced approach may be beneficial for established unit operations (e.g., freeze drying,
2299   chromatography) that are directly scalable and where the concept of design space can be
2300   exploited for changes linked to established licensed processes. In this case, manufacturers and
2301   regulators alike are encouraged to partner and understand each other's expectations
2302   concerning the application of the enhanced approach in a regulated environment.
2303

2304   ## 9.5.   Business Case for Patient

2305   The business case from the patient's perspective for the enhanced approach is shown by the
2306   mind map in Figure 9-2. The key levers identified for the patient are: reduced cost of treatment,
2307   availability of treatment supplies, reproducibility and consistency of the drug product, assurance
2308   that the product is safe and efficacious, and the highest consistent quality of the product. The
2309   improved patient value delivered through the enhanced approach may not be readily apparent
2310   to the individual consumer. In general, patients benefit directly from the value delivered to the
2311   regulators and manufacturers. Thus, no further work was done specifically on the patient
2312   business case.
2313

2314   **Figure 9-2: Mind Map of Business Case Levers for Patient**
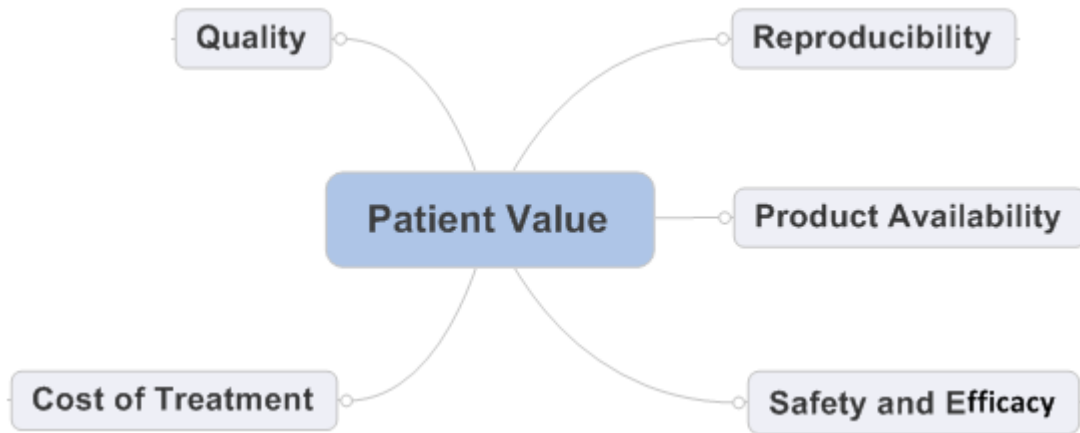
2315
2316
2317   The enhanced approach could become a hardship for consumers if the additional workload
2318   substantially slows the development of new therapies or unreasonably limits regulatory
2319   approval of products already produced with the traditional approach.
2320

2321   ## 9.6.   Business Case for Manufacturer

2322   The business case for applying the enhanced approach to vaccine development was constructed
2323   from the vaccine manufacturer's perspective. The thought process used was first to identify and
2324   prioritize the appropriate levers, then to determine the benefits and advantages of the
2325   enhanced approach for the levers specific to vaccine process development. Next, the
2326   implementation costs associated with the enhanced approach were evaluated. Comparing these
2327   costs along with the benefits, a vaccine-specific manufacturer's business case was constructed.
2328

2329   The levers impacting vaccine manufacturers were brainstormed based on the experience of the
2330   team members involved, and are depicted in a mind map (Figure 9-3). It is recommended that a
2331   company- and product-specific value stream brainstorming exercise be conducted in a cross-
2332   functional manner using risk management principles.
2333
2334   **Figure 9-3: Mind Map of Business Case Levers for Manufacturers**
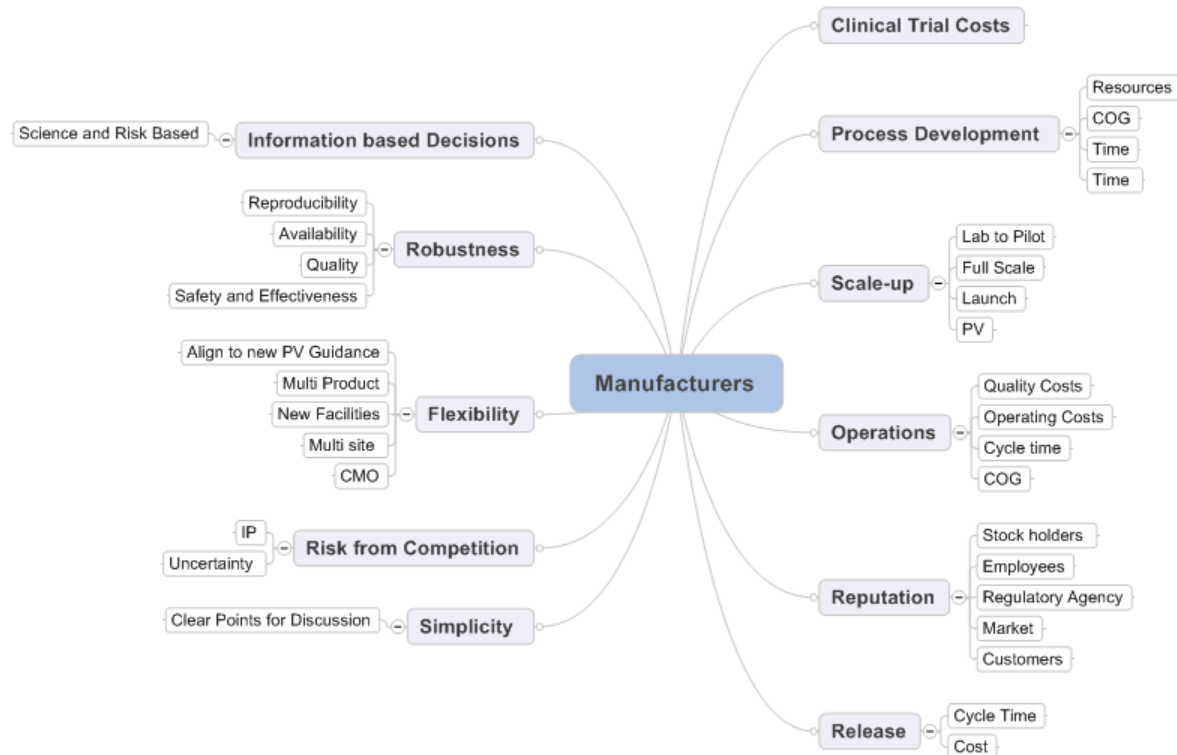
2335
2336
2337   Each of the 11 resulting lever categories was defined to assist in subsequent priority ranking
2338   (Table 9-2).
2339
2340   **Table 9-2: Description of High-Level Levers for Manufacturers**

High-Level Lever for Manufacturer	Definition
Release	• Release test selection (safety, efficacy, physicochemical characteristics, development), qualification during the course of development and validation, specification setting • Real-time release technology; batch release process simplification (electronic batch release process)
Flexibility	• Process definition achieved to maintain a high level of compliance/quality while being able to make changes within predetermined limits on the shop floor; streamline change control • Greater regulatory flexibility; ability to implement changes with

High-Level Lever for Manufacturer	Definition
	minimum regulatory burden and expedited time to approval
Clinical Trial Costs	• Ability to ensure clinical material is of the quality required to meet patient needs • Improved product CQA understanding in the clinic • Reduction of clinical bridging studies
Robustness	• Capability of the process to maintain acceptable ranges of quality and process attributes while operating within the predefined design space • Better assess manufacturability and achieve process/method reliability.
Operations	• Technical procedures driving production, release, and supply of product • Reduce or eliminate number of reworked batches, failures, atypical, OOSs, etc.
Process Development	• Ability to define the production methods, equipment, operating ranges, and specifications (process, product) that can be transferred into a manufacturing environment • Knowledge management - capture the associated know-how and know-why
Scale-up	• Ability to use appropriate scale-down models and comparability methodologies to develop successful large-scale procedures and operating ranges
Simplicity	• Similar to flexibility; greater process understanding and ability to relate inputs to resulting outputs
Reputation	• Capacity to speed up registration through strong partnership with agencies • Best-in-class supply chain (shorter cycle time, no recalls, etc.)
Risk from Competition	• Potential patent protection, ability to get to market (licensed) on time
Information-Based Decisions	• Data-driven decisions for process/analytical development, product release/resolution of atypical and overall scientifically sound decision-making processes

2341
2342   Prioritization ranking was accomplished, again based on the vaccine development and
2343   manufacturing experience of the team members involved, followed by team discussion, and
2344   documentation of the rationale behind the designated priority estimation (Table 9-3). During
2345   the prioritization, similar lever categories were merged to obtain seven remaining levers.
2346

2347 **Table 9-3: Prioritized Drivers for Manufacturers and Associated Rationale**

Business Case Lever	Estimated Priority	Rationale
Robustness	High	Prevention of process drift and improved capability for CQAs ensure product availability.
Process Development (PD) Scale-up	High	Defined steps for PD ensure effective experiments are executed. Better process understanding and therefore simpler tech transfer/scale-up.
Flexibility/Simplicity	High	Improved facility utilization. Opportunities for process improvement/adaption, transfer, multi-products use, and comparability.
Information-Based Decisions	Medium	Management decisions based on process and product knowledge improve success rate.
Clinical Trial Costs	Medium	This is the most expensive part of development, so any opportunity to improve success rate has high return.
Release/Operations	Medium	Release costs are high as a result of nature of test, amount of testing, and timing for release. Enhanced approach with parametric release can allow simplification of release process. Enhanced approach application during development definitively simplifies operation on a daily basis (less nonconformances, less out-of-specifications, parametric release).
Reputation/Risk from Competition	Low	Companies embracing QbD may be able to demonstrate success and improved value.

2348
2349 The example prioritization criteria, although not formalized, were considered effective since
2350 there was a reasonable split among all three priority levels. The three high-priority levers were
2351 robustness, process development/scale-up, and flexibility/simplicity. The three medium-priority
2352 levers were information-based decisions, clinical trial costs, and release/operations. The sole
2353 low-priority lever was reputation/risk from competition. All levers were considered important,
2354 regardless of their ranked prioritization. Individual companies should complete this evaluation
2355 for each unique application.
2356
2357 The benefits of the enhanced approach were developed specifically for the high-priority levers
2358 only, and compared with drawbacks and pain points of traditional approaches (Table 9-4). Direct
2359 benefits largely were related to COGs and impacted high-priority levers such as robustness. Low
2360 COGs was particularly important to vaccine manufacturers to enable more global access to
2361 vaccines. Indirect benefits largely were intangible and impacted lower priority levers such as
2362 reputation. Intangible elements were particularly important to vaccine manufacturers because
2363 perceptions may reduce sales of vaccines, limiting illness prevention in target populations.
2364

2365   **Table 9-4: Comparison of Traditional and Enhanced Approaches for Vaccine Development for**
2366   **the Key Levers for Manufacturers**

	Traditional	Enhanced
Robustness	• Interaction and impact of parameters not always explored	• Experiments and data from laboratory and nonclinical studies are used to derive specifications • Interactions are better understood • Less sensitivity to raw material and parameter input variations
Process Scale-up/ Development	• Manufacturing constraints not always integrated in the early development • Studies linked to development are process specific; transfer of data across multiple unit operations is rare • Work from laboratory experimental design is not always predictable; leads to resource and cycle time constraints	• Use of appropriate DOE and other statistical models allows appropriate key specifications linked to the target product/process to be derived; this also eventually offsets the upfront increase in cost of development • Better understanding of multivalent interactions (first order, second order, etc.) • Better use of PAT models
Flexibility/ Simplicity	• Limitations around changes and process improvements • Licensure-based changes lengthy in some cases • Limited risk assessments	• Potential changes can be made within the design space without need to extensive change control and regulatory oversight • Simplified comparability protocol or technical transfer

2367
2368   The costs of the enhanced approach for vaccines were the pre-investment — specifically, the
2369   effort and time involved implementing and performing enhanced approach activities. Most of
2370   the additional cost was associated with the following three tasks: establishment of a
2371   multivariate design space, adoption of advanced control strategies such as PAT, and
2372   performance of extensive analytical characterization. Since the extent of application of the
2373   enhanced approach for a vaccine was readily customizable based on previously identified risk
2374   areas, these costs were able to be readily managed to ensure a sufficient level of derived
2375   benefit.
2376

2377   Costs and benefits were then compared to develop the business case. This comparison was
2378   considered in the general sense here, and more specifically in a later section. A few key
2379   principles were noteworthy: (1) Since enhanced approaches were an investment by
2380   development for manufacturing, the same part of the company did not always both spend the
2381   resources and reap the benefit. (2) Notable quality or supply interruption tended to limit vaccine
2382   sales more than expected based on the vaccine manufacturing costs associated with these
2383   events. (3) Most of the world has limited access to vaccines compared with the developed
2384   world. These three principles suggest that that application of enhanced approaches has been
2385   different for vaccines compared with other pharmaceutical products, and also likely different for
2386   specific vaccines.
2387

9.7.   Business Case for Regulator

2389   The business case for applying the enhanced approach to vaccine development also was
2390   constructed from the vaccine regulator's perspective. The thought process used to identify and
2391   prioritize the appropriate regulatory levers was similar to those used for the manufacturer's
2392   business case. For the regulator's business case, the assessment of advantages of the enhanced
2393   approach includes a focus on scientific knowledge desired to maintain or improve the vaccine's
2394   safety and efficacy for the patient. A comparison of the costs together with the benefits to the
2395   regulator's business case was constructed.
2396

2397   The levers impacting regulators also were brainstormed based on the experiences of the team
2398   members from the working group and categorized using a mind map (Figure 9-4).
2399

2400   **Figure 9-4: Mind Map of Business Case Levers for Regulators**

2401
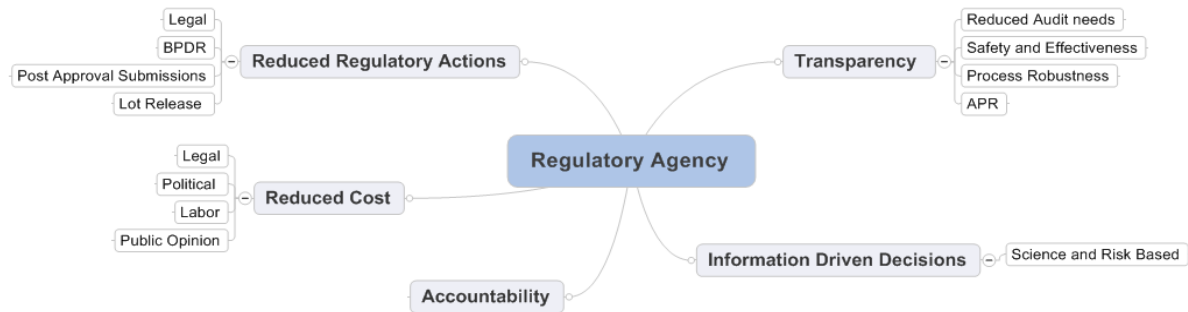
2402
2403
2404   Each of the five resulting lever categories was defined to assist in subsequent priority ranking
2405   (Table 9-5).
2406

Table 9-5: Description of High-Level Levers for Regulators

High-Level Lever for Regulator	Definition
Reduced Regulatory Action	• Interactions with regulatory agencies during development and post-licensure, including annual inspections and post-licensure amendments • Fewer inspections both PAI or general GMP • Classification of submissions from PAS to CBE-30 or CBE or to annual reportable • Reduced review time due to transparency of decision rationale and associated knowledge • Refocus resources to reduce oversight on lower-risk products/processes in favor of higher ones
Reduced Cost	• Lower costs resulting from "for-cause" inspections • Reduced cost associated with scheduled inspections and submissions/review for manufacturers and regulators • Enhanced approach filing could reduce filing review effort when submitting process changes within design space, etc.
Accountability	• Responsibility for decisions during development and care of the process post-licensure clarified for the regulators • Enhanced approach filing would help demonstrate that a reasonable level of product/process knowledge has been generated • Regulators assuring public that manufacturers met regulations for vaccine production. Enhanced approach filing provides regulators with knowledge they need to make their assessments.
Transparency	• Overt linkage of decisions made by manufacturers during development and post-licensure to prior knowledge or data for the current process • Encourages manufacturers to develop open and honest knowledge-driven relationship with regulators regarding inspections and submissions on manufacture of vaccine • Manufacturers notifying regulators if a problem or concern exists with vaccine production & distribution and the extent of its impact based on enhanced product/process understanding
Information-Driven Decisions	• Linking decisions to sound science based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale

2409   Prioritization ranking was accomplished based on informal interactions and discussion with
2410   regulators and manufacturing experiences of the team members involved, followed by team
2411   discussion and documentation of the rationale behind the designated priority (Table 9-6).
2412
2413   The regulator's business case for the enhanced approach offers some attractive advantages
2414   driven by the improved "ability to predict" from the knowledge developed from the enhanced
2415   over the traditional approach. Regulators can use the value stream approach presented to
2416   consider which applications might benefit from the additional investment in the enhanced
2417   approach. The traditional approach to process changes and product development often can be
2418   an effective path for managing product life cycle. However, some processes do not benefit as
2419   much as others from the additional knowledge provided by the enhanced approach to be
2420   robust, cost effective, efficacious, and safe. Considerations regarding the enhanced approach
2421   should be evaluated along with the expected value returned to regulators. Each project using
2422   the enhanced approach offers regulators, as well as manufacturers, unique opportunities and
2423   oversight challenges.
2424
2425   The approach outlined offers regulators a tool to prioritize important value stream goals relative
2426   to the specific situation under evaluation. In the case of a new or first-in-class vaccine, when
2427   prior knowledge is relatively low, regulators might highly value the improved transparency and
2428   clear information-driven decisions associated with the extensive process development of
2429   enhanced approach and thus be willing to invest additional resources to help guide
2430   manufacturers toward aligned expectations.
2431
2432   In the case where a manufacturer is entering a well-established market where there is a large
2433   body of prior knowledge, regulators may highly value the focus of the enhanced approach on
2434   risk-based knowledge gaps, ensuring robustness for this commodity product. Product entry into
2435   this market might offer competition and pricing benefits to patients, and the enhanced
2436   approach could minimize cost increases for regulatory agency oversight by ensuring process
2437   robustness. The enhanced approach offers additional knowledge that may offer manufacturers
2438   and regulators an improved ability to predict performance (and thus reliable resupply), but to be
2439   part of a sustainable business model, this approach must offer benefits over the traditional
2440   approach to both parties.
2441
2442

2443   **Table 9-6: Prioritized Levers for Regulators and Associated Rationale**

Business Case Lever	Estimated Priority	Rationale
Reduced Cost	High	• Fewer supply interruptions and associated oversight actions • Cost associated with reduced number of "for-cause" inspections and submissions reviews • Enhanced approach filing could reduce review times when submitting process changes within design space, etc.
Reduced Regulatory Action	High	• Effective and consistent interactions with manufacturers during development and post-licensure • Risk-focused approval and general GMP inspections • Fewer supplements by re-classification of some post-licensure submissions from approval supplements to annual reports
Information-Driven Decisions	High	• Linking decisions with scientific judgment based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale
Accountability	Medium	• Clear process decisions during development and planning for process verification post-licensure • Enhanced approach filing would help demonstrate that best effort for product/process knowledge has been generated • Enhanced approach filing would provide regulators with product and process knowledge they need to make assessments
Transparency (incorporated in information-driven decisions lever)	Low	• Linking decisions with scientific judgment based on available knowledge and understanding • Transparent justification of decisions with supporting data and risk-based rationale • Enhanced approach filing would provide regulators with product and process knowledge they need to make assessments

2444
2445   The prioritization criteria, although not formalized, were the same as those used for the
2446   manufacturer's drivers. It was considered effective for the regulator's drivers since there was a
2447   reasonable split among three priority levels. The three high-priority levers were: reduced cost,

2448   reduced regulatory action, and information-driven decisions. The medium-priority lever was
2449   accountability. The low-priority lever was transparency. When the team revaluated these
2450   decisions, it was decided that Transparency was not a separate category, since it provided
2451   overlapping benefit within the information-driven decision lever. All levers were considered
2452   important, regardless of their ranked prioritization. The costs and benefits of the enhanced
2453   approach were developed specifically for the high-priority levers for manufacturers and for
2454   regulators. These were combined and compared with drawbacks and pain points of traditional
2455   approaches for vaccines (Table 9-7).
2456
2457   **Table 9-7: Comparison of the Traditional and Enhanced Approaches for Vaccine Development**
2458   **for the Key Levers for Regulators**

	Traditional	**Enhanced**
Operations	• Supply to market is sometimes uncertain because of slower resolution of manufacturing and testing issues • Product release and in-process controls based on battery of tests that are redundant in some cases, leading to increased cycle time and higher costs • Release based primarily on attribute testing	• Improvements can be made by reducing testing and utilization of key inputs linked to PAT models, cycle time, and oversight costs • Better (product/process) understanding of why certain procedures are being implemented • Improved process understanding and more well-characterized products leads to better evaluation of the impact of optimization and flexibility changes
Cost of Product Regulatory Oversight	• Periodic process redesign at development and commercial scales (analytical and clinical comparability), resulting in more complicated filings	• Potential improvements in product/process understanding, leading to fewer development iterations through licensure and straightforward development history • Process knowledge and design space provides clear guidance for determining quality impact of deviations from normal operating range • Human and physical resource savings required to assess manufacturer's provided information because of improved transparency

Information- Based Decisions	• Knowledge and technology transfer to manufacturing not always efficient because of fewer direct links with identified risks	• Decisions can be traced to supporting data and risk-based rationales for reviews throughout product life cycle • Improved transparency of experimental work, since development data is readily accessible for review

2459

2460 The benefits of the enhanced approach require a high degree of collaboration and exchange of
2461 information between the manufacturer and the regulator to attain the ultimate goal of
2462 providing a safe and efficacious vaccine product. A few of the benefits highlighted in the
2463 comparison of the traditional and enhanced approaches for both the manufacturers and
2464 regulators are: (1) better understanding of certain procedures being implemented for the
2465 product and process, (2) possible reduction in testing based on PAT models, (3) potential cost
2466 savings of human resources for assessment of information, and (4) potential to avoid repeats of
2467 earlier experimental work, since developmental data is more readily available. All of these
2468 benefits for the enhanced approach are obtained only by the manufacturers and regulators
2469 partnering and gaining an understanding from each other linked to the application of the
2470 enhanced approach in a regulated environment.

2471

2472 Costs of the enhanced approach increase if the manufacturers do not partner with the
2473 regulators and provide the initial pre-investment for implementation of the enhanced approach.
2474 The pre-investment cost for the enhanced approach consists of the time and effort for
2475 regulators to understand the implementation of enhanced approach activities in a regulated
2476 environment. These initial costs to regulators could relate to understanding: (1) the impact of a
2477 manufacturer's changes within the design space on the vaccine product, (2) changes in
2478 regulatory submission information from the manufacturer when initially implementing the
2479 enhanced approach, and (3) whether changes to improve processes or the product impact
2480 previous product/process characterization work conducted for that product. Partnering
2481 between the manufacturers and regulators for initial implementation of enhanced approach
2482 decreases the costs to both parties. The collaborative exchange of information outweighs start-
2483 up costs over time and results in an improved vaccine product, maintaining the safety and
2484 efficacy of the product as the ultimate goals.

2485

2486 ## 9.8.   Specific Business Cases for Implementation of the Enhanced
2487 Approach

2488 A relative rating system was constructed to evaluate implementation costs and enhanced
2489 approach benefits.

2490

2491   **Equation 9-1: Relative Return on Investment (ROI factor)**
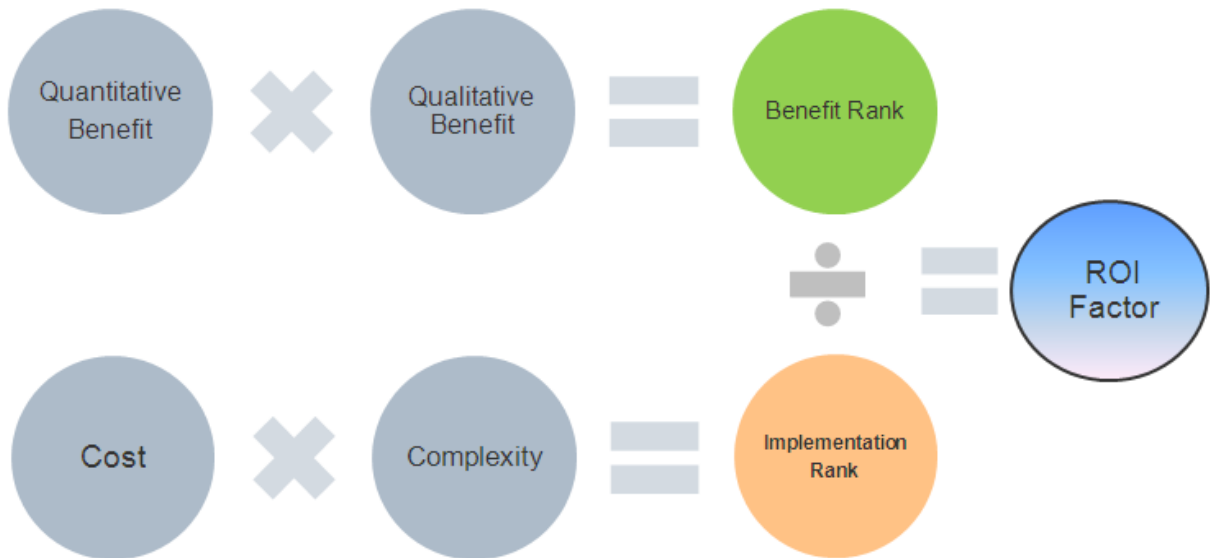
2492
2493   The relative return on investment (ROI factor) was defined as: ROI factor α [benefit
2494   rank/implementation rank], where the benefit rank was defined as: Benefit rank = [quantitative
2495   x qualitative], and the implementation rank was defined as: Implementation rank = [cost x
2496   complexity].
2497   Quantitative benefits were easily calculated savings, such as lower OOS costs and fewer failed
2498   batches. Qualitative benefits were harder to quantify and included good will with patients and
2499   regulators.
2500
2501   For the enhanced approach, a ranking score that includes the relative quantitative benefit is
2502   multiplied by the relative qualitative benefit to obtain a benefit ranking. A score of 1 represents
2503   the least relative benefit rank, whereas a score of 25 represents highest relative benefit rank.
2504   Thereby, the relative benefit rank for the enhanced approach (vs. the traditional approach) can
2505   be evaluated for the degree of benefit.
2506

2507   **Table 9-8: Benefit Rank Definition**

5 (very high)	**5**	**10**	**15**	**20**	**25**
4 (high)	**4**	**8**	**12**	**16**	**20**
3 (same)	**3**	**6**	**9**	**12**	**15**
2 (low)	**2**	**4**	**6**	**8**	**10**
1 (very low)	**1** (very low)	**2** (low)	**3** (same)	**4** (high)	**5** (very high)

Increasing qualitative benefit (vertical axis label)

Increasing quantitative benefit

Rank Score	Comments in terms of benefit ranking
15 to 25	Operations with high benefit – Flexible/favorable benefit ranking - High.
7 to 12	Operations with average benefit – Moderate benefit ranking - Med
1 to 6	Operations with less or negative benefits – Marginal benefit ranking - Low

2508
2509   Cost was defined as including cost of staff, equipment, and other materials for the process and
2510   associated analytical development, as well as production for that activity. It also included the
2511   time for the activity. For the examples in this case study, costs of additional clinical studies that
2512   might specifically be needed to support the enhanced approach were excluded, the base cost
2513   was the traditional cost, and the enhanced approach was believed to be able to lower as well as
2514   raise net costs, depending on the specific situation.

2515
2516   Complexity was defined according to whether the activity is new (not been done by any
2517   organizations to the best of our knowledge), unique (been tried by only a few companies, and
2518   only a few have had success), difficult (been tried by many companies and generally has had
2519   several challenging aspects), or simply semi-complex or noncomplex (routine).

2520
2521   For the enhanced approach, a ranking score that includes the relative level of complexity
2522   associated with the implementation is multiplied with the relative costs for implementation to
2523   obtain an implementation ranking. A score of 1 represents the least relative implementation
2524   rank, whereas a score of 25 represents highest relative implementation rank. Thereby, the
2525   relative implementation rank for the enhanced approach (vs. the traditional approach) can be
2526   evaluated for the ease of implementation.

2527

2528   **Table 9-9: Implementation Rank Definition**

Increased complexity of implementation		1	2	3	4	5
	5 (cutting edge)	5	10	15	20	25
	4 (unique)	4	8	12	16	20
	3 (difficult)	3	6	9	12	15
	2 (semi-complex)	2	4	6	8	10
	1 (noncomplex)	1	2	3	4	5
		1 (0.6 X base cost)	2 (0.8 X base cost)	3 (1 X base cost)	4 (1.25 X base cost)	5 (1.5 X base cost)
		Increasing Cost				

Rank Score	Comments in terms of implementation ranking
1 to 5	Operations with ease of implementation – Flexible/favorable ranking - High
6 to 12	Operations with average ease of implementation – Moderate ranking - Med
15 to 25	Operations with implementation linked to increased documentation practices – Marginal ranking - Low

2529

2530
2531   **Table 9-10: ROI Factor Definition**

Benefit Rank		5	10	15	20	25
	25	5	2.5	1.67	1.25	1
	20	2.5	2	1.33	1	0.8
	15	3	1.5	1	0.75	0.6
	10	2	1	0.67	0.5	0.4
	5	1	0.5	0.67	0.25	0.2
		5	10	15	20	25
		Implementation Rank				

Based on the above ranking system, relative ROI factors were obtained and interpreted according to the following framework:

Figure 9-5: Rank Score for Relative ROI
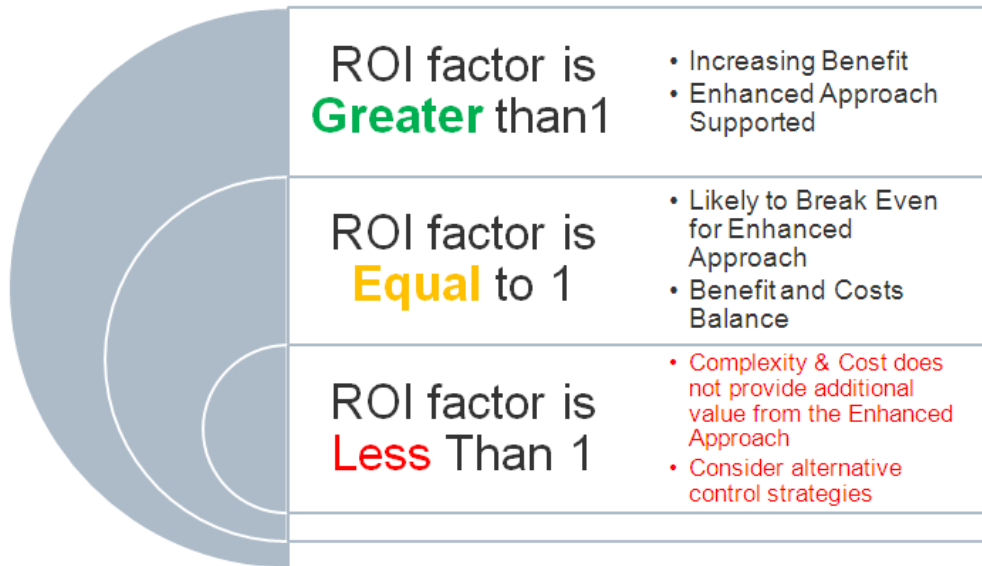
>1 ROI factors that were greater than 1 represented a benefit rank greater than the implementation rank and were more likely to produce gains.

=1 ROI factors that were about 1 represented a benefit rank about equal to the implementation rank and were considered to be "break even."

<1 ROI factors that were less than 1, represented a benefit rank that was less than the implementation rank and were less likely to produce gains (and may produce losses).

The ROI factor approach was next applied to establish specific business cases for three example steps or activities from the A-VAX case study. The examples selected were:

- Scale-up of a virus-like particle (VLP) conjugation time reduction by five hours, increasing manufacturing capacity of 24x7 operating plant by 20% for bottleneck process step
- Source change for enzyme for polysaccharide extraction to reduce cost by 5% by improving enzyme purity
- Site change for drug product lyophilization to increase industrial capacity

For each example, it was first decided whether it was appropriate to evaluate based on the aggregate activity or to divide the analysis into sub-activities (i.e., scale-up, tech transfer, validation, licensure) to evaluate the incremental ranking. If sub-activities were invoked, then the implementation investment was credited for subsequent activities, resulting in lower ROI factors. The individual, incremental ROI factors can then be averaged with appropriate weighting (not done here) or compared directly in a decision analysis.
Factors such as process development, technology transfer and scale-up, process validation, batch processing, and release of product were mapped to specific manufacturer and regulator

2548   levers from Table 9-3 and Table 9-6. Relative rankings were based on quantitative/qualitative
2549   benefits and complexity/costs for implementation expected when the enhanced approach was
2550   applied for the example activity.
2551
2552   **Scale-up of VLP conjugation time reduction example**
2553   The VLP conjugation process scale change example, when considered in four incremental
2554   activity stages, showed benefits of the enhanced approach in these stages. These steps reduced
2555   VLP conjugation time by five hours, increasing manufacturing capacity of 24x7 operating plant
2556   by 20% for bottleneck process step. The example clearly illustrated that every development
2557   activity showed benefit when using the enhanced approach. The ROI factor was higher than 1 in
2558   all cases (ROI factor = 1.2, 2.2, 1.8, 3.3) and generally increased for the activities positioned
2559   closer to commercial manufacturing. Although the implementation cost was higher for the early
2560   activities, even then there was a favorable ROI factor.
2561
2562   **Table 9-10a: ROI Factor Rankings for Scale-Up of the VLP Conjugation Time Reduction**
2563

Key Levers (High-Priority Levers)		Step or Activity Example	Effort Level of Complexity	Implementation Cost	Implementation Rank[a]	Quantitative Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (Benefit/ Cost)
Business	Regulatory								
Process Dev't scale-up	Info-driven decisions	VLP conjugation-scale-up	3 Not much different	4 Added cost of dev't)	12	4 Improved scale-up success	4 More information available for the process	16	1.3 Despite higher costs, ROI factor still favorable
Process Dev't scale-up	Info-driven decisions	VLP Conjugation-tech transfer	3 Process remains the same	3 No additional investment	9	5 Additional dev't work supporting tech transfer	4 Helps achieve prerequisites for launch	20	2.2 ROI higher since leverage scale-up investments
Process Dev't scale-up	Info-driven decisions	VLP conjugation-process validation	3 Process remains the same	3 No additional investment	9	4 Fewer runs overall during PV than tech transfer	4 Helps achieve prerequisites for launch	16	1.8 ROI reduced since less benefit in PV of leveraging scale-up investment
Process Dev't scale-up	Info-driven decisions	VLP conjugation-licensed operation	3 Process remains the same	2 Lower costs of enabling licensed operation	6	5 Realize full benefit of investment (e.g., reduced losses, cycle time improvements)	4 Improved customer satisfaction	20	3.3

2564

2565

2566   **Enzyme source change example**

2567   This analysis also can be used to compare different change proposals to rank their expected ROI
2568   factors, helping to prioritize them. An example of the value stream analysis for three possible
2569   approaches for enzyme replacement source is shown below in Table 9-11B. The enhanced
2570   approach using small-scale DOE models provides the highest potential benefits (ROI factor = 6).
2571   This indicated that the value returned to stakeholders was higher than the traditional approach
2572   of full-scale process development and process validation (ROI factor = 0.8). Thus, there is clear
2573   advantage to implementing the enhanced approach for the enzyme replacement with a
2574   recombinant enzyme source.
2575
2576   An intermediate scenario was also explored because the enzyme replacement was a
2577   recombinant version of the enzyme rather than just an enzyme supplier change using a similar
2578   manufacturing process. The enhanced approach relies on application of product and process
2579   knowledge from the DOE used to determine the design space for the nonrecombinant enzyme
2580   at the small scale. Rather than checking the equivalence of the current and new enzymes at
2581   reference manufacturing-scale process conditions, the enhanced approach addresses whether
2582   the design spaces for the two enzymes overlap in the qualified scale-down model. The ability of
2583   small-scale models to predict manufacturing scale process performance with the recombinant
2584   enzyme is a critical consideration because the licensed design space was demonstrated with the
2585   nonrecombinant enzyme source.
2586
2587   Risk assessments should consider the potential for scale-up risk based on the small-scale model
2588   qualification and recombinant enzyme DOE studies. A compromise approach may be needed,
2589   where enhanced process development is performed at small scale but results are verified at full
2590   scale to confirm the recombinant enzyme design space. In this case, the value returned is much
2591   less (ROI factor = 1.3 vs. 6 without full-scale verification). This reduction was driven by the
2592   additional costs of a full-scale run and the lost opportunity for manufacturing runs while the
2593   facility is changed over for engineering or validation run activities. However, the scale-up
2594   uncertainty was mitigated and some value increase remained over the traditional approach,
2595   where traditional full-scale process validation was required because the ROI factor increased by
2596   0.5 over the traditional approach ROI = 0.8.
2597
2598   When considering risk, manufacturers must balance their approach so that the project can be
2599   successfully delivered in an acceptable amount of time. Every project involves some risk and
2600   uncertainty that must be considered and mitigated by project teams. To provide value, teams
2601   cannot mitigate against all uncertainty, so teams must manage some level of residual risk for all
2602   projects. When considering the enhanced approach, teams must not only consider the risks they
2603   are mitigating, but also the additional value returned over more traditional methods.
2604
2605   In the enzyme replacement example, the team decided to execute the enhanced approach
2606   because the risk associated with enzyme replacement was relatively low and the step was well-
2607   understood and -documented through its established design space. Using this approach, an ROI
2608   factor as high as 6 was possible if the team was confident about its approach and could defend
2609   its rationale to regulators.
2610
2611   When risk is low, performing unnecessary full-scale activities causes the ROI factor to drop
2612   significantly because of the cost of full-scale runs and the lost opportunity for manufacturing

2613 runs while the facility is changed over for engineering or validation run activities. In this case,
2614 the project team is doing much more work than needed for success. The incremental reduction
2615 in risk comes at a significant reduction in value returned (ROI factor = 1.3). The longer
2616 implementation timeline and lower ROI factor may force the company to consider abandoning
2617 the improvement altogether, unfortunately providing no value to stakeholders.
2618
2619 When used with risk management tools as outlined in ICH Q9, the value stream approach can
2620 help prioritize risk mitigation projects to ensure that implementation of the enhanced approach
2621 retains sustainable value.
2622
2623 **Table 9-11B: Manufacturers' ROI Factor Rankings for Enzyme Source Change**

2624

Manufacturers' ROI Estimate:
Enzyme Source Change with Enhanced Approach and Process Monitoring Verification

Key Levers (high priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2 Investment in small scale DOE in initial filing	1 No FS runs, but higher costs for DOE	2	4 Reduced RM cost	3 Improved purity and consistency	12	6
Complexity: Low for DOE small scale studies Imp Cost: Low for DOE studies in comparison with full scale Development runs but DOE costs still higher than traditional small scale experiments					Benefits: Faster realization of reduced raw material costs & improved purity/consistency over original enzyme source which may also improve process robustness				

2625
2626

2627

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	3 Added facility change over	3 FS run cost & lost facility time	9	4	3	12	1.3
Complexity: Increases for full scale verification because of added logistics for manufacturing facility scheduling and change-over. Imp Cost: Increases for full-scale verification because of full-scale run cost and lost facility time.					Benefits: No additional benefits of adding full scale engineering run to Enhanced approach but introduces additional costs and complexity				

2628

2629

2630

Manufacturers' ROI Estimate:
Traditional Approach with Full Scale PV / Commercial

2631

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	4 Scheduling of manufacturing facility, lack of small-scale data	4 FS PV runs costs and down time of manufacturing facility	16	4	3	12	0.8
Complexity: Higher for traditional approach since potential lack of adequate small scale model for trouble shooting and optimization would require development studies and PV at full scale. Costs: Significantly higher because licensed manufacturing facility must undergo change over for full-scale development and PV runs, resulting in lost manufacturing time and increased materials cost.					Benefit: Unchanged benefits but added complexity and cost of traditional method reduce ROI for implementation of improved enzyme source change.				

2632

2633   **Table 9-12C: Regulator ROI Factor Rankings for Enzyme Source Change**

2634   This analysis also can be used by regulators to compare different change proposals and rank
2635   their expected ROI factors from the regulator's prospective. An example of the value stream
2636   analysis for the three possible approaches for enzyme replacement source is shown below in
2637   Table 9–11c. The enhanced approach using the qualified small-scale model to confirm the
2638   design space for the recombinant enzyme still provides the highest potential benefits from the
2639   regulator's prospective (ROI factor = 4). The regulator's cost in this case is lower than the
2640   manufacturer's ROI because regulators do not incur the costs associated with the process
2641   development and full-scale activities, the latter of which are avoided with the enhanced
2642   approach. The regulator's ROI factor still indicated that the value returned to stakeholders is
2643   higher than the traditional approach (ROI factor = 1.5). Thus, there appears to be clear

2644   advantage to implementing the enhanced approach for the enzyme replacement with a
2645   recombinant enzyme source, from both the manufacturer's and regulator's view points.
2646   The expected ROI factors for the intermediate scenario from the regulator's view also were
2647   explored. Since the enzyme replacement was a recombinant version of the enzyme rather than
2648   just an enzyme supplier change, there may be potential for scale-up risk, because the filed
2649   qualified scale design space was demonstrated with the nonrecombinant enzyme source. An
2650   assessment of risks associated with small-scale model qualification only with the
2651   nonrecombinant enzyme in this case may suggest that a compromise approach might be
2652   needed, where the enhanced process development is performed at small scale but the result is
2653   verified at full scale. The small-scale model design, qualification, and correlation with full-scale
2654   operations are not covered in this case study, but this information should be considered when
2655   evaluating the scale-up risk.
2656
2657   If properly executed and documented, the enhanced approach provides clear rationale and
2658   supporting data to reinforce the decision to proceed with the enzyme change without full-scale
2659   run verification. In situations where the regulator's risk assessment indicates that the small-
2660   scale model data is not sufficient, then a discussion of the potential risks and ROI factors
2661   achieved for each of the proposed scenarios might support a compromise positions. For
2662   example, the enhanced process development could be performed at small scale, with an
2663   engineering run conducted for full-scale verification, but once success is demonstrated, then
2664   traditional process validation would <u>not</u> be executed. The value returned to regulators and
2665   manufacturers in this case is less (ROI factor = 2.7 and 1.3), but value is still returned to
2666   all stakeholders.
2667
2668   The value stream tool introduced in this case study provides process knowledge and
2669   implementation data that can improve the decision process when considering where to
2670   implement the enhanced approach. Manufacturers and regulators are encouraged to use formal
2671   value determination tools, such as this one, to ensure efficient and effective resource utilization.
2672   Each application should be customized for the manufacturer, the regulator, and
2673   the product.

<div align="center">

Regulators' ROI Estimate:
Enzyme Source Change with Enhanced Approach and Process Monitoring Verification

</div>

2674

Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Review Resources and Time	Enzyme Source Change	2 DOE data reviews, trained resources	2 Focused review based on approved DS	**4**	4 Fewer investigations	4 Less supply interruptions	**16**	**4**

Enhanced Approach but with addition of a Full Scale Eng Run Verification

Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2	3 Additional data from eng run to review	**6**	4	4	**16**	**2.7**

Traditional Approach with Full Scale PV / Commercial

Key Levers (high priority levers)		Step or activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant benefit	Qualitative benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Process Development Scale-up	Reduced Resources and Time	Enzyme Source Change	2	3	**6**	3	3	**9**	**1.5**
					Benefits: Reduced owing to delay of enzyme change implementation owing to manufacturers' additional cost and complexity associated with Traditional Approach				

2675

Site change for drug product lyophilization

The site change for drug product lyophilization to increase manufacturing capacity was considered in one activity stage. Breaking down the discrete items, such as facility, technology transfer, and comparability elements, was not pursued because site transfer to use additional capacity is a current industry practice. However, the cycle times associated with such transfers are lengthy and equivalency models are not equally nor consistently applied. With the utilization of the enhanced approach employing design space concepts linked to equipment and product comparability, such changes are expected to be facilitated and associated effort with cycle development and validation exercises significantly reduced. Based on the analysis and descriptors above, the ROI factors based on relative benefit and implementation costs scores yields are favorable.

2676
2677
2678
2679
2680
2681
2682
2683
2684
2685
2686
2687

2688   **Table 9-13c: ROI Factor Rankings for Site Change for Drug Lyophilization**

Key Levers (high-priority levers)		Step or Activity Example	Effort Level of Complexity	Implement Cost	Implement Rank	Quant Benefit	Qualitative Benefit	Overall Benefit Rank	ROI Factor (benefit/cost)
Business	Regulatory								
Flexibility	Reduced regulatory action	Site change for DP	3 Process remains the same	3 Costs dominated by facility and equipment	9	5 Tangible gains from increased speed of transfer and approval	4 Meeting increased market demand reliably, patient supply	20	2.2 Validation then becomes more straight-forward

2689

2690
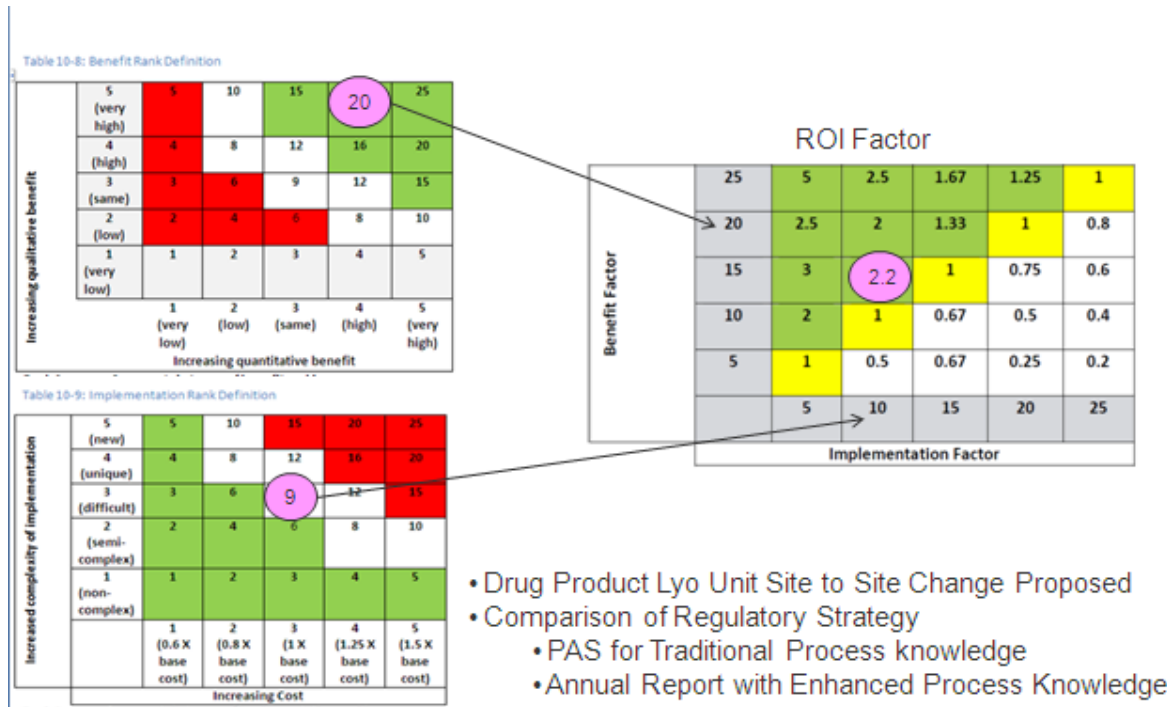
2691

2692   Overall, the main benefits of the enhanced approach for these three examples are: formalized
2693   assessment of risk, linkage of high-risk inputs to subsequent experiments for process
2694   understanding and/or subsequent control strategy, streamlined number of experiments through
2695   use of DOE, use of a scale-down model appropriate for manufacturing, and development of
2696   process models and quantification of variability to depict process understanding.

2697

2698   DOE experimental design was used in an integrated manner by linking studies to high-risk inputs
2699   and designing space studies with the goal of defining and understanding an appropriate design
2700   space. Consistent linkage also was made to proposed critical quality or key process attributes.
2701   Repeat of earlier work because of inefficient data and information (knowledge) management
2702   practices was minimized.

2703

2704   With an established design space that is relevant for manufacturing conditions, tech transfer is
2705   streamlined. Risk analysis is updated to generate prioritized experiments to fill identified gaps.

2706   Using the enhanced approach, facility and equipment specifications as well as process batch
2707   records are developed faster.
2708
2709   Sufficient scale-down model studies resulted in scale-up success. Classical three-lot process
2710   qualification at the center point was replaced by single-lot confirmation at scale. Process
2711   understanding resulted in reasonable ranges for eventual manufacturing, translating into fewer
2712   atypicals. Process validation effort (new FDA guidance: stage 1, 2, and 3) is reduced since many
2713   documents generated through the enhanced approach can be directly applied to these
2714   deliverables.
2715
2716   Overall, the main implementation investments for the enhanced approach for these three
2717   examples fall into two categories:

2718   •   Equipment — The appropriate type and number of scale-down systems are needed to
2719   permit DOE and other types of experimentation in a timely manner. Although high
2720   throughput and/or miniature systems are not required, their use would assist in maximizing
2721   information obtained during available timeframes, if that were desirable. Analytical
2722   equipment is needed to match the process equipment to provide prompt assessment of
2723   product/process quality.

2724   •   Business processes — The enhanced approach is not about generating more information;
2725   rather, it is focused on generating the right type of information. Effective business processes
2726   need to be established to promote accurate assessment of risk, robust experimental design,
2727   leveraging of prior knowledge, etc. Process and analytical scientists need to be prepared to
2728   spend additional time discussing and planning their work in a cross-functional manner, then
2729   evaluating whether the results obtained generate the appropriate product/process
2730   understanding.

2731   Of course, both of these categories require staffing. Whether it is more staffing or less staffing
2732   overall has been hard to ascertain. Many companies have staffing models where staffing
2733   estimates are incorporated. Many companies have time systems where staffing actual numbers
2734   are recorded. Few companies have been able to bridge the estimates to the actual within an
2735   accuracy of better than 10% to 20%. Thus, it can be difficult to evaluate changes in net staffing
2736   demand with the enhanced approach.
2737

2738   ## 9.9.   Business Case Customization Frameworks for Management
2739   Consideration

2740   Companies need to figure out their specific implementation costs and benefits, and ROI factors.
2741   The total capitalized cost estimate of a new product is $802 million (year 2000 dollars) as
2742   published by DiMasi et al. (2003), with a base case out-of-pocket cost per approved new drug of
2743 $402 million. Furthermore, it is estimated for small molecules that nearly 25% of the classical
2744   (i.e., traditional) pharmaceutical industry expenses are incurred in product manufacturing,
2745   where waste and sampling/yield losses can be as high as 50% and that 5% to 15% of product loss
2746   occurs in later phases of operations (Better by Design, Sven Stegemann. World Pharmaceuticals
2747   Frontiers. 2010. Volume 1. pp 76 to 78). Similar values might be applicable for vaccine
2748   manufacturing. Accordingly, the cost incurred through product loss in manufacturing can add
2749   significantly to the cost of goods and present limitations to effective product turnaround.
2750

2751   Based on the numbers shown above, the cost to bring an entity to market is significant.
2752   However, after such investments, in some cases performance at industrial platforms shows a
2753   wide variance in write-offs as a result of product waste and loss. Although losses are not broken
2754   down by category, the limitations of traditional models may account for a significant portion of
2755   such losses. In the traditional model, batches are tested at several stages in the manufacturing
2756   process (i.e., raw materials, in-process material, and end product) against a number of
2757   parameters and quality attributes. Where a batch does not meet a required specification, it is
2758   typically discarded as out of specification, resulting in product loss and unavailability, which can
2759   lead to patient supply constraints.
2760
2761   Quality and performance are achieved primarily by imposed compliance with limited flexibility in
2762   the manufacturing process. Product specifications may be derived using test data from a limited
2763   number of development batches, which is not always based on a statistically significant
2764   sampling and can be a source of variability. Under this framework, process success is linked to
2765   the inherent variability of the process and the type of validation strategy executed and the
2766   limited development (design) characterization detailed in the license.
2767
2768   In contrast, where applied, the enhanced approach has the potential to offer a method that can
2769   improve overall manufacturing performance, reduce cost of goods, and assure compliance
2770   across the defined design space. It represents a scientific, risk-based approach to
2771   pharmaceutical process and product development with deliberate design considerations across
2772   the product development life cycle to final commercialization. (Refer to the key drivers linked to
2773   implementation and benefit ranking for the enhanced approach.)
2774
2775   **Value Stream Decision to Go Beyond the Traditional Approach**
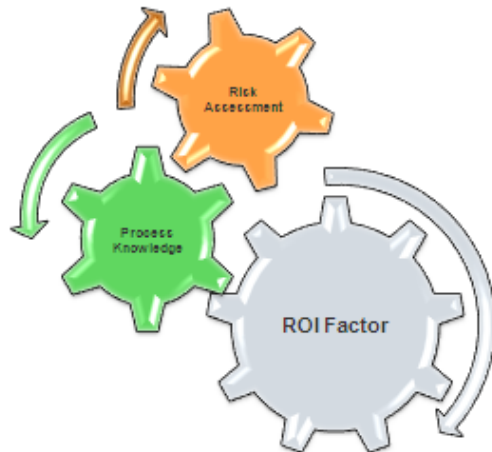
2776
2777   The decision to supplement the traditional approach is complex, and implementation tools such
2778   as those introduced here should be developed to help support effective investment in the
2779   enhanced approach. The ROI factor is just one element in the decision to implement the

2780   enhanced approach. A successful implementation strategy depends also on a rigorous
2781   demonstration of risk assessment rationale and process knowledge.
2782
2783   The enhanced approach may increase the upfront cost of development, but there will be ROI
2784   with better throughput for manufacturing operations, better efficiency, and more predictable
2785   controls via fewer deviations and reduced cost of goods (e.g., cycle time and reduced testing
2786   using PAT models). Application of the enhanced approach ensures predictability and the ability
2787   to consistently meet predefined product quality attributes by process control and
2788   understanding.
2789
2790   Furthermore, the enhanced approach promises to ultimately contribute to improving the safety
2791   of drugs compared with existing practices. With a product developed using the enhanced
2792   approach, there is continuous monitoring of critical parameters, and the ability to make changes
2793   to key process parameters based on feed-stream variability (e.g., raw material changes,
2794   equipment issues) is permissible based on data and scientific rationale. Also, control of
2795   operations is linked to technology-driven models where monitoring ensures the required
2796   product-critical attributes are achieved. It also provides efficiencies in investigations for out of
2797   specifications and allows for process simplifications.
2798
2799   An additional consideration is that the enhanced approach serves as the basis of a robust and
2800   detailed regulatory dossier. In that way, parameters and quality attributes that are linked to the
2801   clinical performance are understood. This linkage may allow for ease of implementation across
2802   sites when the necessary prerequisite elements are in place.
2803
2804   ## 9.10. Key Implementation References

2805   The price of innovation: new estimates, of drug development costs. Joseph A. DiMasi., Ronald
2806   W. Hansen, Henry G. Grabowski. Journal of Health Economics 22 (2003) 151–185
2807
2808   Better by Design, Sven Stegemann. World Pharmaceuticals Frontiers. 2010. Volume 1. pp 76 to
2809   78. http://www.worldpharmaceuticals.net/editors_choice_march10.htm

CMC-VWG

10.   Applying QbD to Live Vaccines (Upstream - LAIV)

2810

10.1. Introduction for Viral-Based Vaccine Upstream

2811

2812

2813   Vaccines based on viral components represent an important segment of the vaccines available
2814   on the market including influenza, poliovirus, and hepatitis A.

2815

2816   Because of their viral composition, these vaccines present process requirements that must be
2817   taken into account during their development to establish robust manufacturing. Such
2818   specificities include the biological complexity inherent in viruses, with an impact on the
2819   definition of suitable analytical tools for characterization, the use of several particular cell
2820   substrates susceptible to the virus to be produced (i.e., non-tumorigenic adherent cell lines such
2821   as vero cells), and the presence of certain process steps (e.g., production of viral seed stocks,
2822   viral infection and propagation steps during the production process).

2823

2824   These process constraints make the establishment of a process platform as for monoclonal
2825   antibodies' processes more challenging, with potentially less process history data and less prior
2826   knowledge to draw on in some cases.

2827

2828   Having these specificities in mind, the section of this case study dedicated to viral-based
2829   vaccines will illustrate how QbD methodology can be applied to their development. To illustrate
2830   this section, the proposed process is based on an adherence Madin Daby Canine Kidney (MDCK)
2831   cell line grown in static and dynamic conditions (microcarriers) using animal-free media
2832   formulations for the production of an influenza virus at the final bioreactor scale of 2,000 liters.

2833

10.2. Executive Summary

2834

2835

2836   The focus is put on specific process steps such as viral infection and the final cell growth in a
2837   bioreactor. It was decided not to address the question of tumorigenicity and adventitious agents
2838   in this case study because their control is complex and still relies on intensive testing of the
2839   different cell banks, viral stocks, raw materials, and process intermediate steps.

2840

2841   This section illustrates how to consider in parallel critical quality attributes (CQAs) and key
2842   process attributes (KPAs) during the development of a viral vaccine. A specific risk assessment
2843   methodology considering CQAs and KPAs is proposed.

2844

2845   It is also important to carefully consider the variability of the analytical tools used during the
2846   development of such a vaccine. Some assays in the early stages of the product development
2847   might present variability too high to be suitable for DOE applications. The proposed strategy to
2848   define the design takes into account this analytical variability.

2849

2850   This section also illustrates the use of one-factor-at-a-time (OFAT)/univariate analysis for some
2851   of the process parameters, such as media stability evaluation.
2852
2853   A methodology is proposed to ensure the definition of an efficient way to scale up the
2854   bioreactor scale with the establishment of a scale-down bioreactor model taking into account
2855   the specificity of microcarrier-based cell culture (impact on mixing and shear stress).
2856
2857

10.3. Process Description (Phase II Process)

2858
2859

2860   The production of an influenza virus on an adherent cell line has been chosen for the QbD
2861   analysis in this case study. A process flow diagram, as well as a brief description of the different
2862   process steps at the end of the phase II development, is presented in this chapter.

Unit operation 1

Cell thawing

Step 1

Unit operation 2

Cell expansion - static
T-flask and Multitray
37°C - 5% CO2
4 passages alternatively at day 3 and day 4

Step 2

Unit operation 3

Final cell growth - dynamic - 5 days
200 L Bioreactor
Micro-carrier 3 g/L Cytodex 1
Batch culture 37°C
pO2 20% - pH 7.2 - P 0.1 bar
Stirring 25 rpm
Daily glucose and glutamine adjustment

Step 3

Unit operation 4

Viral seed thawing →

Infection - Viral production - 5 days
MOI 10^{-3} - 33°C
200 L Bioreactor
Micro-carrier 3 g/L Cytodex 1
Batch culture
pO2 20% - pH 7.2 - P 0.1 bar
Stirring 25 rpm
Daily glucose and glutamine adjustment

Step 4

Unit operation 5

Harvest - clarification
Micro-carrier sedimentation
Supernatant collection
Micro-carrier washing
Centrifugation

2863
2864
2865

2866   Cell culture

2867   • Unit operation 1: **Cell thawing**
2868   The adherent cell line MDCK, stored in liquid nitrogen, is thawed at 37°C and seeded at
2869   20.000 cells/cm² in T175 cm² followed by incubation at 37°C with 5% CO_2 during four days.

2870   • Unit operation 2: **Cell expansion – static (multitray)**
2871   The production of the biomass necessary for the launch of the final bioreactor using an

2872   animal-free proprietary medium is assured by four cell passages performed every three and
2873   four days respectively at 20.000 and 15.000 cells/cm². Cells are detached with a non-animal-
2874   origin enzyme and incubated at 37°C with 5% CO_2.

2875 • Unit operation 3: **Final cell growth – dynamic culture in bioreactor**
2876   The production of the biomass required for the viral infection step in a stainless steel 200 L
2877   bioreactor is performed for five days. Cells are seeded at 150.000 cells/ml and grown on
2878   Cydotex 1 at 3g/L at 37°C. A daily glutamine (2 mM) and glucose (1 g/L) adjustment is
2879   performed. Bioreactor regulations are as follows:

2880 • Regulation: pO2 20% - pH 7.2 - P 0.1 bar.

2881 • Stirring: 25 rpm.

2882 • Viral production

2883 • Unit operation 4: **Infection and viral production**
2884   Infection is performed five days after the bioreactor seeding, when the cell density reaches
2885   at least 2.5×10^6 cells/ml. Growth medium is replaced by a viral production medium, and
2886   temperature is decreased to 33°C. The wild type influenza virus is activated by addition of a
2887   serine protease at 100IU for 30 minutes and added at a multiplicity of infection (MOI) of 10^{-3}
2888 ^{3}. Viral replication is allowed for five days with the same bioreactor regulations as for cell
2889   growth except for the temperature regulation, which is maintained at 33°C. Again a daily
2890   glucose and glutamine adjustment is performed as well as a daily addition of serine protease
2891   at 2IU/day for viral activation.

2892 • Unit operation 5: **Harvest and clarification**

2893 • This step is performed after five days of viral replication. The microcarriers are sedimented
2894   and the supernatant is harvested before clarification by centrifugation. The clarified harvest
2895   is then transferred for downstream purification.

2896 • This phase II process will be the starting point for the different analyses described in
2897   sections 3, 4, and 5.

2898

2899

10.4. Unit Operations Selected

The unit operations selected for this case study will be identified by ranking their theoretical impact on chosen critical quality attributes (CQAs) and key process attributes (KPAs).

10.4.1.   Identification of CQAs and KPAs

CQAs are output parameters linked to the quality of the product (safety and efficacy). Those considered for this case study are:

- Protein content: Total protein was chosen at this step of the process as a purity indicator that can be linked to host cell protein content.

- The virus integrity on crude harvest is assessed via the ratio hemagglutinin (HA) attached to the virus on total HA. The HA content is analyzed by SRD (single radial immunodiffusion), and the HA linked to the virus is measured after performance of an analytical sucrose density gradient.

Remark: Host cell protein and DNA are also critical at this stage. However, they are eliminated by the purification process steps. Purification of Phase I and II process was efficient enough to ensure a residual DNA and host cell protein content of the purified bulk significantly below the specifications. Therefore, the purification process capacity to ensure these impurities' removal will be checked after the Phase III process definition.

KPAs are output parameters linked to process consistency and business aspect (e.g., supply issue, production delay, cost impact). Those considered for this case study are:

- Antigenic titer (SRD): hemagglutinin (HA) content
- Cell density at the end of growth

10.4.2.   Selection of the Unit Operations

The tool used for this selection is a cause-and-effect matrix. The theoretical impact of each unit operation (= input) on each above identified critical attribute (= output) will be scored according to the table below. The theoretical impact is estimated within the conditions usually encountered.

Rank/Weight	Input Process Step to CQA and KPA
10	Strong relationship known
7	Strong relationship is expected/likely
4	Not-so-strong relationship or not expected
1	Known to not have a relationship

2934 It should be noted that an additional operation unit has been included (unit operation 2b: cell
2935 expansion – dynamic with a bead-to-bead transfer), as this additional step will be necessary to
2936 ensure the scale-up of the process.
2937 This scoring will lead to a ranking of the different operation units as shown in the following
2938 table. The rankings reflect the link between the unit operations (input) and critical attributes
2939 (output).
2940

	CQA		KPA		
	Total protein (HCP, DNA)	Virus integrity	Antigenic titer	Final cell density (end of growth)	Total
Unit operation 1: Cell thawing	1	1	1	7	10
Unit operation 2: Cell expansion - static (multitray)	1	1	5	7	14
Unit operation 2b: Cell expansion - dynamic (microcarriers, bioreactor)	5	1	5	7	18
Unit operation 3: Final cell growth - dynamic (microcarriers, bioreactor)	7	7	7	10	31
Unit operation 4: Infection - viral production (microcarriers, bioreactor)	10	10	10	na	30
Unit operation 5: Harvest and clarification	10	5	10	na	25

2941
2942 According to this analysis, three steps are identified as having more impact on the CQAs and
2943 KPAs. However, for illustration purposes, in this case study we will concentrate on two steps:
2944 final cell growth and infection and viral production.
2945

2946 ## 10.5. Identification of Prior Knowledge (from Work Done Prior to the End
2947 of Phase 2 and from Other Processes)
2948

2949 All parameters linked to the cell expansion in the stationary phase and in the bioreactor,
2950 including the bead-to-bead transfer, were developed to support other products and can be
2951 considered as referring to a generic process. This process platform was implemented for this
2952 project with only minor adjustments.
2953
2954 Following are parameters that were developed during phase II and will not require further
2955 optimization:
2956 • Composition of culture medium for cell growth and viral infection.
2957 • Bioreactor seeding density: 150.000 cells/ml was selected during screening of this
2958   parameter based on antigenic titer and cell density at the end of cell growth.
2959 • Cytodex concentration: Several concentrations were tested, and 3g/L was selected based on
2960   cell density at the end of cell growth and antigenic titer.
2961 • Temperature during cell growth and infection: For cell growth, the range 36–38°C was
2962   screened and showed no impact on antigenic titer and cell density at cell growth end.
2963   During viral replication, the range 32–34°C was studied, showing no impact on antigenic
2964   titer.
2965 • pO2 during cell growth and infection: Between 10% and 50%, the pO2 was shown to have
2966   no impact on growth and viral production.

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2967 • Pressure during cell growth and infection (has been taken from other cell culture processes
2968 using the same equipment)

2969 • Cell infection duration: It was optimized to maximize yield; the impact on total protein and
2970 hence specific productivity was not investigated.

2971 – Seed thawing is independent of final scale and was defined for other flu processes.

2972 10.6. Process Risk Assessment at the End of Phase II

2973 QbD is a continuous approach, and risk assessments will be performed all along the process. At
2974 the end of Phase II, the risks are based on the Phase II process and anticipation of the risks
2975 resulting from scale-up. The risk assessment will be repeated when the final-scale phase III
2976 process is developed.

2977 10.6.1. Identification of High-Risk Process Parameters (Phase II 200 L Scale)

2978 10.6.1.1. Lists of Parameters for Growth and Infection

2979 First, all parameters of the final cell growth and infection/viral production steps having a
2980 potential impact on the CQAs and KPAs have been listed using the fishbone matrix. Manpower,
2981 however, has not been evaluated because it is not considered to be specific for this case study.

2982
2983

Final growth on microcarriers

Method: bead to bead

- Cell detachment enzyme type, enzyme activity, enz. inhibitor, inhibitor conc, buffer composition, T, duration, pre-washing and washing
- Seeding cell density
- Additives
- Beads type and concentration
- Beads conditionning
- Inoc ratio
- Cell tranfer: pump speed/pressure, duration
- Cell attachment agitation, duration

Method/material: growth in bioreactor

- pH
- pO2
- T
- pCO2
- pressure
- Headspace aeration rate
- Sparger aeration (via pO2 regulation)

- Agitation speed: impeller
- Duration
- Scale-up
- Bioreactor design (engineering)
- Additives

CQA:
HCP
DNA
Total protein,
Virus integrity
KPA:
Cell density end growth
Antigenic titer (SRD)

- Raw material control
- Media preparation
- Media storage/shelf-life (T, duration, light protection)
- Media filtration
- Media preheating
- Medium concentration
- Medium composition
- Feed (time, volume, conc)

- Gas environnement

- Cell count / viability
- Metabolites
- pO2, pH, pCO2, T

Environment/ Raw material

Measure (in-process testing)

2984

Infection – viral production

Method: infection

- Cell characteristics (passages)
- Seed characteristic
- Seed thawing
- MOI
- Seed activation (concentration of protease and duration of action)
- Additives
- T
- Cell: density and physiological state

Method/material viral replication

- pH
- pO2
- T
- pCO2
- pressure
- Headspace aeration rate
- Sparger aeration (via pO2 regulation)

- Agitation speed: impeller
- Duration
- Scale-up
- Bioreactor design (engineering)
- Stabilizer

CQA:
HCP
DNA
Total protein,
Virus integrity
KPA:
Cell density end growth
Antigenic titer (SRD)

- Raw material control
- Media preparation
- Media storage/shelf-life (T, duration, light protection)
- Medium concentration
- Medium composition
- Feed (time, volume, conc) or/and perfusion (time, flow rate, conc)

- Cell count / viability
- Metabolites
- pO2, pH, pCO2, T

Environment/ raw material

Measure (in-process testing)

2985
2986

2987   **10.6.1.2.   Identification of Potential Critical Parameters**

2988   The tool chosen for this identification is called the FMEA approach (failure modes and effect
2989   analysis). The process parameters are ranked based on the RPN (Risk Priority Number). In this
2990   example of a process design phase, the knowledge is rated in the RPN to improve process
2991   understanding to assure a greater process robustness and manufacturability. The RPN is the
2992   product of four scores:
2993
2994   RPN = Severity * Knowledge * Occurrence * Detection
2995   The severity factor itself is the product of the impact of a process parameter on the critical
2996   attributes and the criticality of the attributes. Higher scores for severity and knowledge were
2997   used than for occurrence and detection because:

2998   •   RPN places less emphasis on the occurrence, which may not always be scored reliably
2999   because of the limited number of data sets available at the end of phase II.

3000   •   Detection still largely relies on in-process and release testing.

3001   Each step of the FMEA is described hereunder.
3002   Step 1: Scoring of process outputs
3003   Each CQA and KPA will be scored according to the following table.
3004

Rank / Weight	Critical Quality Attribute (CQA)	Key Process Attribute (KPA)
10	Established or expected direct relationship to product quality (safety or efficacy)	
5	No knowledge on the impact product quality (safety or efficacy)	High supply issue or discontinuity, business loss
3		Significant production delay, high cost impact, rejection of product
1	No product quality (safety or efficacy) impact expected	No consistency impact expected

3005
3006
3007   Step 2: Cause-and-effects matrix for severity calculation (S)
3008   For the two unit operations selected (cell growth and infection/viral production), all process
3009   parameters are listed and scored according to their relationship with the CQAs and KPAs (see
3010   following table).
3011
3012

Rank / Weight	Input Process Parameter to CQA or KPA
10	**Strong relationship known**
5	**Unknown relationship/ weak**
1	**Known to not have a relationship**

3013
3014 Only negative and theoretical impacts should be considered (and not based on knowledge of the
3015 process). The impact should also be evaluated within the conditions usually encountered.
3016 After this scoring, a severity S factor will be calculated according to the formula blow:
3017

$$S= \frac{\sum(\text{"CQA or KPA scoring" X "process parameter/CQA or KPA relationship scoring"})}{\text{"number of CQA or KPA"}}$$

3018
3019

3020 This severity score is finally normalized to obtain a final S score of 10, 7, 4, or 1.
3021
3022 Step 3: FMEA
3023 In this step, the S' score of each process parameter will be modulated to manage and decrease
3024 the "potential" risk. Three different modulation levels exist:
3025
3026 Level 1: Knowledge scoring (L)
3027

1	**High**	**DOE/OFAT. Evaluations are "fit to purpose" (OFAT choice is justified). "Strong" bibliography or commercial retrospective data.** **= Existing Whole Design Space**
5	**Medium**	**Incomplete data/view (ex: Monovariate experiments performed for parameters needing a multivariate approach or retrospective commercial data) ➔ OFAT for interdependent parameters** **= Incomplete Design Space**
10	**Low**	**Low Knowledge. Absence of data or few data, which do not allow conclusion.** **= No Design Space**

3028

3029 Level 2: Occurrence scoring (O)
3030

5	High	**No historical data**(? 30 batches) **Commercial/historical data**: frequency (OOS, OOC, RD …)X ? 3 %
3	Medium	**Commercial/historical data**: frequency (OOS, OOC, RD …) 1% ? X ? 3 %
1	Low	**Commercial/historical data**: frequency (OOS, OOC, RD …) X ? 1 %

3031

3032
3033 *OOS: out of specification*
3034 *OOC: out of consistency*
3035 *RD: deviation report*
3036
3037 It has been decided to score all relationships with an occurrence of 1 so as not to create an
3038 artificial difference since the historical data available for all parameters is more or less
3039 equivalent.
3040
3041 Level 3: Detection scoring (D)
3042

1	High	**Input**= *relevant*control (alarm, device control, check on due time) + PAT, OR **output** = real-time detection, alarm and method of measurement variability: X ? 10 %
3	Medium	**Input** = *control with an appropriate measurement variability* , OR **output** = no real-time detection and method of measurement variability: 10 ? X ? 20 %
5	Low	**Input**= no control of theinput norcontrol on due time, OR **output** = method of measurement variability: X > 20 %

3043

3044 Finally, a Risk Priority Number (RPN = S' x L x O x D) is calculated and will classify the input
3045 process parameters according to criticality.
3046
3047 All this information is gathered in the FMEA table, which is presented on the next page

OUTPUT PARAMETER LABEL		cell density end of growth	Antigenic titer	total protein	virus integrity					
LINK TO (CQA/CPPA)		KPA	KPA	CQA	CQA					
SCORE		5	5	5	10					
INPUT PARAMETER LABEL	FAILURE MODE					S"	L	D	O	RPN"
Growth										
pH	low negative impact outside range	10				10	10	1	1	100
daily glucose addition (concentration)		10				10	10	1	1	100
daily glutamine addition (concentration)		10				10	10	1	1	100
inoculation cell density	Lower: -> yield impact Upper: medium faster depleted	10				10	1	3	1	30
agitation speed (rpm)	mixing study done at 200L scale	10				10	1	1	1	10
incubation temperature	well defined for phase I-II process	10				10	1	1	1	10
air flow in head space (L/min)		1				1	5	1	1	5
media preheating T	Media preheated between 35 and 39°C	1				1	1	1	1	1
transfer time of preculture suspension	well controlled range no impact observed within range	1				1	1	1	1	1
final volume (L)	Weight measure	1				1	1	1	1	1
pO2 (%)	phase I/II showed no impact between 10-30 %. No issue to regulate at 20 % at 200 L.	1				1	1	1	1	1
culture duration		10				10	10	1	1	100
Infection										
cell density end of growth			5	5	1	4	5	3	1	60
pH			5	1	1	4	10	1	1	40
pO2 (%)	phase I/II showed no impact between 10-30 %. No issue to regulate at 20 % at 200 L.		1	1	1	1	1	1	1	1
seed activation by serine protease (concentration)	Scoring for lower failure mode		10	5	1	7	10	1	1	70
seed activation duration by serine protease			10	5	1	7	10	1	1	70
Multiplicity of Infection	Low: risk of virus degenerescence High: lower yield		10	5	5	10	5	1	1	50
daily serine protease addition (activity)	Scoring for lower failure mode		10	5	1	7	5	1	1	35
infection duration			5	1	5	7	5	1	1	35
daily glucose addition (concentration)			5	10	1	7	5	1	1	35
daily glutamine addition (concentration)			5	10	1	7	5	1	1	35
infection temperature			5	5	5	7	1	1	1	7
working seed thawing temperature	Thawing procedure defined		1	1	1	1	1	1	1	1
agitation speed (rpm)	mixing study done at 200L scale		5	5	1	4	1	1	1	4
air flow in headspace			1	1	1	1	1	1	1	1
working seed thawing duration	Thawing procedure defined		1	1	1	1	1	1	1	1

3048

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3049   To select the critical parameters of each operation unit, all process parameters are ranked
3050   according to the RPN. The potential critical parameters are associated with those bars that are
3051   "exceptional signals" compared with the other bars considered as "noise signals." Bars
3052   associated with noise increase uniformly (like a staircase), while bars associated with a signal
3053   increase significantly in magnitude (like a wall). The critical parameters selected are highlighted
3054   in the blue boxes in the figures below.
3055

Effect of growth parameters

3056
3057

Effect of infection parameters
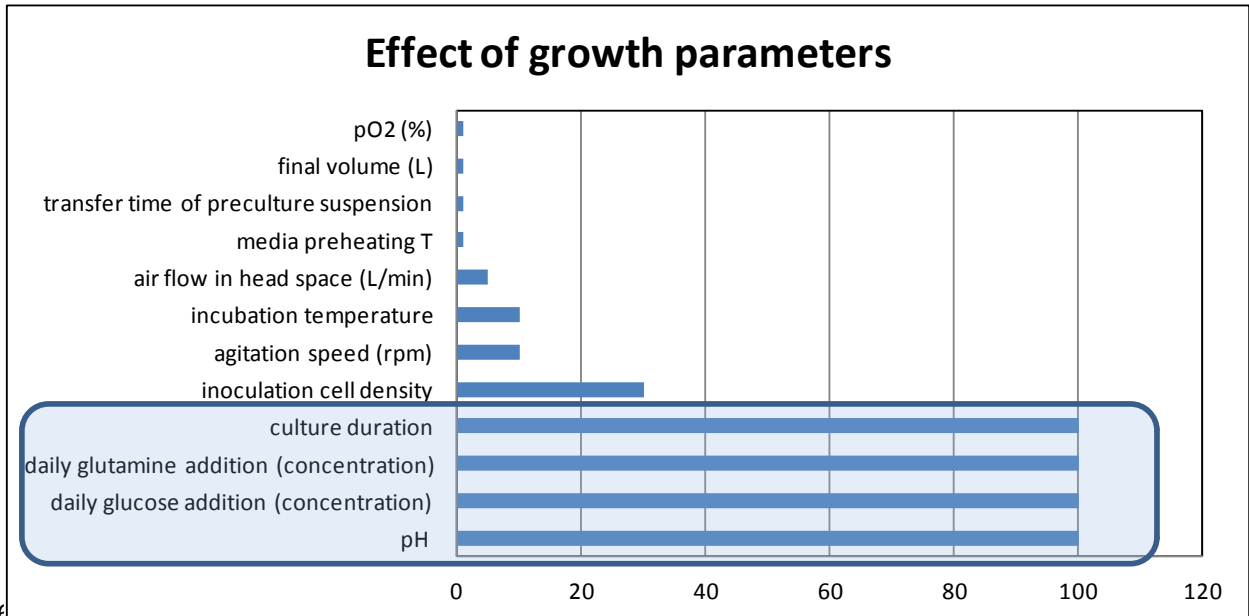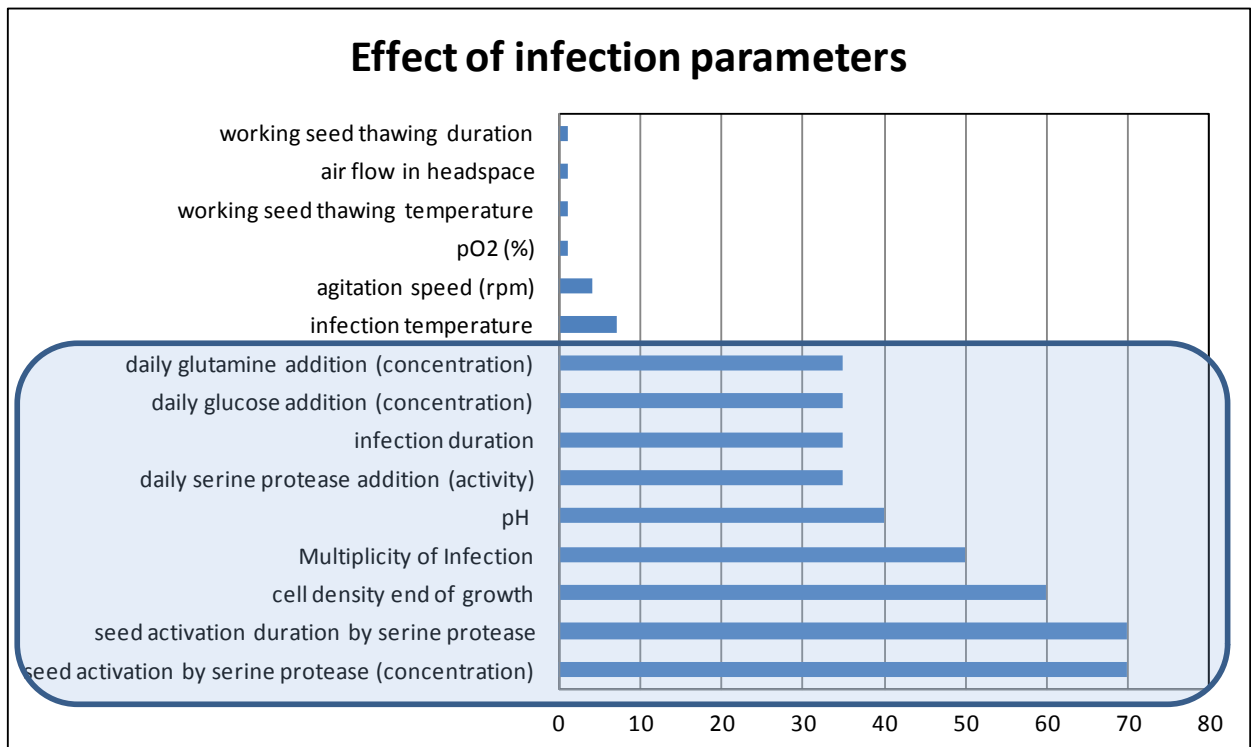
3058
3059   Four growth parameters (duration, glutamine and glucose concentrations, pH) and nine
3060   parameters for infection (glutamine and glucose concentrations, infection duration, activation

3061   by serine protease activity and duration, pH, MOI, cell density at end of growth, daily
3062   concentration of serine protease) are identified as high-risk process parameters from the FMEA
3063   analysis and will be further studied.

3064   10.6.2.   Identification of Phase III Scale-up Risks

3065

3066   The phase III development includes the final optimization of the process and the scale-up to the
3067   final process scale (2,000 L bioreactor).
3068   The critical parameters identified in previous section are scale independent and will be studied
3069   at a scale-down model at 10L (section VII.B).
3070   However, some additional parameters should also be studied to cover a successful scale-up of
3071   the process:

3072   •   Media preparation and filter size.

3073   •   Media stability.

3074   •   Implementation of a bead-to-bead cell passage required between the 200 L and the 2,000 L
3075   bioreactor (not described in this case study as it is part of prior knowledge).

3076   •   Addition of a final expansion step in a 2,000 L bioreactor (dynamic conditions) to reach the
3077   final biomass and infection at 2,000 L with the constraints linked to the scale-up.

3078   •   Scale-up of agitation: described in section VII.

3079   •   Addition of a shear protective additive (see section VIII).

3080   ## 10.7. Scale-up and Scale-down Models

3081

3082   An appropriate scale-up should assure that the process performances are similar at all scales.
3083   One specificity of adherent cell lines grown on microcarriers, in animal-free media, is their shear
3084   sensitivity. Therefore, the mixing is probably the biggest challenge for the scale-up to 2,000 L
3085   scale, justifying the rationale for its description in this section. An inadequate scale-up would
3086   impact the CQA and KPA (e.g., a higher shear at large scale could affect the cell density, the
3087   protein content in the harvest, and the antigenic titer).

3088

3089   Geometrical similarity is maintained for the design of the bioreactors from bench to pilot and
3090   manufacturing scales. That means that they all have the same shape, one being a uniform
3091   scaling (enlarging or shrinking) of the others (i.e., the ratio of all corresponding dimensions is
3092   equal).

3093

3094   10.7.1.   Scale-up of Mixing

3095   **10.7.1.1.**   **Maintain Microcarriers in Suspension**

3096   Microcarriers must stay in suspension during culture. The minimal speed required to maintain
3097   them in suspension follows the Corpstein law:

3098

3099   Equation 10-1: Minimal Agitation Speed to Suspend Microcarriers

3100

3101
3102

$$N_{\min} = k.\left(\frac{\rho_s - \rho_l}{\rho_l}.v_{chute}\right)^{0.3}.f(d/D).(D/D0)^{-n}\ with: 0.5 < n < 1$$

3103   Where:
3104   Nmin   = minimal agitation speed to suspend microcarriers [rps]
3105   k   = constant depending on the agitator type and the microcarriers'
3106   concentration
3107 ρ = density (ρ_s for microcarrier, ρ_l for liquid) [kg/m³]
3108 v_{chute} = microcarriers' settling speed [m/s]
3109   d   = impeller diameter [m]
3110   D   = vessel diameter [m]
3111   f(d/D) = coefficient depending on the impeller type and diameter
3112   D/D0   = ratio of vessel diameter at the two scales studied (scale factor)
3113   A security factor of 10% is taken on the minimal required speed to take into account the
3114   accuracy of the Corpstein relation.

3115   **10.7.1.2.   Liquid Homogeneity**
3116   The mixing time is a good indicator of liquid homogeneity. For vessels in geometric similarity
3117   and in a turbulent hydrodynamic regime, the mixing time (tm) is maintained constant if the
3118   agitation speed (N) is conserved at all scales.

$$N \, tm = cst$$

3119   **10.7.1.3.   Shear**
3120   The shear is much more detrimental for cells grown on microcarriers than for cells in
3121   suspension. Indeed, in turbulent flows, eddies are formed in the liquid. Larger eddies transfer
3122   their kinetic energy to smaller ones. These small eddies end up by dissipating their kinetic
3123   energy into heat. The cells are affected if their size (for cells in suspension) or the size of the
3124   microcarriers (for adherent cells) is of the same order of magnitude as the smallest eddies. The
3125   size of the smallest eddies depends on the specific volumetric power (P/V) input; high P/V leads
3126   to very small eddies and potentially more cell damage.

Acceptable shear stress if
Eddy's dimension **>** particle dimension
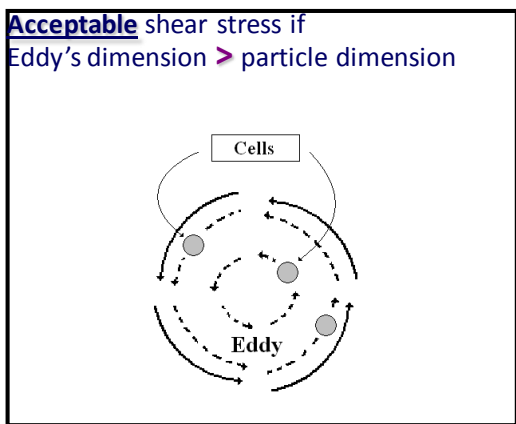
Suspension cell culture
➔ **small particles (cells)**
➔ **small microeddies acceptable**
➔ ***high P/V* is acceptable**

Critical shear stress if
Eddy's dimension **<** particle dimension
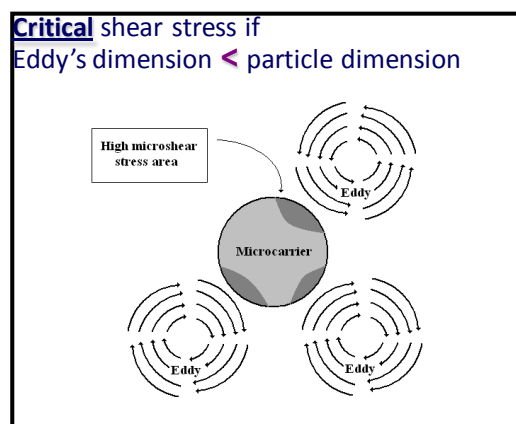
Microcarrier cell culture
➔ **big particles (microcarriers)**
➔ **only big microeddies acceptable**
➔ ***P/V becomes critical according to this theory***

3127

Numeric exemple :

– Cell diameter ➔ ≈ 20 µm (=λ_1) = microeddies' critical dimension

– Microcarrier diameter ⋅⌣ ≈ 200 µm (=λ_2) = microeddies' critical dimension

$$\lambda = \left(\frac{\rho \cdot \upsilon^3}{P/V}\right)^{1/4}$$ ⟹ $$\frac{\lambda_1}{\lambda_2} = \left(\frac{(P/V)_2}{(P/V)_1}\right)^{1/4} = 0.1$$ ⟹ $$\frac{(P/V)_1}{(P/V)_2} = 10\,000$$

$$P/V = N_P \cdot \rho \cdot N^3 \cdot d^5$$ ⟹ $$\frac{(P/V)_1}{(P/V)_2} = \left(\frac{N_1}{N_2}\right)^3$$ ⟹ $$\frac{N_1}{N_2} \approx 20$$

3128
3129 Where:
3130 λ = particle diameter [m]
3131 ρ and ν = fluid density and viscosity [kg/m³] and [m²/s]
3132 P/V = volumetric power dissipated in the liquid [W/m³]
3133 Np = power number (characteristic of the impeller, constant in turbulent
3134 regime)
3135 N = agitation speed [rps]
3136 d = impeller diameter [m]
3137 **=> In the same mixing configuration, cells in suspension can be agitated 20 times faster than**
3138 **cells on microcarriers without damage!**
3139 The scale-up criteria to reproduce the same eddy sizes at various scales would be to keep a
3140 constant volumetric power (P/V).
3141 In turbulent flow, the volumetric power is calculated by:

$$P/V = N_P \cdot \rho \cdot N^3 \cdot d^5$$

3142
3143 P/V is a good indicator of the mean shear. On the other hand, the maximal shear, produced at
3144 the edge of the impeller, can be correlated to the tip speed (vp = Π.d. N).
3145

3146 **10.7.1.4.   Mixing Scale-up Strategy**
3147 Three scale-up strategies are compared:
3148 • Agitation fixed at each scale to maintain microcarriers in suspension
3149 • Agitation calculated to achieve same mixing time at all scales
3150 • Agitation kept at constant volumetric power
3151 The starting point is the 200 L bioreactor operated at 25 rpm (phase I/II process).
3152
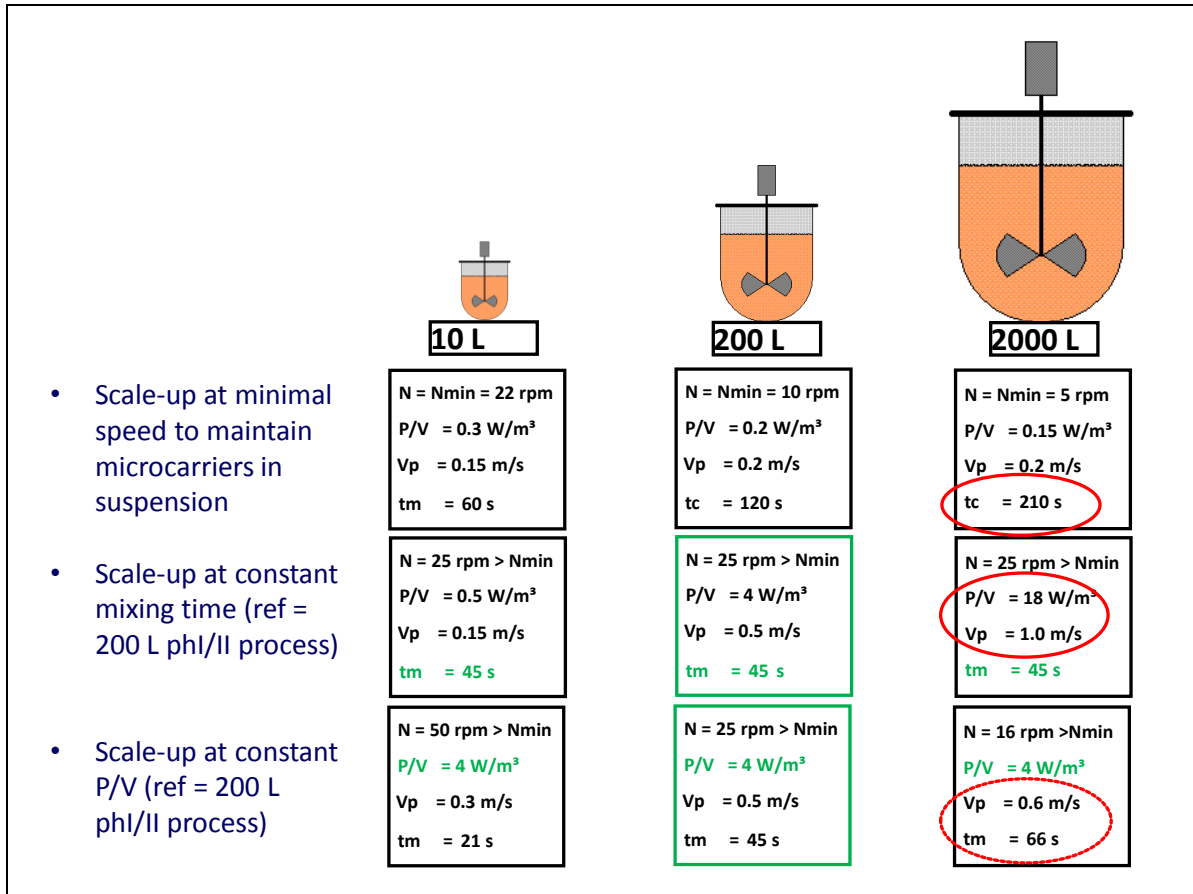
3153
3154
3155   The scale-up at the minimal speed to get microcarriers suspended is not optimal because the
3156   mixing time is increasing at large scale. Local nonhomogeneities — for example, during pH
3157   adjustment with base — could affect the cells.
3158
3159   The scale-up that keeps the mixing time constant (i.e., assuring the same liquid mixing
3160   efficiency) requires more power per volume at large scale. This can lead to cell damage and poor
3161   growth.
3162
3163   The preferred option is to perform the scaling-up at constant volumetric power. The starting
3164   point is the 200 L bioreactor. When the bioreactor is scaled up to 2,000 L, the agitation speed is
3165   fixed at 16 rpm; the tip speed (maximal shear) and the mixing time are only slightly increased.
3166

3167   10.7.2.   Scale-down Models
3168

3169   Process optimization and process validation can be done to some extent at small scale. The lab
3170   bioreactors have a capacity of 10 L and are similar in geometry to the 200 L and 2,000 L
3171   bioreactors. The scale-up is performed at constant power per volume (4 W/m³). The
3172   corresponding speed at 10 L scale is 50 rpm. This is above the minimal speed required to
3173   maintain the microcarriers' suspension. There is no impact of the scale on the CQAs and KPAs as
3174   shown here.

3175   Summary of process and quality attributes for small scale and 200 L scale for the phase II
3176   process:

3177

	10 L scale	200 L scale
CQA		
Protein content (g/L)	1.37	1.33
Virus integrity (%)	84	80
KPA		
Antigenic titer (µg/ml)	91	97
Cell density end of growth (cells/ml)	$2.6 \; 10^6$	$2.8 \; 10^6$

3178

3179   The performances of the process at the 2,000 L manufacturing scale will be shown after the DOE
3180   section, on the Phase III optimized process.

3181

3182   10.8. Strategy for Phase III Process Optimization

3183   10.8.1.   OFAT Analysis

3184   For some factors, an OFAT approach (one factor at a time) is appropriate for process
3185   understanding and/or optimization. The factors described here are investigated in univariate
3186   studies.

3187   • Media preparation – filter resizing:

3188   - Increase of filtrated medium volume per filtration area for the scale-up from 200 L to
3189   2,000 L to avoid an oversized area for the 2,000 L scale.

3190   - Tested on cell growth and viral production steps.

3191   • Media stability – shelf life:

3192   - Powdered basal medium tested for two years to meet commercial constraints.

3193   - Rehydrated media tested for two months and validated for a one-month expiration that
3194   is in line with commercial-unit constraints.

3195   - Tested on cell growth and viral production steps.

3196   • Addition of an additive to avoid cell damage at 2,000 L scale. The influence of the nontoxic
3197   additives on several physicochemical parameters such as $k_l a$, foam, and bubble coalescence
3198   was studied. Six additives were screened for their physicochemical properties. Results are
3199   summarized in the following table:

3200

Additives	Concentration [% w/w]	Toxicity	Surface tension [mN/m]	Bubble coalescence [%]	k_la impact [min^{-1}]	Foam
Water (reference)			73	10	0,06	Reference
Additive 1	0.05	OK	63	8	≈	More stable
	0.1	TOX	63	9	+	
	0.15	TOX	63	9	++	
Additive 2	0.05	LTOX	62	0	-	More stable
	0.1	LTOX	62	0	++	
	0.15	TOX	62	13	++	
Additive 3	0.05	OK	63	0	≈	More stable
	0.1	TOX	62	5	≈	
	0.15	TOX	62	20	-	
Additive 4	0.05	OK	68	18	+	Less stable
	0.1	OK	68	21	++	
	0.15	LTOX	68	17	++	
Additive 5: poor solubility	0.05	OK	/	/	- - -	Much more stable
	0.1	OK	/	/	- - -	
	0.15	LTOX	/	/	- - -	
Additive 6	0.05	OK	60	-30	- - -	Much more stable
	0.1	OK	59	0	- - -	
	0.15	OK	57	14	- -	

3201 TOX=significant cytotoxic effect – LTOX = low cytotoxic effect
3202
3203 Additives 4 and 6 were further studied in culture at a concentration of 0.1%. Agitation and
3204 aeration were increased to highlight a potential shear-protecting action of these two additives
3205 during cell growth. The growth was monitored according to cell count and LDH assay in the
3206 supernatant. Additive 6 was confirmed as the best shear protector against agitation and
3207 aeration and thus selected for the 2,000 L process.

3208 10.8.2.   DOE Analysis

3209 The high-risk process parameters selected in a risk assessment (FMEA; see Section VI.B) are
3210 investigated in a multivariate study. The growth phase and the virus production phase will be
3211 studied in the same DOE. The experiments are performed at 10 L scale; the scale-down models
3212 were qualified as representative of the final process scale (Section VII.B). Twelve parameters
3213 stand out in the risk assessment (the cell density at the end of the growth phase will be
3214 considered as an output of growth phase and not as a parameter).
3215
3216 The design of a model with 12 parameters would require overwhelming work; therefore,
3217 parameters are first tested in a screening study. The significant parameters are then further
3218 studied in a response surface design to establish the mathematical relationship between the
3219 process parameters and the critical attributes.
3220
3221 The process expectations are defined in terms of acceptable ranges of the critical attributes
3222 (CQAs and KPAs) detailed in the table below. The table also mentions the analytical variability of
3223 the tests.
3224
3225

	Target	Acceptable range	Analytical variability
Total protein	1.2 g/L	< 1.5 g/L	10%
Virus integrity	80%	> 70%	20%
Antigenic titer	100 µg/ml	> 80 µg/ml	10%
Cell density end of growth	$3.0\ 10^6$ cell/ml	$2.5\ 10^6$–$3.5\ 10^6$ cell/ml	20%

3226

3227   **10.8.2.1.   Screening for High-Risk Process Parameters**

3228   The Folded Plackett & Burman design is performed to select from the 12 high-risk process
3229   parameters those having a significant impact on the CQAs and KPAs. The factors are tested in a
3230   Minimum Run Equi-replicated Resolution IV Screening Design.
3231
3232   In this design, each factor is varied over only two levels. The resolution IV design allows
3233   estimation of main effects in a linear model while two-factor interactions will be aliased with
3234   other two-factor interactions. In this study, the testing of the 12 factors requires 26
3235   experiments.
3236
3237   The 26 experiments are performed at 10 L scale. The investigated ranges of the parameters are
3238   fixed based on phase II process setpoints and knowledge built during phase II process
3239   development.
3240

Parameters	Setpoint Ph II process	Range DOE
Growth		
. pH		6.8
. Daily glucose feeding		1–3
. Daily glutamine feeding	2	2–4
. Duration	5 days	4–6
Infection		
. pH		6.8
Multiplicity of Infection	10^{-3}	10^{-3}
. Virus activation: activity of serine protease	100 IU/ml	50–200 I
. Virus activation: contact duration of serine protease	30 min	15–60

Time of harvest	5 days	3–6 days
Daily addition of serine protease	2 IU/ml	1–10 IU/ml
Daily glucose feeding	1 g/l	1–3.0 g/l
Daily glutamine feeding	2 mM	2–4 mM

3241

3242   The results of the experiments were statistically analyzed. The first important finding is that out
3243   of the 12 potential high-risk process parameters, collectively five had a significant effect on the
3244   CQAs and KPAs: growth duration, multiplicity of infection, activity of the serine protease at the
3245   activation step, concentration of the daily addition of serine protease, and duration of infection.
3246   This is represented graphically in Pareto charts and normal plots.

3247

3248   In the Pareto chart, the effects and the interactions are ranked by decreasing amplitude of
3249   significance based on a Student t-test. The parameters above the black horizontal line are each
3250   significant at the 95% confidence level.

3251

3252   In the normal plot graph, the nonsignificant parameters should be distributed as noise and
3253   should be aligned in the Gaussian arithmetic scale. Significant effects are highlighted out of the
3254   line.

3255

3256   For both types of graph, the significant positive effects between a process parameter and a
3257   critical attribute are plotted in red, and the negative effects are plotted in blue.

3258

3259   Effect of process parameters on total protein:
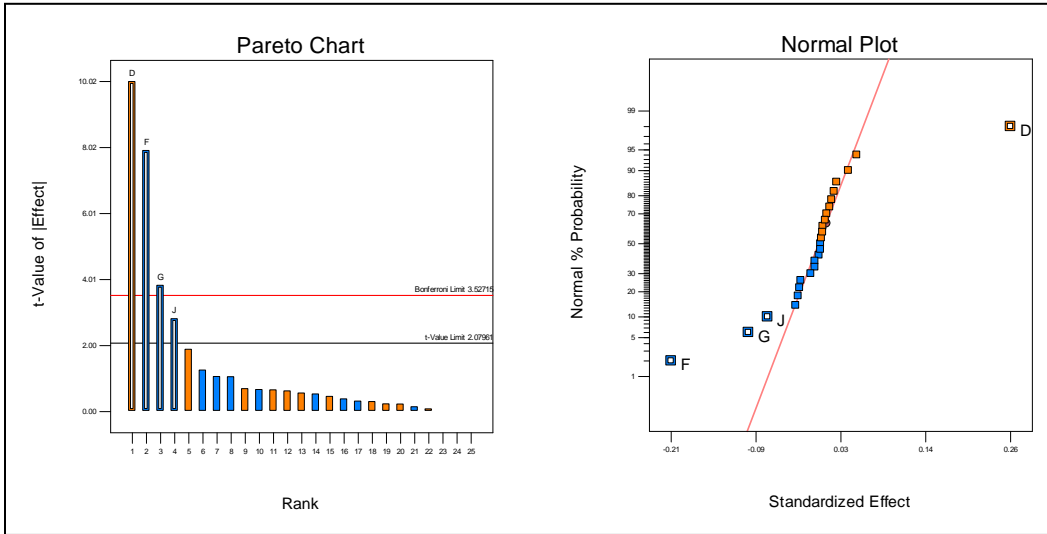
3260
3261

3262   Effect of process parameters on virus integrity:
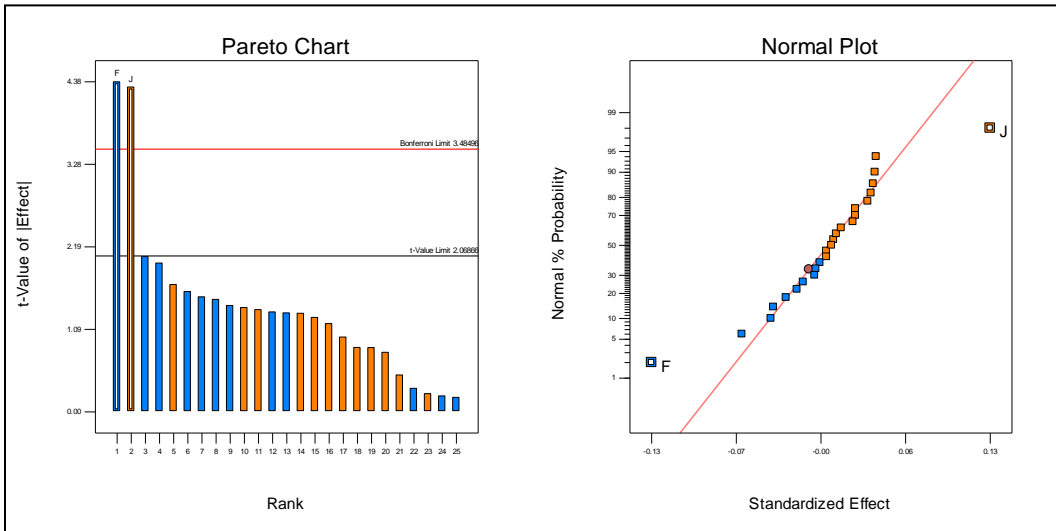
3263
3264
3265
3266

3267   Effects of process parameters on antigenic titer
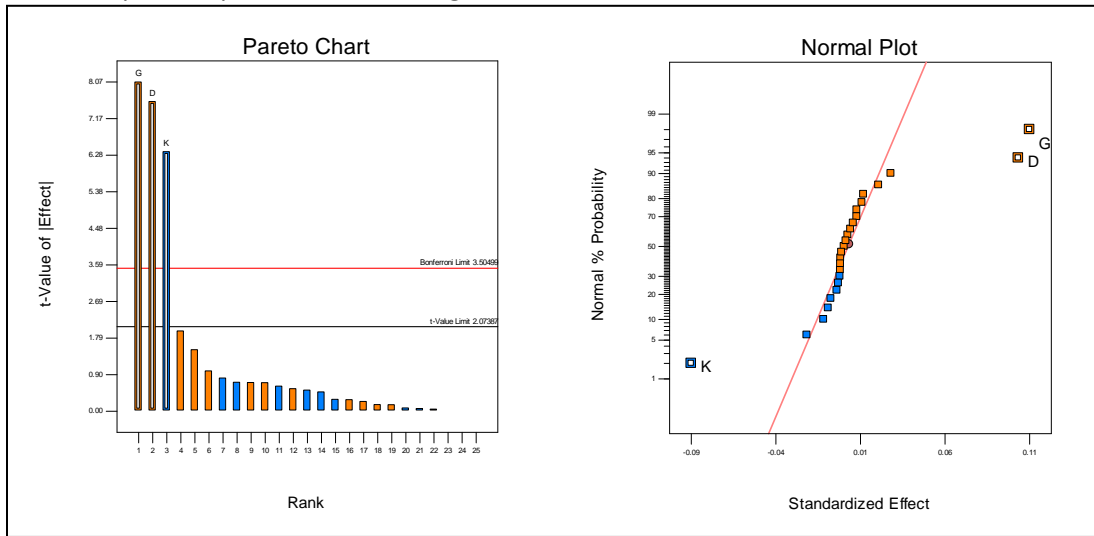
3268
3269
3270   Effects of process parameters on cell density end of growth
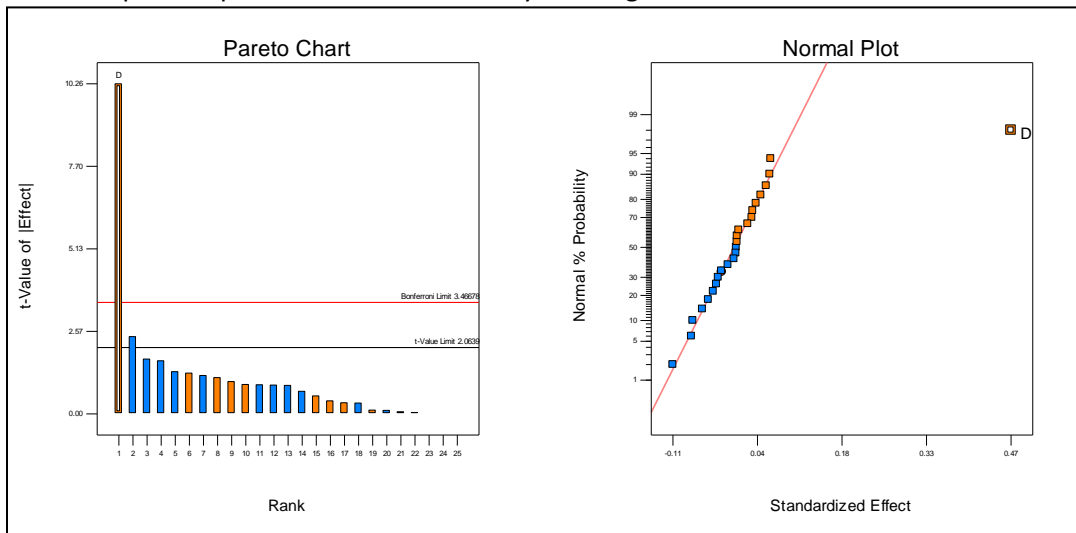
3271
3272   The effects of the five significant process parameters are represented in the following graphs.
3273   For each critical attribute (total protein, virus integrity, antigenic titer, and cell density), a first
3274   graph shows the distribution of the values of the critical attributes for the 26 experiments. The
3275   data are then sorted by process parameters, each of which was tested in the DOE at two levels.
3276   If an effect is statistically significant, a linear trend links the process parameter and the critical
3277   attribute, showing the amplitude of the effect.
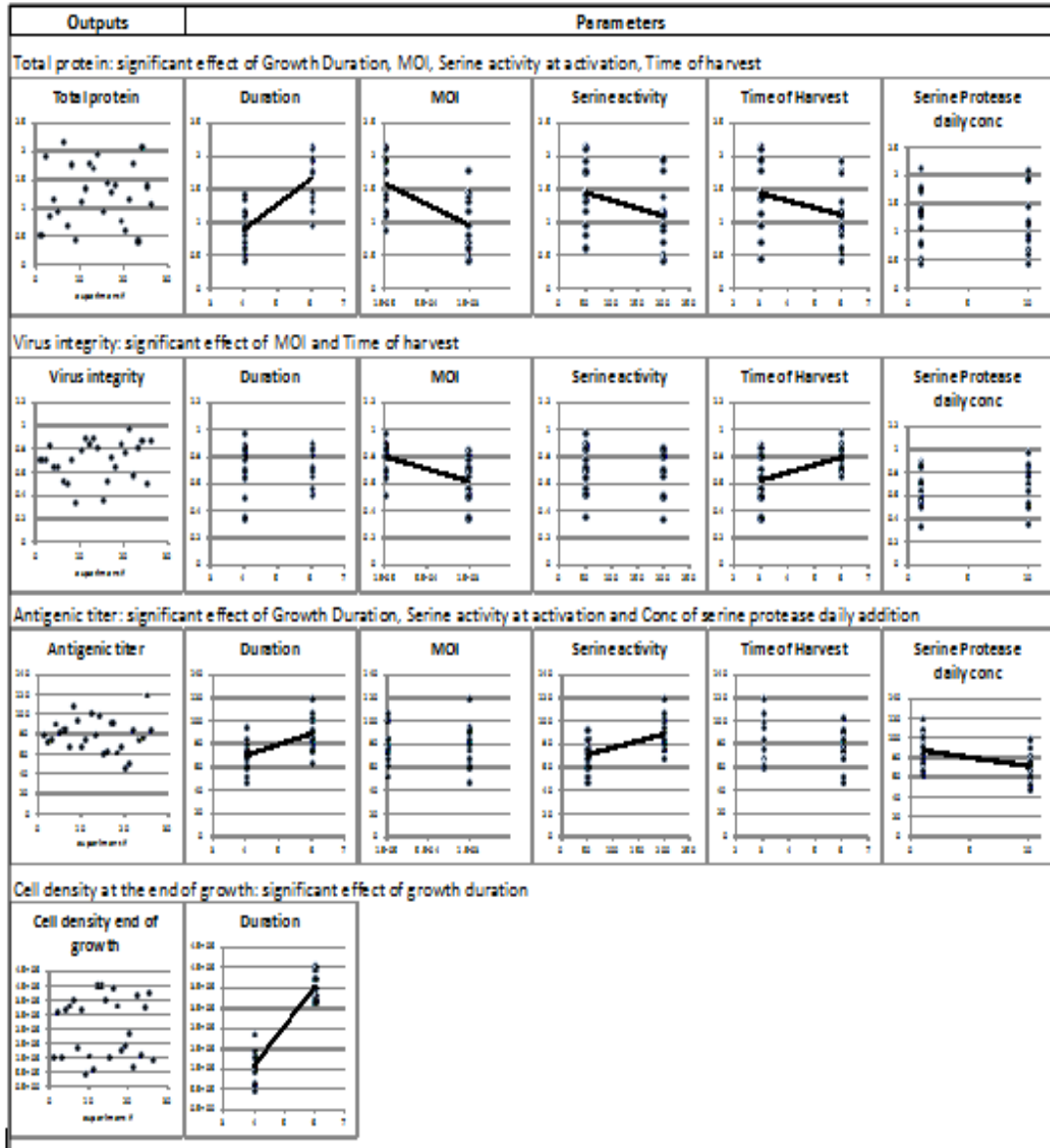
3278

10.8.2.2.   Response Surface for Process Optimization

3279

3280   The screening study identified five parameters having a significant effect on the CQAs and KPAs:
3281   growth duration, multiplicity of infection, activity of the serine protease at the activation step,
3282   concentration of the daily addition of serine protease, and duration of infection. The effects of
3283   those five process parameters are further studied using the Response Surface Methodology
3284   (RSM). With the RSM, the responses of interest are expressed as a second-order polynomial
3285   function of all the process parameters and their interactions; it will allow prediction of
3286   responses in the whole studied domain.

3287

3288   Five-factor, 29-run, face-centered central composite design is used, each factor being varied
3289   over three levels; it requires 29 experiments. The cultures are performed in 10 L bioreactors.
3290   The screening study has already shown the trends for the impact of the critical process

3291   parameters on the critical attributes; consequently, the ranges of some parameters are adapted
3292   for the surface response DOE to achieve better performance. The upper limit of the multiplicity
3293   of infection was increased; the activity of the serine protease at viral activation was focused on
3294   high values, while the activity for the daily addition of serine protease was moved toward lower
3295   values.
3296

Parameters	Setpoint Ph II process	DOE range
Growth		
Duration	5 days	4–6 days
Infection		
Multiplicity of Infection	10^{-3}	10^{-5}–10^{-2}
Virus activation: activity of serine protease	100 IU/ml	100–200 IU/ml
Time of harvest	5 days	3–6 days
Daily addition of serine protease	2 IU/ml	0.3–5 IU/ml

3297
3298   The results of the 29 experiments were statistically analyzed. The significant effects of the
3299   process parameters on the responses are shown by the p-value tables. A table is created for
3300   each response (i.e., critical attribute). The process parameters are listed in the lines and
3301   columns. The diagonal of the table represents the significance of the parameters on the selected
3302   response (first-order linear effect and second-order quadratic effect). The cells above the
3303   diagonal represent the significance of the interactions between two parameters on the
3304   response.
3305
3306   Significant effects are in red if the effect or synergy is positive (positive contribution to the
3307   output when variables are increasing) and in blue if the effect or synergy is negative (negative
3308   contribution to the output when variables are increasing). The threshold for a statistically
3309   significant effect is p-value <0.05.

==Coefficients p-values== from ANOVA (analysis of variance), for single effects (linear and quadratic), and two-way interactions.

Positive effect/synergy in the studied range

Negative effect/antagonism in the studied range

Total Protein

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. <0.001 Quad. 0.019	0.236	0.431	0.049	0.564
MOI		lin. <0.001 Quad. 0.09	0.101	0.164	0.194
Serine protease activity			lin. 0.004 Quad. 0.021	0.043	0.298
Time of harvest				lin. 0.035 Quad. 0.071	0.094
Conc. daily serine protease					lin. 0.034 Quad. 0.028

Virus integrity

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. 0.234 Quad. 0.453	0.462	0.241	0.497	0.378
MOI		lin. 0.01 Quad. 0.15	0.131	0.664	0.632
Serine protease activity			lin. 0.354 Quad. 0.575	0.369	0.564
Time of harvest				lin. 0.464 Quad. 0.069	0.697
Conc. daily serine protease					lin. 0.294 Quad. 0.642

Antigenic titer

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. <0.001 Quad. <0.001	0.128	0.234	0.151	0.324
MOI		lin. <0.001 Quad. <0.001	0.043	0.037	0.049
Serine protease activity			lin. <0.001 Quad. 0.043	0.021	0.303
Time of harvest				lin. 0.621 Quad. 0.324	0.013
Conc. daily serine protease					lin. 0.033 Quad. 0.019

Cell density end of growth

	Duration	MOI	Serine protease activity	Time of harvest	Conc. daily serine protease
Duration	lin. 0.03 Quad. 0.07	NA	NA	NA	NA
MOI		NA	NA	NA	NA
Serine protease activity			NA	NA	NA
Time of harvest				NA	NA
Conc. daily serine protease					NA

3310

CMC-VWG

3311   The relation between process parameters and critical attributes can be plotted in surface
3312   response graphs, as seen in the following figure example:

Design-Expert® Software
Factor Coding: Actual
Total protein
1.54667
0.333333

X1 = A: Duration
X2 = D: Time of harvest
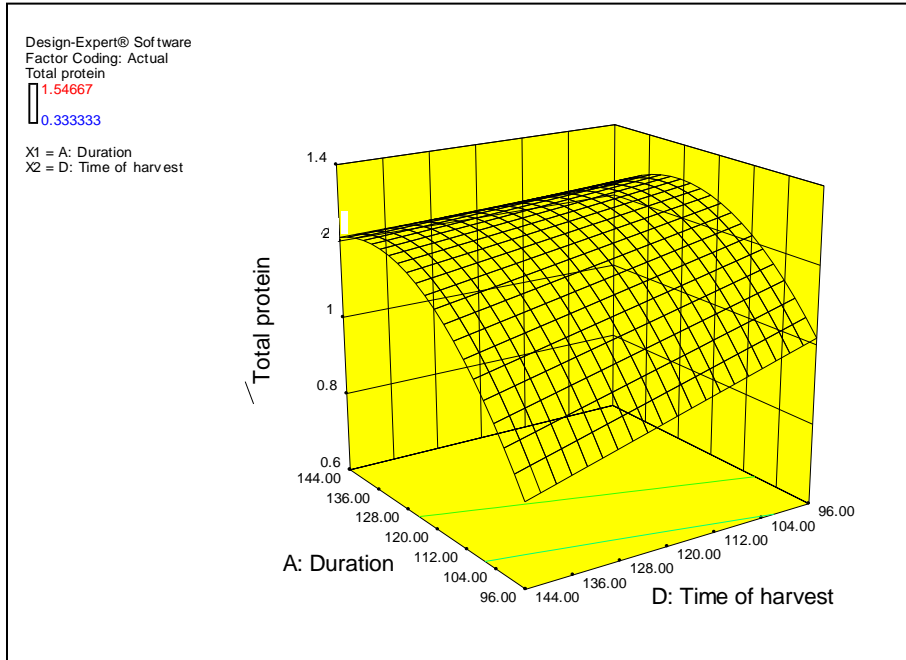
3313
3314   The response surfaces can be visualized for all critical attributes in function of two process
3315   parameters. Other examples of surface responses are shown in the design space section of this
3316   chapter. The phase III process setpoints were redefined based on the DOE results.
3317

Parameters	Setpoint Ph II process	Optimum setpoint Ph III
Growth		
Duration	5 days	5 days
Infection		
Multiplicity of infection	10^{-3}	10^{-4}
Virus activation: activity of serine protease	100 IU/ml	200 IU/ml
Time of harvest	5 days	5 days
Daily addition of serine protease	2 IU/ml	1 IU/ml

3318

3319

3320   **10.8.3.   Phase III Process Validation at Final Scale**

3321   The process optimizations were performed at 10 L scale. The DOE allowed the team to optimize
3322   the process and to predict the critical attributes at the reference conditions (Phase III process
3323   setpoints):
3324

	Parameter	Phase III
Process	Duration (h)	120
	MOI (-)	10^{-4}
	Serine protease activity at activation (IU/ml)	200
	Time of harvest (h)	120
	Cc daily addition serine protease (IU/ml)	1
Responses	Total protein (g/L)	1.1
	Virus integrity (%)	88
	Antigenic titer (µg/ml)	104
	Cell density end of growth	3.2E+06

3325
3326   These optimizations were implemented at the 200 L and 2,000 L scales. No difference was
3327   observed within the scales.
3328
3329   Summary of process and quality attributes for the three scales for the phase III process:
3330

	10 L scale	200 L scale	2,000 L scale
CQA			
Protein content (g/L)	1.13	1.17	1.22
Virus integrity (%)	90	82	84
KPA			
Antigenic titer (µg/ml)	103	96	107
Cell density end of growth (cells/ml)	$3.2 \cdot 10^6$	$2.9 \cdot 10^6$	$3.3 \cdot 10^6$

3331
3332
3333

3334

3335   10.8.4.   Updated Process Description Based on Process Changes between End of
3336   Phase II and Final Process — Final Scale

3337
3338   Based on the OFAT and DOE optimizations and on the validation of those conditions at 2,000 L
3339   scale, the Phase III process is defined:
3340

Parameter	Phase II (200 L)	Phase III (2,000 L)
Cell growth		
Cell passage	NA	**Bead-to-bead passage (200 L > 2,000 L)**
Cytodex concentration	3 g/L	3 g/L
Shear protective additive	-	**0.1%**
Seeding density	150.000 cells/ml	150.000 cells/ml
Temperature	37°C	37°C
pO$_2$	20%	20%
pH	7.2	7.2
Pressure	0.1 bar	0.1 bar
Stirring	25 rpm	**16 rpm**
Daily glucose adjustment	1 g/L	**2 g/L**
Daily glutamine adjustment	2 mM	**3 mM**
Growth duration	5 days	5 days
Viral infection		
Minimum cell density	2.5 10^6 cells/ml	**3 10^6 cells/ml**
Cytodex concentration	3 g/L	3 g/L
Temperature	33°C	33°C
pO$_2$	20%	20%
pH	7.2	7.2
pressure	0.1 bar	0.1 bar

Stirring	25 rpm	**16 rpm**
Daily glucose adjustment	1 g/L	**2 g/L**
Daily glutamine adjustment	2 mM	**3 mM**
MOI	10^{-3}	**10^{-4}**
Viral activation: serine protease activity	100 IU	**200 IU**
Viral activation: serine protease contact duration	30 min	30 min
Daily addition of serine protease: concentration	2 IU	**1 IU**
Viral replication duration	5 days	5 days

3341

10.9. Design Space and Control Space

3343
3344 ### 10.9.1.   Critical Process Parameters
3345

3346 The design space must be determined in order to predict robust process conditions and
3347 demonstrate assurance of quality in the ICH definition of "design space": *"the multidimensional*
3348 *combination and interactions of input variables and process parameters (e.g. material*
3349 *attributes) that have been demonstrated to provide assurance of quality."*
3350
3351 The criticality of process parameters is reevaluated based on all the knowledge generated
3352 during phase III process development. The reevaluation uses a process risk assessment and
3353 takes into account the capability to control the process parameters.
3354
3355 In the following table, blank spaces indicate that parameters do not affect critical attributes in
3356 the ranges studied. Green and red denote parameters that affect critical attributes. Green
3357 indicates that capability of controlling the parameters is robust and effective. Red indicates that
3358 the range in which the parameters can vary before a CQA is potentially affected is close to the
3359 control capability.
3360
3361 Note that during Phase II process development, the optimal infection temperature was defined
3362 at 33°C, and this parameter did not come out of the risk assessment performed at the end of
3363 Phase II. Nevertheless, data available after Phase III process optimization suggested that the
3364 growth and the infection duration could interact with the infection temperature to affect
3365 antigenic titre.
3366

3367

Parameter	Quality attributes		Process attributes		Risk mitigation
	Total protein	Virus integrity	Cell density end growth	Antigenic titer	
Cell passage					Studied during Ph II development
Cytodex concentration					Studied during Ph II dvpt
Shear protective additive					OFAT study done during Ph III process optimization
Seeding density					Studied during Ph II dvpt
Temperature cell growth					Studied during Ph II dvpt
pO_2					Studied during Ph II dvpt
pH cell growth					DOE done during Ph III process optimization
Pressure					Studied during Ph II dvpt
Stirring					Scale-up based on Phase II dvpt
Daily glucose adjustment					DOE done during Ph III process optimization
Daily glutamine adjustment					DOE done during Ph III process optimization
Growth duration	🟩		🟩	🟩	**DOE done during Ph III process optimization. Potential interaction with induction T.**
Temperature infection	🟥	🟥	NA	🟥	**Studied during Ph II dvpt, range could be close to control capability. Potential interaction with process duration.**
pH infection			NA		DOE done during Ph III process optimization
MOI	🟩	🟩	NA	🟩	DOE done during Ph III process optimization. No interaction with other studied parameters.
Viral activation: serine protease activity	🟥		NA	🟥	**DOE done during Ph III process optimization. Ph III setpoint at the border of the studied range.**
Viral activation: serine protease contact duration			NA		DOE done during Ph III process optimization
Daily addition of serine	🟩		NA	🟩	**DOE done during Ph III process optimization.**

protease: concentration					**Range redefined around Ph III setpoint.**
Viral replication duration (time of harvest)			NA		**DOE done during Ph III process optimization. Potential interaction with induction T.**
Media preparation – filter size					OFAT study done during Ph III process optimization
Media stability – shelf life					OFAT study done during Ph III process optimization
Viral production media selection					Studied during Ph II dvpt

3368

3369 Since the multiplicity of infection has no interaction with other parameters (see DOE results
3370 during Phase III optimization), the limits of the design space for the MOI are fixed independently
3371 of the other process parameters. The design space for the MOI is fixed from 5×10^{-5} to 9×10^{-3}.
3372 Based on the Phase III optimization DOE results, within this MOI range, 95% of predicted future
3373 results will stay within specifications for CQAs and KPAs (Monte Carlo simulations).

3374

3375 Ideally the design space should be determined at the final step in a DOE combining all possible
3376 influent factors. But MOI has been studied in a pre-Phase III experiment and has shown a single
3377 effect pattern (not interacting with any other parameter). Its predicted impact (on pre-Phase III
3378 exp.) has been considered as an additive to all the further studied process parameters (post-
3379 Phase III exp.). It relies on a hypothesis (all possible interactions with MOI are negligible and its
3380 effect remains the same) that is considered reasonable from a theoretical point of view. This
3381 approach allows for making a profit from previously generated results, preserving them until
3382 final conclusions, without making experiments to retrieve the same information.

3383

3384 The other parameters will be studied in a DOE around the Phase III parameters' setpoints.

3385

Parameter	Phase III process	DOE range
Duration (cell density end growth)	120 h	96–144 h
Temperature at infection	33°C	30–36°C
Virus activation condition (activity)	200 IU/ml	50–300 IU/ml
Daily addition of serine protease (activity)	1 IU/ml	0.3–3 IU/ml
Time of harvest	120 h	96 h to 144 h

3386

3387

	Target	Acceptable range	Analytical variability
Total protein	1.2 g/L	< 1.5 g/L	10%
Virus integrity	80%	> 70%	20%
Antigenic titer	100 µg/ml	> 80 µg/ml	10%
Cell density end of growth	$3.0 \ 10^6$ cell/ml	$2.5 \ 10^6$–$3.5 \ 10^6$ cell/ml	20%

3388 The responses conditioning the design space are the CQAs and KPAs.

3389

3390 A faces-centered fractional central composite design is used; the responses of interest are
3391 expressed as a second-order polynomial function of all the process parameters and their
3392 interactions. It will allow prediction of responses in the whole studied domain. The faces-
3393 centered fractional central composite design requires 29 experiments with the five factors
3394 studied over three levels. The cultures will be performed in the 10 L bioreactors in
3395 representative conditions.

3396

3397 Four out of five parameters were studied by DOE during process optimization with different
3398 ranges for some parameters. Two options could be considered: (1) Enlarge the previous DOE to
3399 integrate the effect of infection temperature and the new ranges of some parameters, or (2)
3400 perform a new DOE. The second option was selected. Indeed, the cost saving with the first
3401 option was marginal (20 new experiments would be required in addition to the 29 performed
3402 during process optimization vs. 29 experiments for a new DOE), and the quality of the design
3403 would be poorer (two blocks of experiments with a long gap in time).

3404

3405 10.9.2.   Prediction Model

3406

3407 For each response, a reduced polynomial model reproduces output variations using a selection
3408 of factor effects and interactions based on an analysis of variance (ANOVA). The response
3409 surfaces are graphical representations of those equations.

3410

3411 The effect of the five process parameters and their interactions on the four responses cannot be
3412 visualized altogether. Graphs in 3D illustrate the effects of two parameters on one critical
3413 attribute.

3414
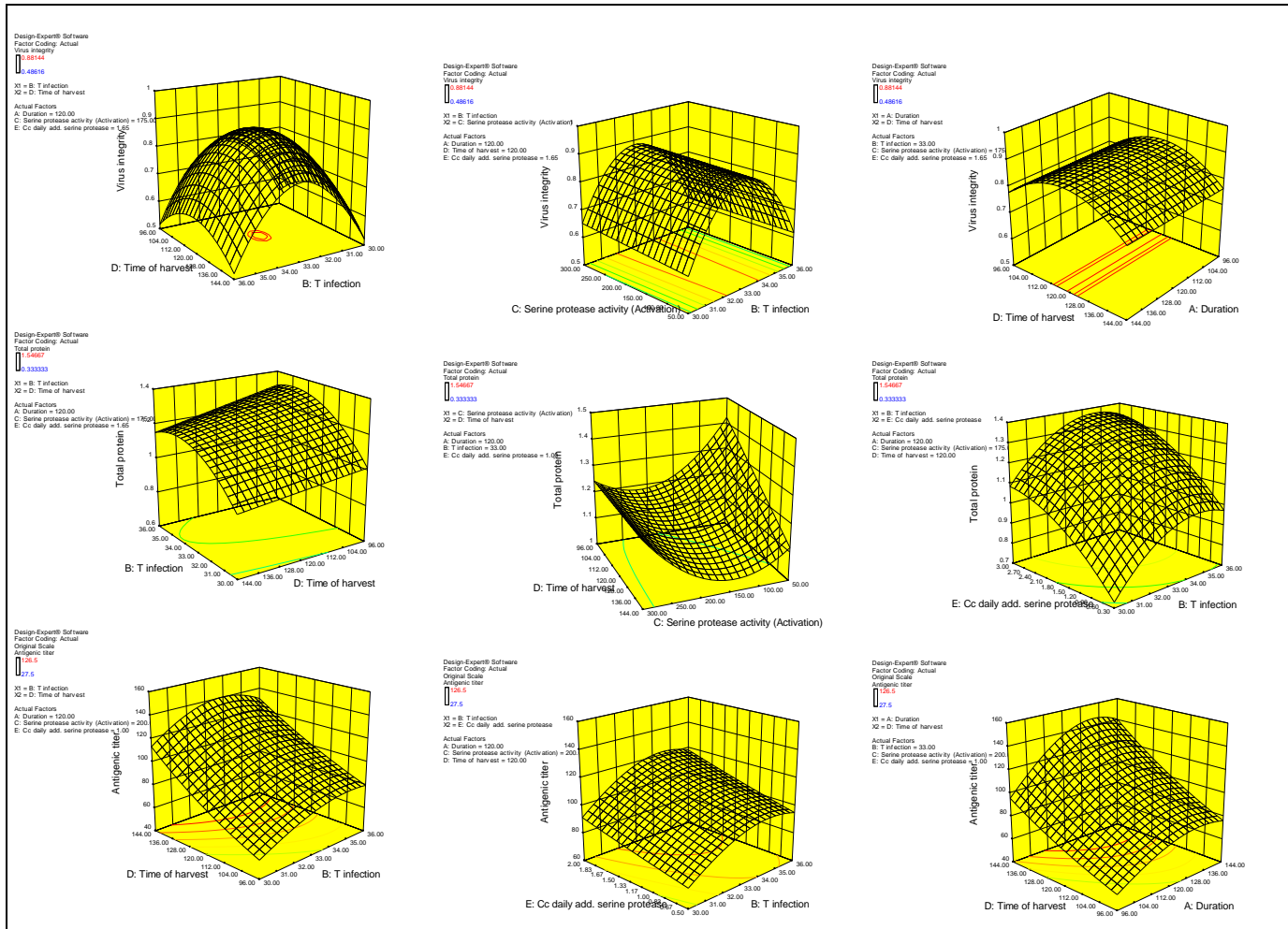
10.9.3.   Optimal Process — Desirability Function

A desirability function is built to calculate the best process parameter region to get the optimal responses. The higher the desirability is, the better the objectives are fulfilled. More weight is given to the quality attributes (virus integrity and total protein). The objectives are:
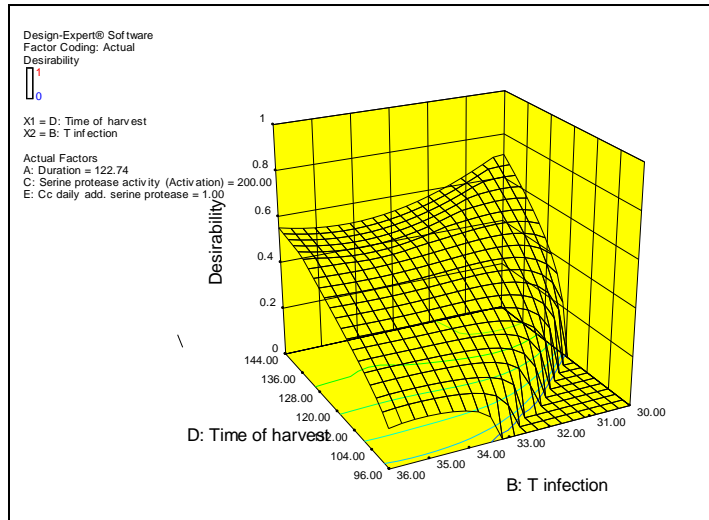
Maximize virus integrity (weight ****)

Minimize protein content (weight ****)

Maximize antigenic titre (weight **)

The final cell growth density is kept between 2.5 10^6 cells/ml and 3.5 10^6 cells/ml.

As an example, the desirability is represented here in function of the harvest time and the infection temperature.

For this particular graph, the desirability is very low for low infection temperature combined
with short infection, with the other parameters being at the reference conditions. It is not an
optimal area for the process. The highest value of the desirability function can be checked
graphically taking the parameters two by two or numerically by maximizing the function. This
method helps to find the optimal process conditions, or in our case to check whether the
conditions fixed for phase III are close to the optimum.

10.9.4.   Design Space

10.9.4.1.   Approach

Numerous statistical approaches can be used to define the design space. The following ones
have been applied and challenged:

An "average overlay plot approach" (as illustrated in ICH Q8)
For each input variable/process parameter, a prediction model is established. Then the design
space (DS) is determined as the subset of the experimental domain where all quality attributes
are predicted to be inside acceptance limits. Unfortunately with this approach, assurance of
quality is not demonstrated because prediction models actually predict average values. In our
case, at the thresholds of overlay plots, 50% of the predicted individual results are outside
acceptance limits.

A "robustified average overlay plot approach"
This approach, very similar to the previous one, consists of adding confidence intervals (CIs) to
overlay plots. DS will then be defined as the experimental domain subset where the
lower/upper 95% CIs (depending if it is a minimal- or maximal-value criteria) of predicted quality
attributes are inside acceptance limits. This method allows taking into account the prediction
model quality, which is an improvement, but still not an assurance of quality for next process
results.

A "tolerance intervals approach"
Instead of confidence intervals, coverage tolerance intervals are calculated around the predicted
threshold for each response, taking into account experimental noise. DS being outside the

3462   tolerance intervals, inside the DS β% of process results are predicted to be inside quality
3463   acceptance limits. Conceptually this approach is adequate to provide assurance of quality;
3464   however, when applying this approach on DOE data sets (by construction aiming to save
3465   experiments), calculated tolerance intervals were very wide, inducing the (almost)
3466   disappearance of the DS. This is probably due to the relatively small degrees of freedom leading
3467   to overly conservative limits with the calculation method used.

3468

3469   **A "% of simulated failure approach"**
3470   If mathematical/statistical calculations failed to determine the DS from a population point of
3471   view (all incoming process results), simulations allow doing so. The experimental domain is
3472   divided into cells; at each intersection (for each response), a huge number of simulations are
3473   made based on previously established prediction models (from DOE), adding random
3474   experimental noise (calculated from residuals). Then for all those locations the proportion of
3475   failed simulation can be calculated. Finally it is possible to determine a sub-domain with a defect
3476   rate below β%. Methodology is (partially) validated in our case by the fact that the 50% defect
3477   rate is exactly the same as the average overlay plot. Therefore, by decreasing the acceptable
3478   defect rate, we make the overlay plot approach more robust.

3479   **10.9.4.2.   Overlay Plot**

3480

3481   Overlay plots show the parameters' values for which the responses are within the specifications.
3482   At the limit of those regions, the responses, calculated by the polynomial relations with process
3483   parameters, are equal to the specifications (edge of failure). It can be represented by 2D graphs
3484   that look at two parameters at a time. For example, two overlay plots are shown here:
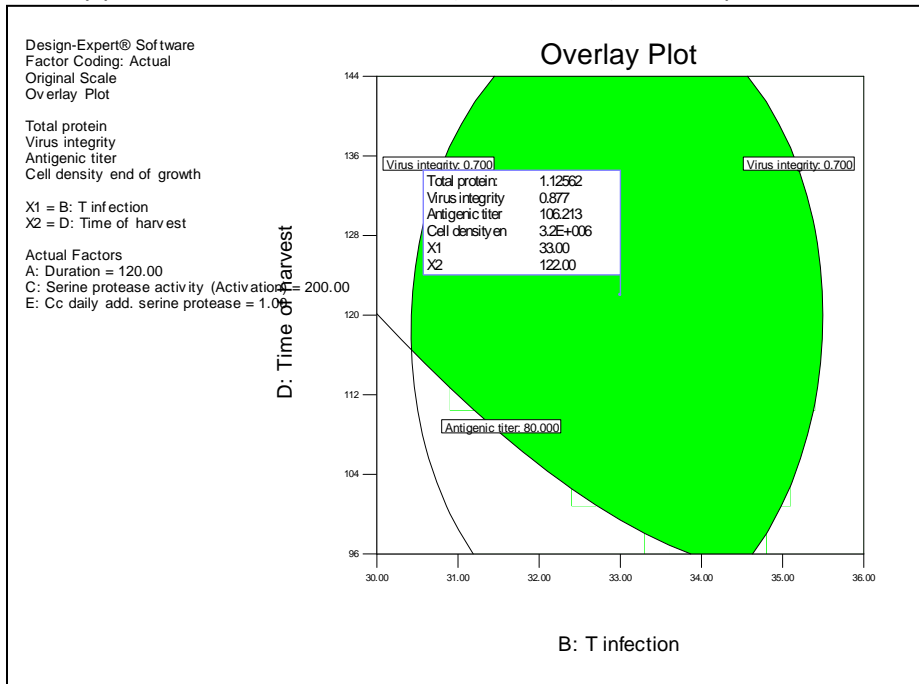3485   •   Green: All specifications are met (in average).
3486   •   White: One or more responses are out of specification.
3487   Overlay plot for duration of growth and duration of infection (harvest time):
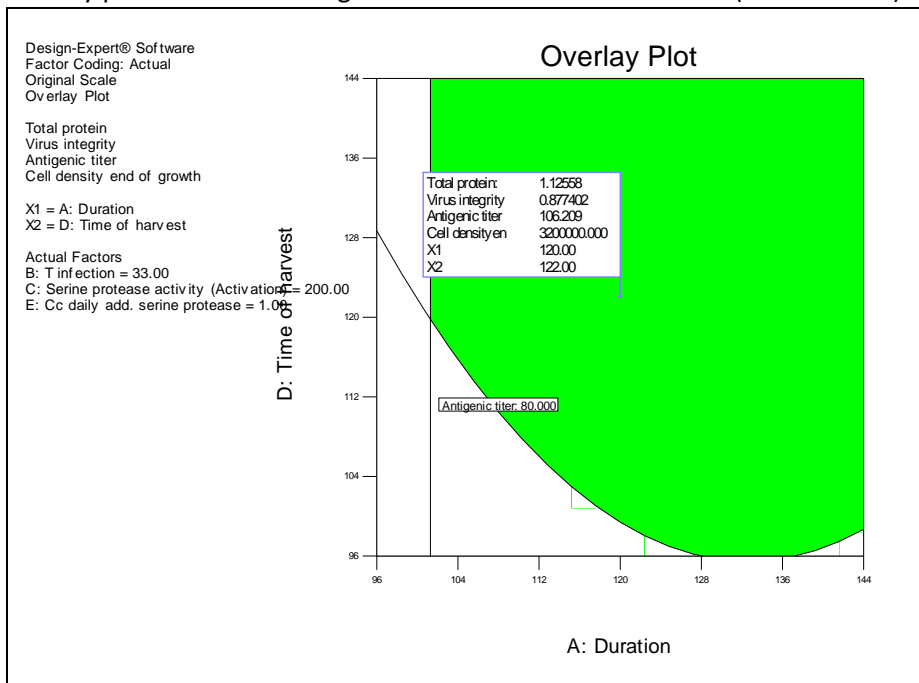
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3490 Overlay plot for duration of infection (harvest time) and temperature of infection:

Design-Expert® Software
Factor Coding: Actual
Original Scale
Overlay Plot

Total protein
Virus integrity
Antigenic titer
Cell density end of growth

X1 = B: T infection
X2 = D: Time of harvest

Actual Factors
A: Duration = 120.00
C: Serine protease activity (Activation) = 200.00
E: Cc daily add. serine protease = 1.00

Overlay Plot

Virus integrity: 0.700   Virus integrity: 0.700

Total protein: 1.12562
Virus integrity   0.877
Antigenic titer   106.213
Cell density en   3.2E+006
X1   33.00
X2   122.00

Antigenic titer: 80.000

D: Time of harvest

B: T infection

3491
3492 The design space could be extracted from the overlay plots, regions where the responses are
3493 within the specifications. In this way of defining a design space, the specifications are met in an
3494 average. This does not take into account the rate of failure because of the uncertainty of the
3495 model or the process variability or the analytical variability. For those reasons, another strategy
3496 will be adopted to determine the design space.

3497 **10.9.4.3.   Design Space Determination**
3498

3499 Rather than taking the limits where the mean responses of the process parameters yield to the
3500 specifications, it can be advantageous to integrate the variability of the responses (known from
3501 the DOE) to predict by simulations the regions where the specifications are met and the defect
3502 rates are acceptable. Those regions constitute the design space.
3503
3504 A failure (defect) is encountered when a batch falls out of the targets:
3505 •   Total protein < 1.5 g/L
3506 •   Virus integrity > 70%
3507 •   Antigenic titre> 80 µg/ml
3508 • $2.5 \ 10^6 \leq$ Cell density end of growth$\leq 3.5 \ 10^6$ cell/ml
3509
3510 The acceptable defect rate is fixed below 5% for the CQAs and below 15% for the KPAs.
3511
3512 The defect profiler tool helps to define the limits of the design space. The defect rate is
3513 represented in function of the process parameters. The reference conditions are shown by the
3514 red dashed vertical lines. The overall risk of failure, the black curve, is the combination of the
3515 failure for each response (illustrated by red curve for proteins, green curve for virus integrity,
3516 blue curve for antigenic titre and orange curve for cell density at the end of growth).

3517
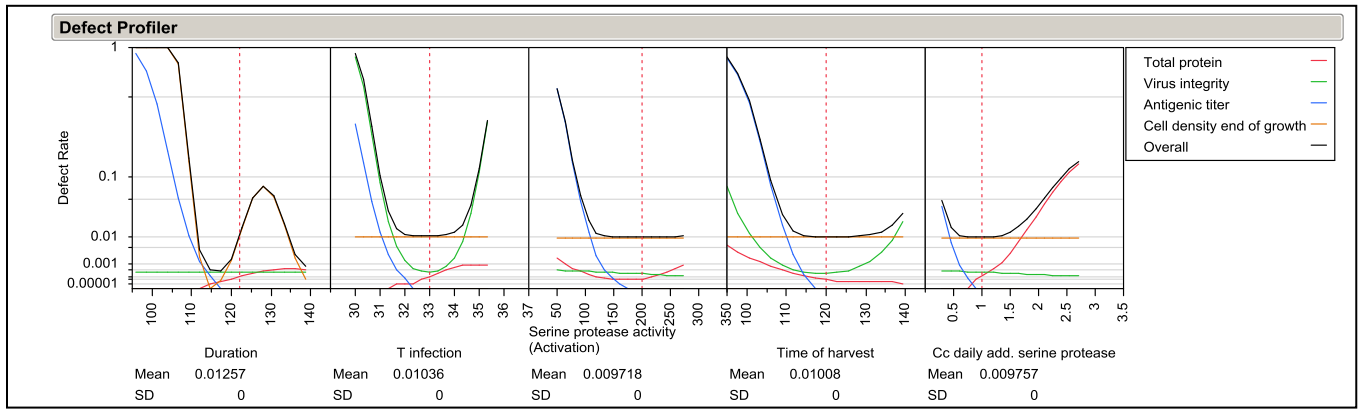
3518
3519
3520 A parallelepiped design space is determined by an iterative algorithm, aiming to maximize
3521 process parameter ranges while keeping the defect rate below 5% for CQAs and 15% for KPAs in
3522 each point of the design space. Simulations are then performed in the proposed design space to
3523 quantify the defect rate.
3524

	Parameter	Ref.	Design space	
			Min.	Max.
Process	Duration	120	116	124
	T infection	33	31.5	34.5
	Serine protease activity (Activation)	200	190	250
	Time of harvest	122	118	126
	Cc daily add. serine protease	1	0.9	1.15
	MOI	10^{-4}	$5\,10^{-5}$	$9\,10^{-3}$
Defect rates	Total protein	0.01%	0.54%	
	Virus integrity	0.03%	1.91%	5% max.
	Antigenic titer	0.00%	14.56%	
	Cell density end of growth	0.96%	3.20%	15% max.
	All	0.99%	14.91%	

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3526
3527 **10.9.5.   Control Space**
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3529 Routine operations will be conducted within the boundaries of the control space. The control
3530 space is included in the design space. It is defined from process knowledge: control capability of
3531 process parameters, technical or equipment constraints, and flexibility of organization.

Parameter	Ref.	Design space	Proposed Range	Control space
Duration (h)	120	116-124	+/- 4	116-124
Multiplicity of infection (-)	10^{-4}	$5\ 10^{-5}$ - $9\ 10^{-3}$	+/- 0.5 LOG	$5\ 10^{-5}$ - $5\ 10^{-4}$
T infection (°C)	33	31.5-34.5	+/- 1.5	31.5-34.5
Serine protease activity at activation (IU/ml)	200	190-250	+/- 10	190-210
Time of harvest (h)	122	118-126	+/- 4	118-126
Cc daily addition serine protease (IU/ml)	1	0.9-1.15	+/- 0.05	0.95-1.05

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3533

3534 As a confirmation, random error on routine process around reference conditions can be added
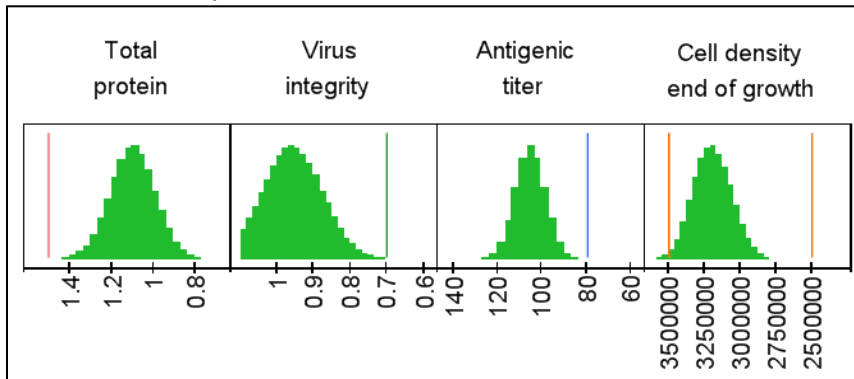3535 to response variability in Monte Carlo simulations.

3536

3537 Distribution of random error on routine process:
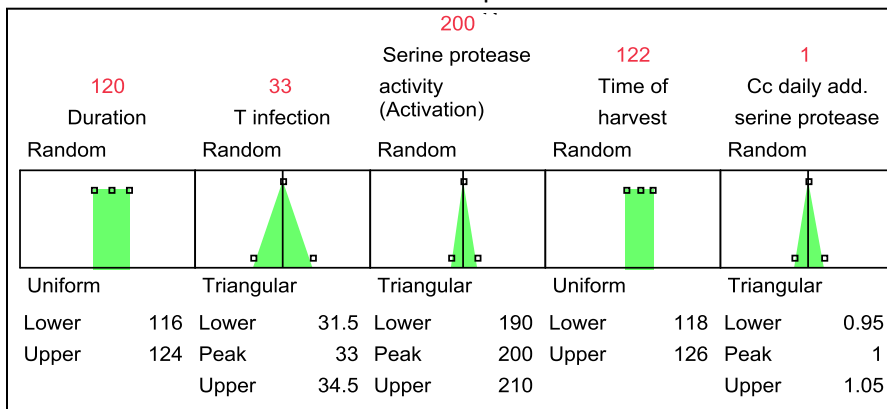
3538
3539 The distribution of the results around the specification is illustrated below; the vertical lines are
3540 the limits of the specifications.


3541
3542 The predicted proportion of out-of-specification results is very low within the control space. The
3543 global defect rate is below 1% in the control space.

| Defect	Rate
Total protein	0.01%
Virus integrity	0.11%
Antigenic titer	0.01%
Cell density end of growth	0.65%
All	0.78%

3544
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3546   The simulations can also show the optimization between the Phase II and the manufacturing
3547   processes in terms of performance and robustness.
3548
3549   Process performances

Predicted responses at reference conditions
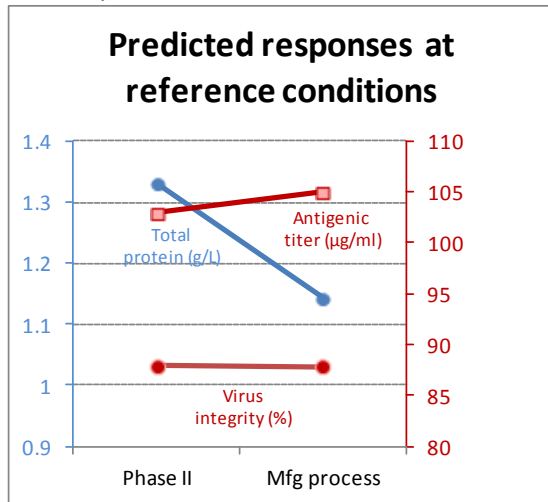
3550
3551   Process robustness

Defect rates at reference conditions
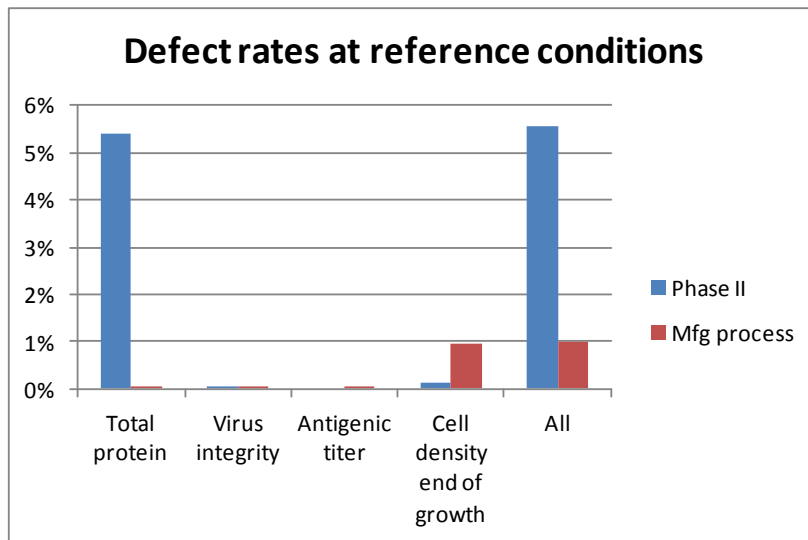
3552
3553
3554   10.9.6.   Categorization of Process Parameters

3555   The process parameters are categorized as noncritical process parameters, critical process
3556   parameters (CPPs), and well-controlled critical process parameters (WC-CPPs). The ICH defines a
3557   CPP as: *a process parameter whose variability has an impact on a critical quality attribute and*
3558   *therefore should be monitored or controlled to ensure the process produces the desired quality.*
3559   A WC-CPP is defined as: a CPP that *has a low risk of falling outside the design space.*
3560   The final assessment of CPPs and WC-CPPs is reevaluated based on the knowledge generated
3561   during design space definition. All critical parameters are WC-CPP.
3562

3563

Parameter	Quality attributes		Process attributes		Risk mitigation
	Total protein	Virus integrity	Cell density	Antigenic titer	
Cell passage					Studied during Ph II development
Cytodex concentration					Studied during Ph II dvpt
Shear protective additive					OFAT study done during Ph III process optimization
Seeding density					Studied during Ph II dvpt
Temperature cell growth					Studied during Ph II dvpt
pO$_2$					Studied during Ph II dvpt
pH cell growth					DOE done during Ph III process optimization
Pressure					Studied during Ph II dvpt
Stirring					Scale-up based on Phase II dvpt
Daily glucose adjustment					DOE done during Ph III process optimization
Daily glutamine adjustment					DOE done during Ph III process optimization
Growth duration	✓		✓	✓	DOE done during design space determination
Temperature infection	✓	✓	NA	✓	DOE done during design space determination
pH infection			NA		DOE done during Ph III process optimization
MOI	✓	✓	NA	✓	DOE done during Ph III process optimization
Viral activation: serine protease activity	✓		NA	✓	DOE done during design space determination
Viral activation: serine protease contact duration			NA		DOE done during Ph III process

					optimization
Daily addition of serine protease: concentration			NA		DOE done during design space determination
Viral replication duration (time of harvest)			NA		DOE done during design space determination
Media preparation – filter size					OFAT study done during Ph III process optimization
Media stability – shelf life					OFAT study done during Ph III process optimization
Viral production media selection					Studied during Ph II development

3564   Noncritical process parameters are blank.
3565   WC-CPP: Parameter impacts an attribute, but is well-controlled.
3566   CPP: Parameter impacts an attribute, but the range is close to the control capability.