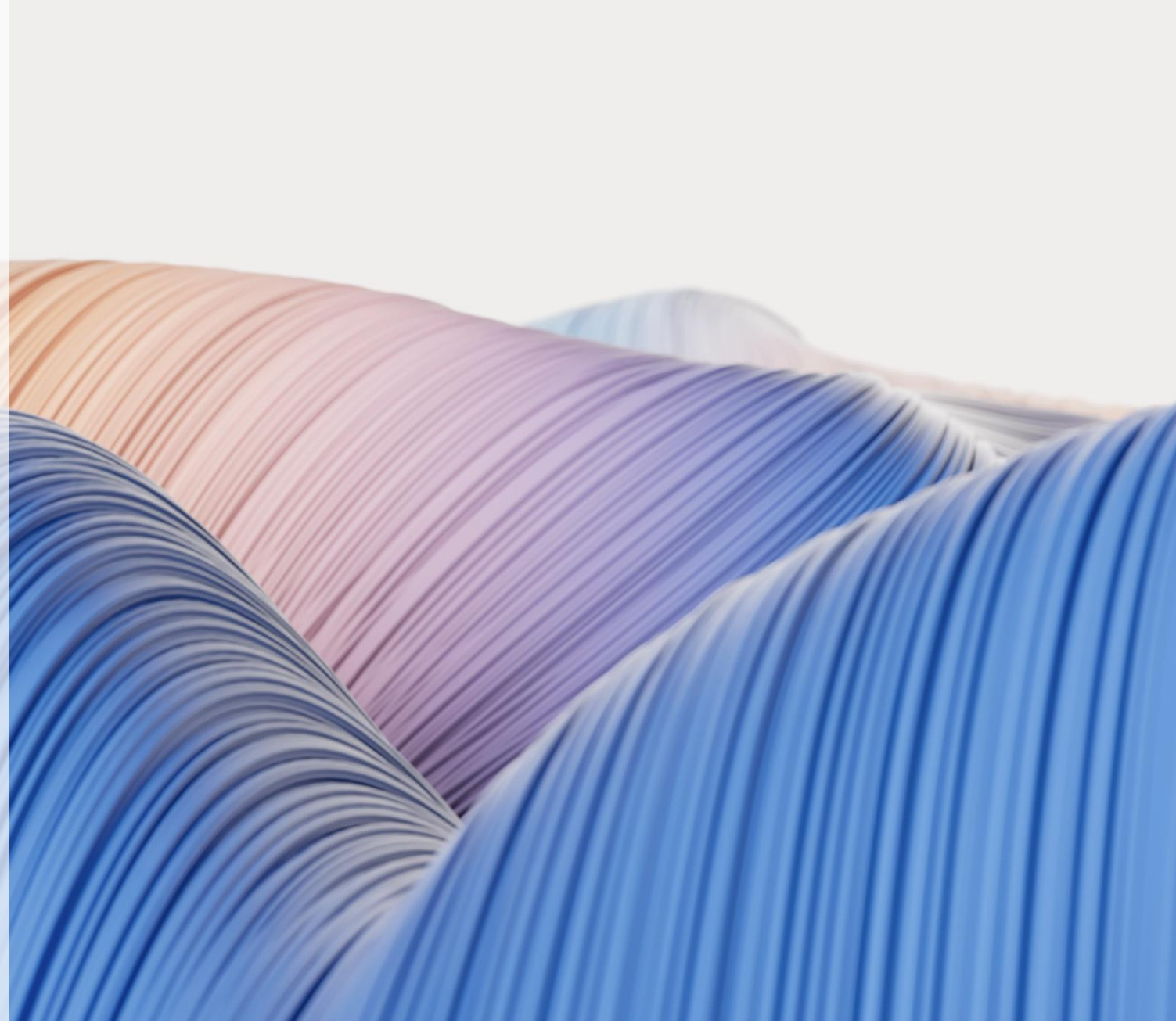
AAV Critical Quality Attributes: Comprehensive Analytical Control Strategies from Release to Characterization



Thomas Powers

Pfizer BTxPS Analytical Research and Development

Organizational Context

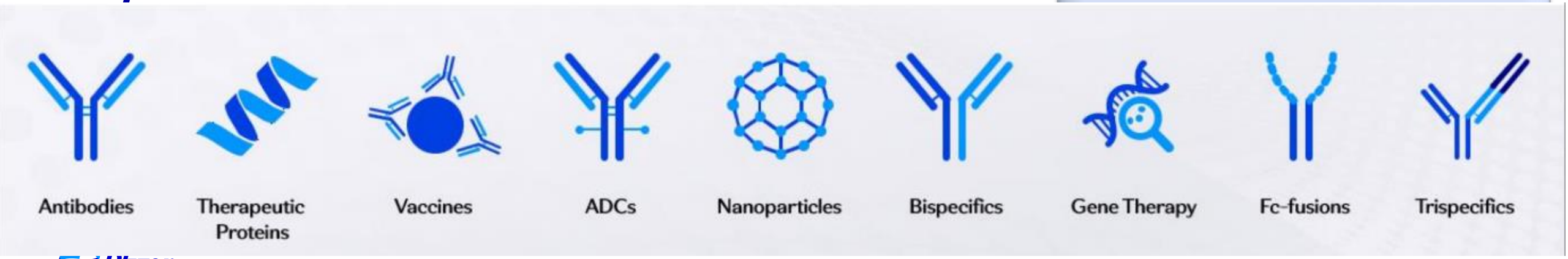
ARD

- Project Progression Lines (PPL)
- Heightened Characterization
- Bioassay and Impurity
- Micro Strategy and Testing

Key Functions: Method development, characterization, process support, qualification/validation, small molecule impurities

Key Technologies: CE, HPLC, NGS, Biochemical techniques, MS

Stage of Development: Pre-clinical to Licensure



Evaluation of the Product's Control Strategy

Pre-clinical	Phase I	Phase II	Phase III

Early Product Knowledge:

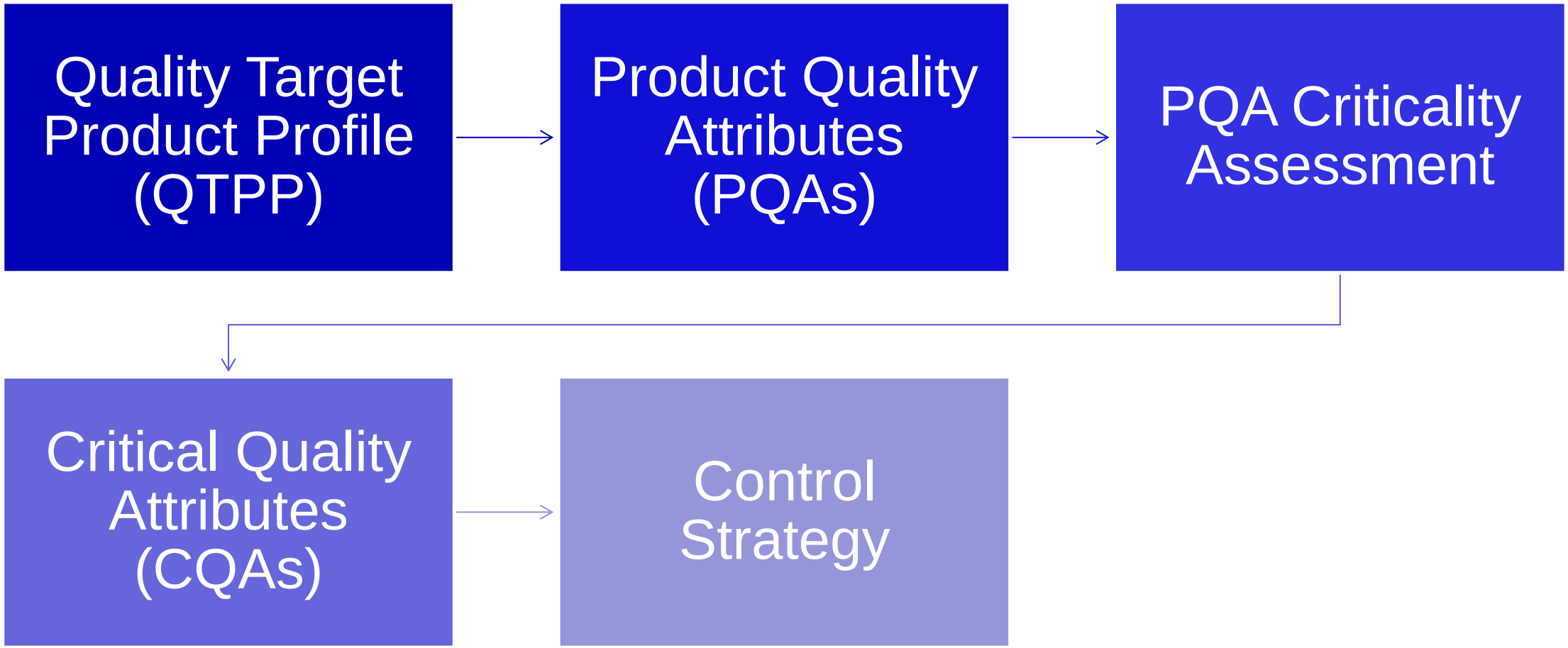
- Research Unit
- Platform Knowledge

Delivery to Commercial Group:

- Well understood product and process
- Specifications and methods
- Control Strategy per ICH Q10

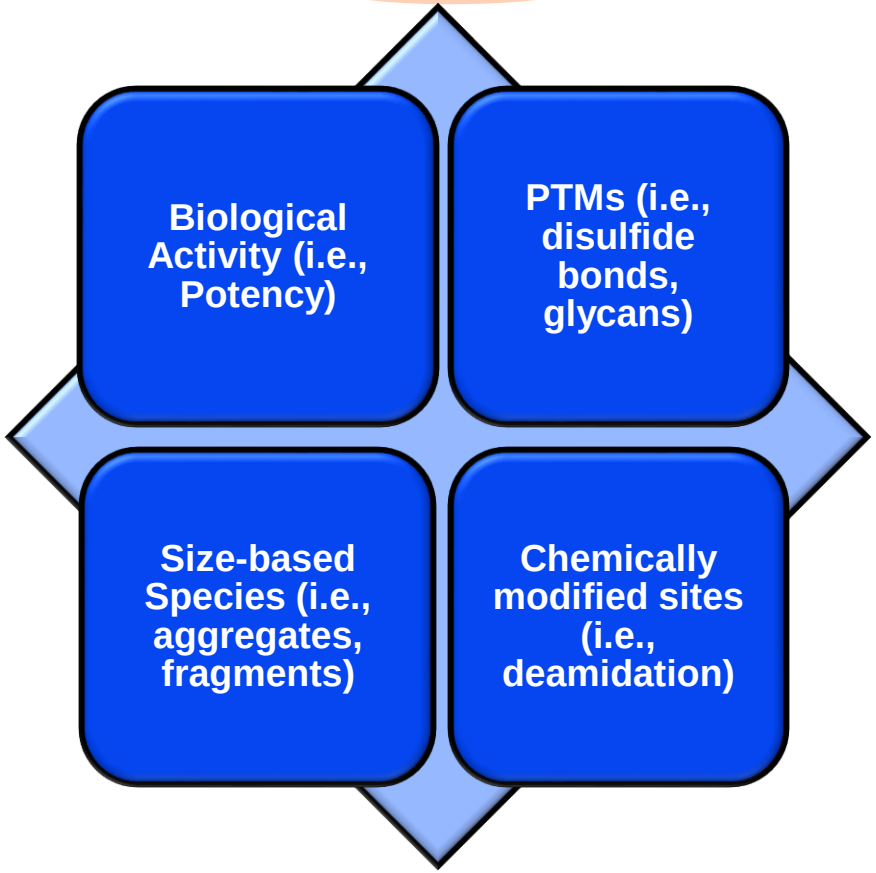
The comprehensive control strategy is essential to ensure appropriate measures are in place to minimize potential patient risks associated with the product.

Overview of Attribute Criticality



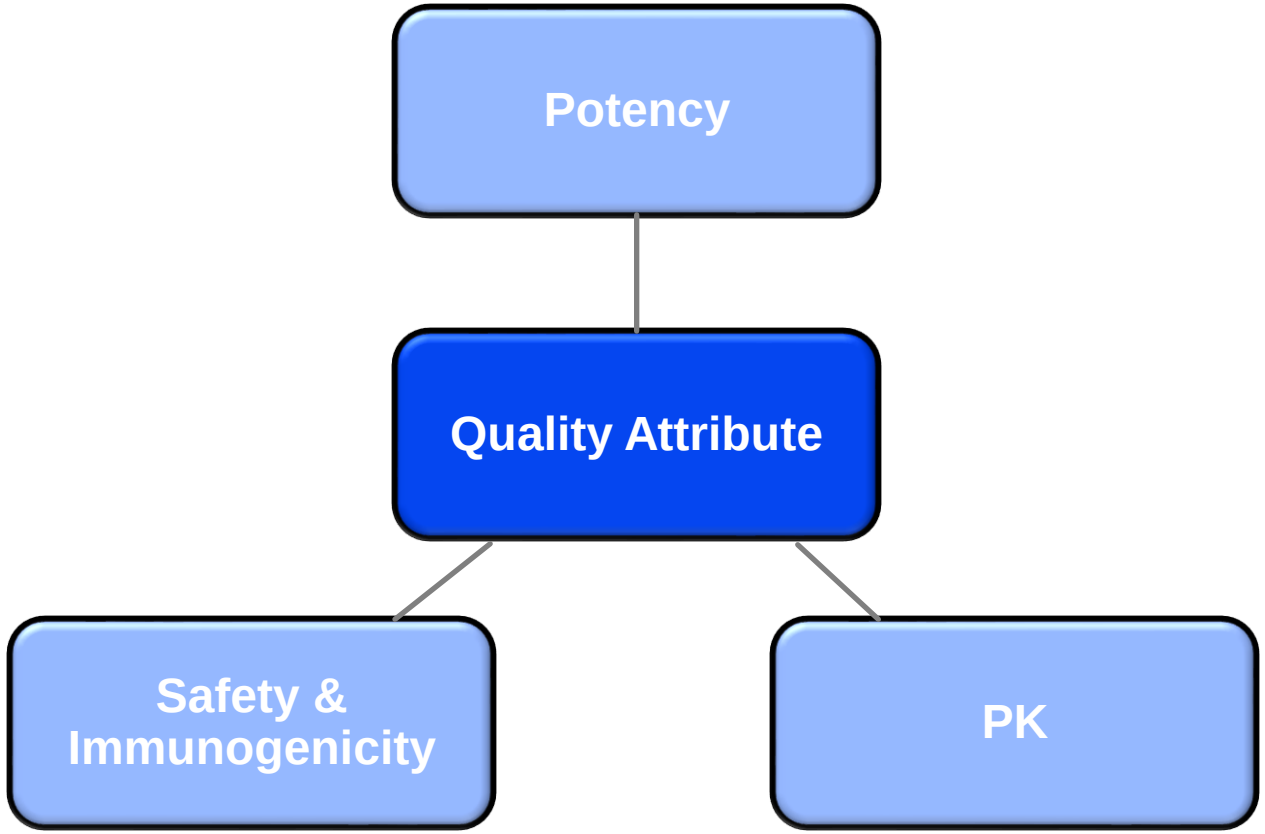
Overview of Attribute Criticality

Product Quality Attribute (PQA)



- Molecular or product characteristics that define the quality of the product
- PQAs have the *potential* to impact product quality

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)

CQA Assessment and Report

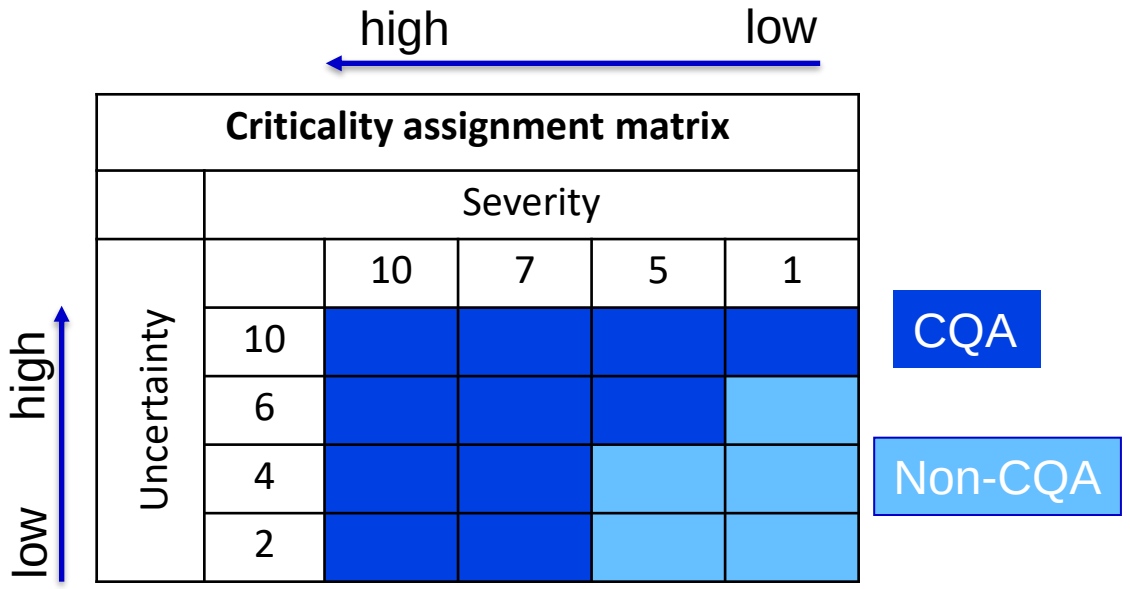
Assess risk to safety and efficacy using a scoring system of severity and uncertainty.

The criticality determination is based on the highest designation of one of the two categories scored.

- The tool automatically applies the scores and determines the criticality.

Sources of information

- Molecule understanding
- Scientific literature
- Prior/platform knowledge
- Structure-function relationships
- Structural elucidation experiments

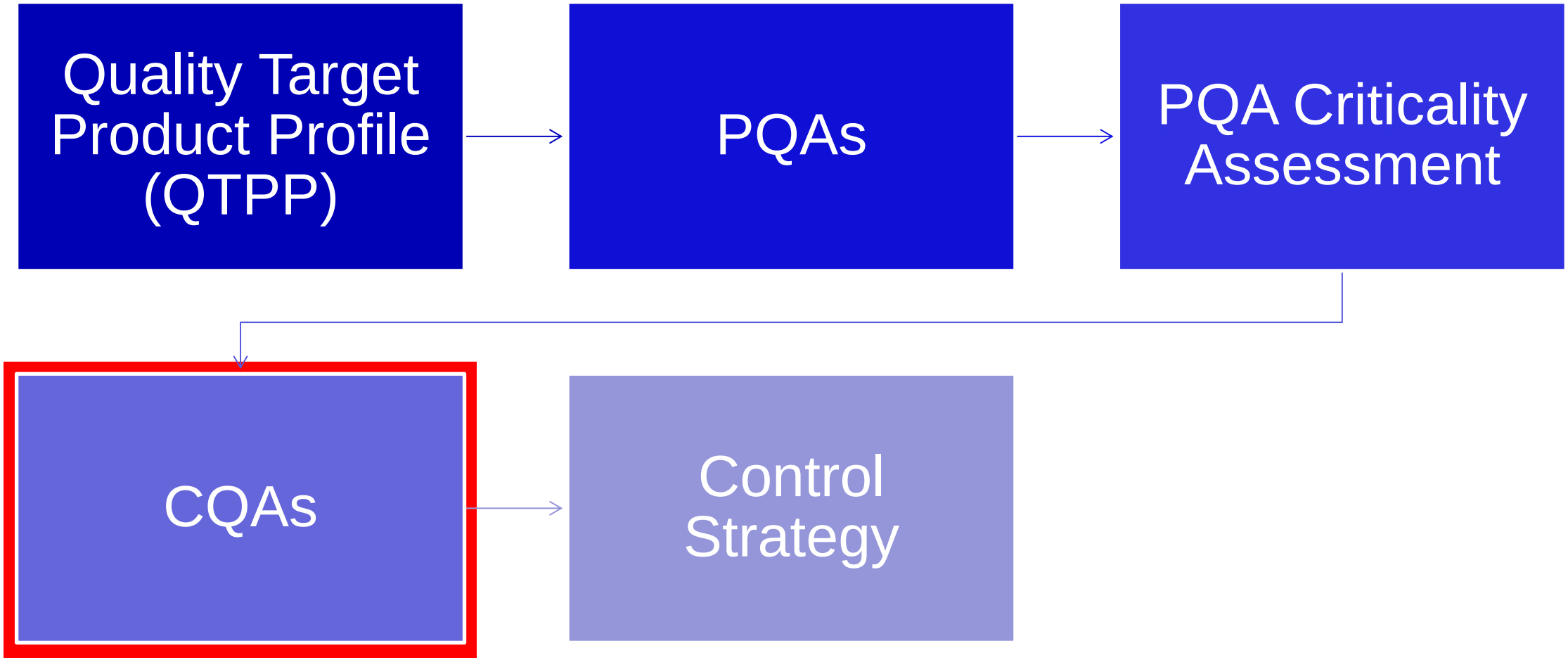


RISK = Uncertainty x Severity

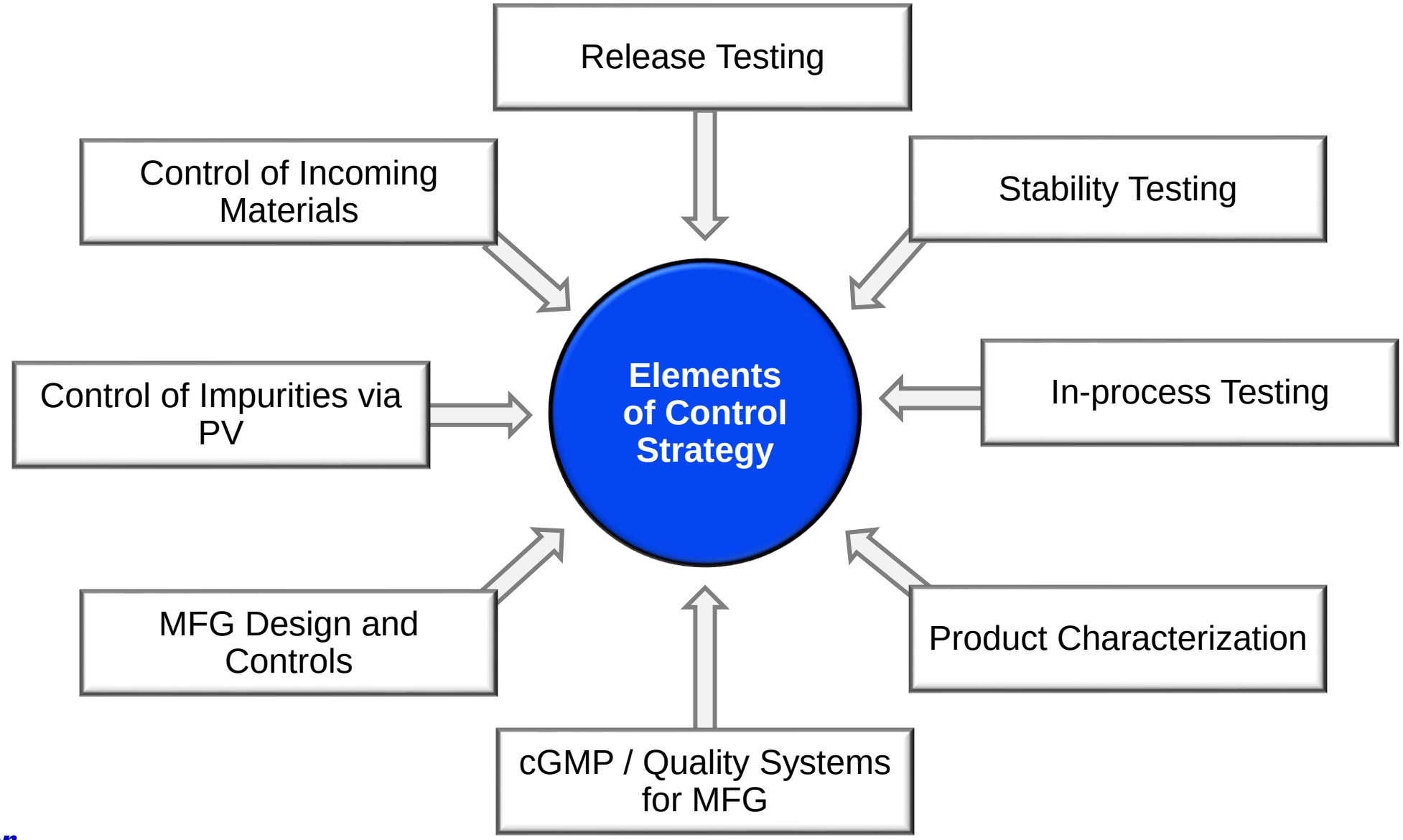
Level of knowledge

Impact to efficacy
Impact to safety

Overview of Attribute Criticality



Elements of the Comprehensive Control Strategy



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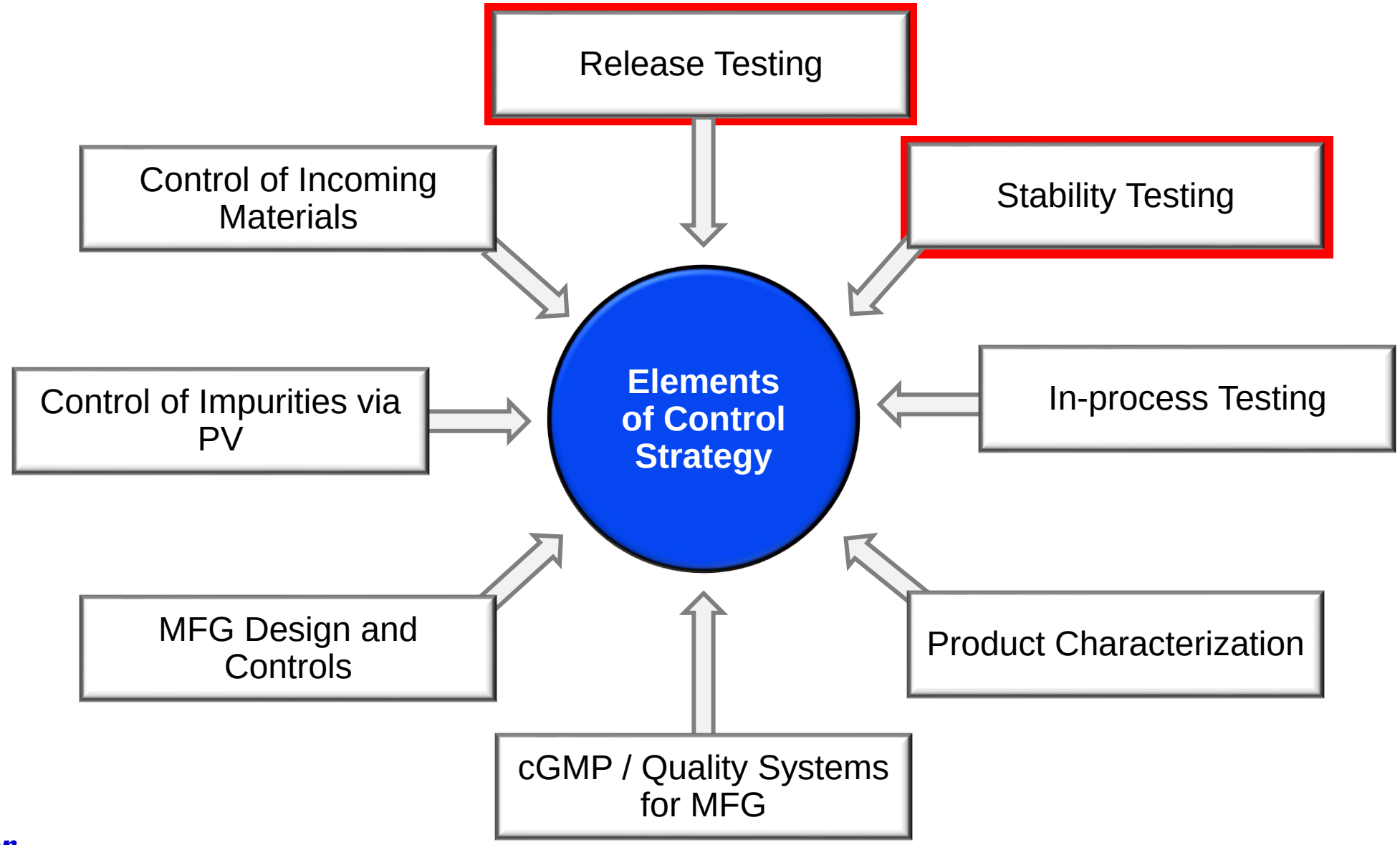


Analytical Control Strategy for AAV Release/Stability Testing

Release/Stability Tests Cover These Attributes

Identity, Strength, Potency	Quality and Purity	Compendial Requirements	Safety
• Capsid Identity	• Capsid Purity and Impurities	• Appearance	• rcAAV*
• Genome Identity	• Genome Impurities	• pH	• Host Cell DNA*
• Genome Titer	• Genome Integrity	• Osmolality	• Host Cell Protein*
• Particle Titer	• Size Distribution	• Volume in Container**	• Plasmid DNA*
• Potency	• Particle Content	• Subvisible Particle**	• Other process-related impurities*
			• Sterility**

* DS Only

** DP Only

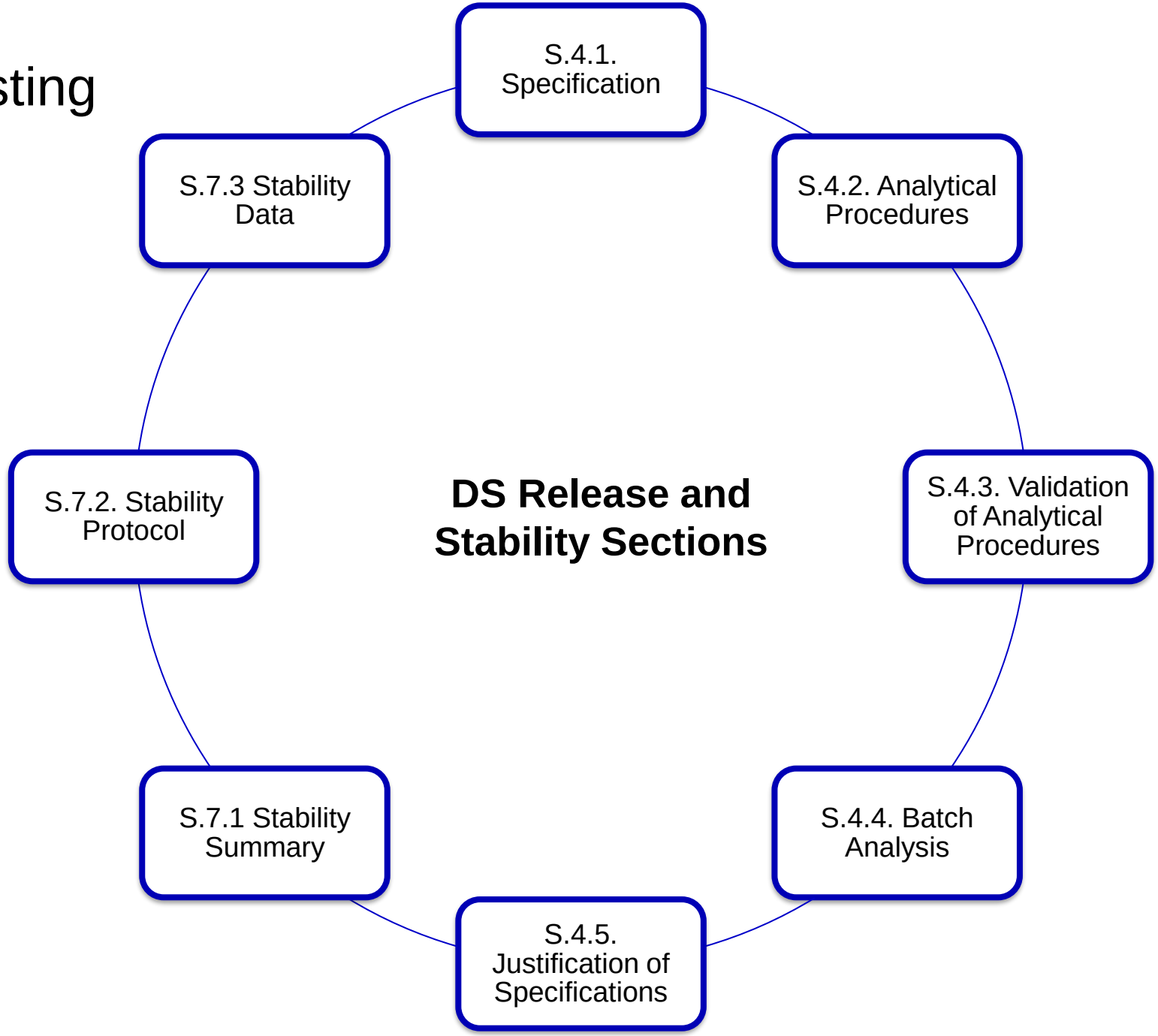
Pfizer has a robust analytical control strategy to support gene therapy programs

Release and stability tests ensure attributes are within acceptable ranges

Release and Stability Testing

Release and stability testing of DS and DP ensure sufficient and appropriate controls are in place to minimize potential risks to patients

- Lab operations following Good Manufacturing Processes (GMP)
- Tested against defined acceptance criteria



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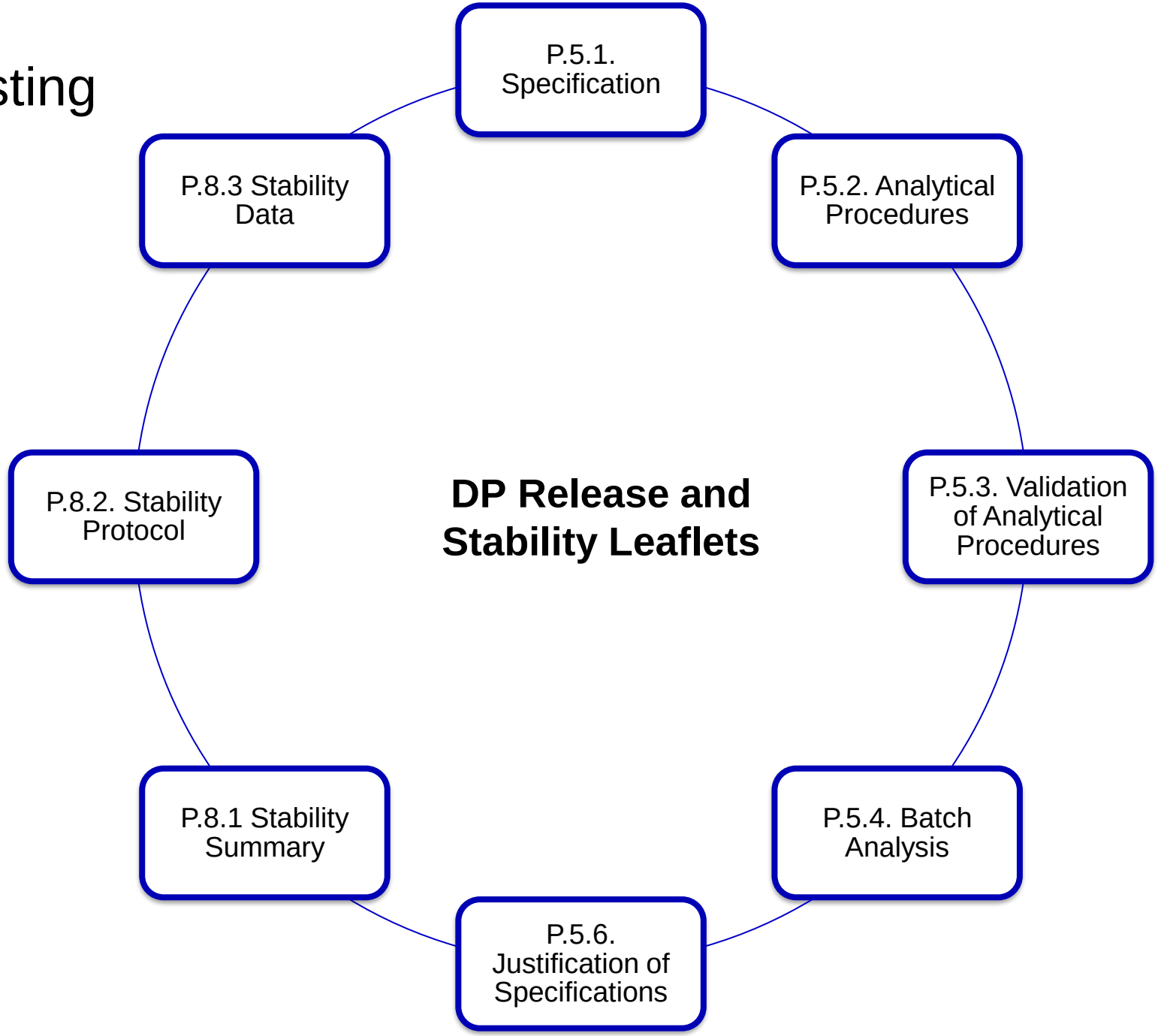
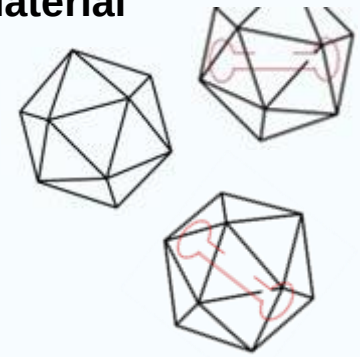


Release and Stability Testing – Vector Genome Titer

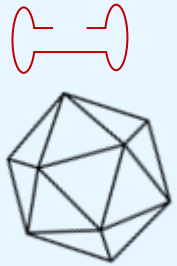
Vector genome titer: commonly implemented dosing assay for AAV gene therapies

AAV Material



Measurement

Included



Not Included

Objectives for Measurement

Reasonable and Support Acceptance Criterion

Limited Type 1 and 2 Errors

Accurate and Precise Method

Development of Novel Procedure (Publication in Draft)

Reproducibility
- ≤ 1.7%

Accuracy
- Within ± 10%

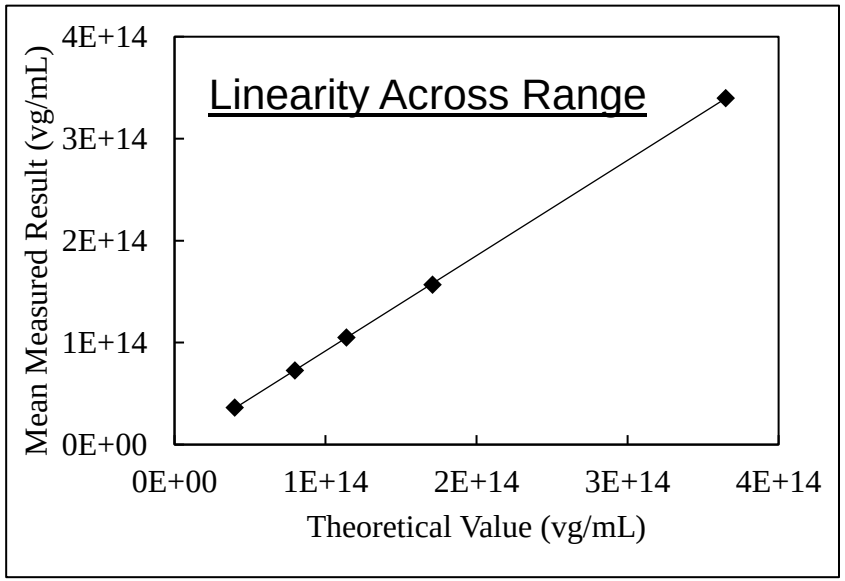
Specificity
- Demonstrated

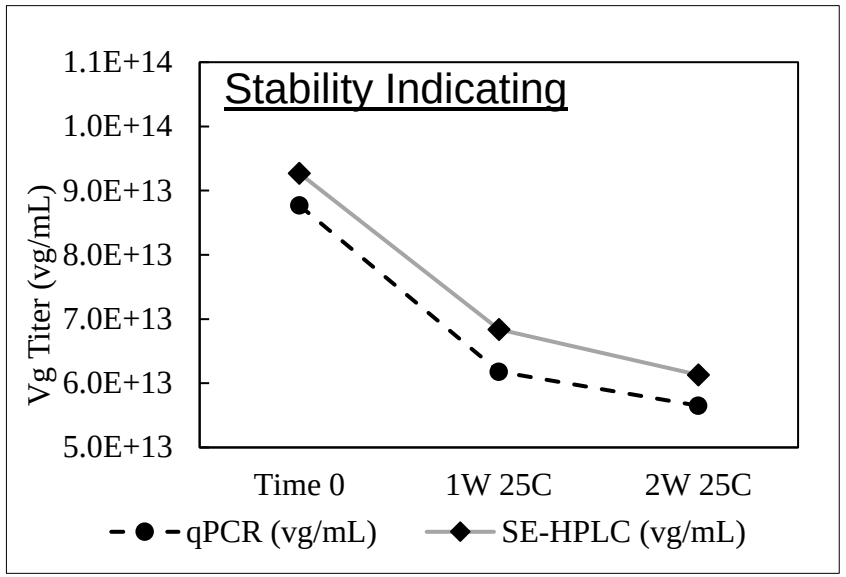
Linearity
- R^2 = 1.00

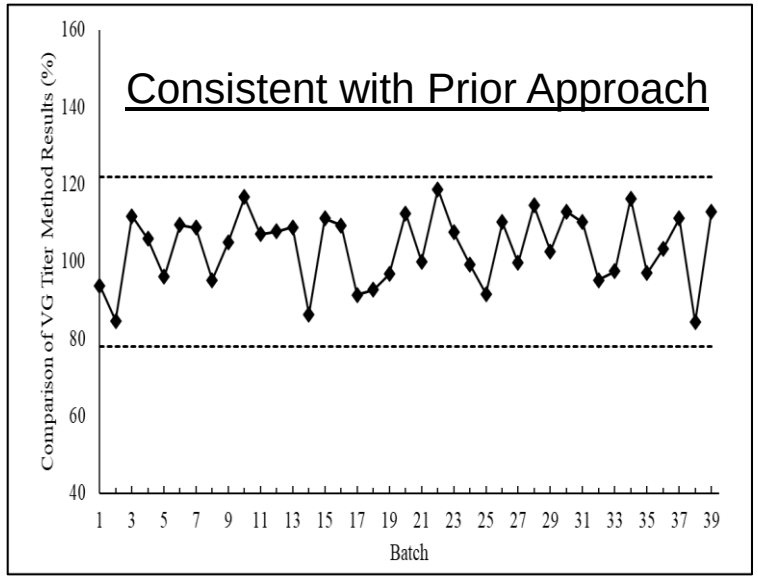
Range
- Suitable range demonstrated for in-process and release testing

Stability Indicating
- Can detect changes on stability

Release and Stability Testing – Vector Genome Titer







Development of Novel Procedure (Publication in Draft)

Reproducibility
- ≤ 1.7%

Accuracy
- Within ± 10%

Specificity
- Demonstrated

Linearity
- R^2 = 1.00

Range
- Suitable range demonstrated for in-process and release testing

Stability Indicating
- Can detect changes on stability

Impact of Procedure Precision on Batch Failure Rate

Analytical Procedure RSD (%)

Acceptance Criterion (+/- X%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
30	0.0	0.0	0.0	0.0	0.1	0.3	0.7	1.2	2.1	4.9	7.1	8.3	12.1	14.6	18.2
29	0.0	0.0	0.0	0.0	0.1	0.4	0.8	1.3	2.1	5.5	7.8	9.7	13.4	16.3	19.7
28	0.0	0.0	0.0	0.0	0.2	0.4	0.8	1.6	2.8	6.2	8.6	10.5	14.5	17.2	21.0
27	0.0	0.0	0.0	0.1	0.3	0.4	1.0	2.3	3.5	7.0	9.5	12.2	16.0	18.0	22.2
26	0.0	0.0	0.0	0.1	0.3	0.4	1.5	2.7	4.2	7.5	11.4	14.0	17.9	19.6	23.2
25	0.0	0.0	0.0	0.1	0.4	0.6	1.8	3.1	5.0	8.4	12.6	15.2	20.4	21.3	24.7
24	0.0	0.0	0.0	0.1	0.4	0.8	2.3	4.1	5.8	9.3	13.7	16.4	22.3	23.3	27.2
23	0.0	0.0	0.0	0.1	0.5	0.8	2.8	4.9	7.0	10.8	15.7	18.3	24.1	25.2	28.9
22	0.0	0.0	0.0	0.1	0.8	1.3	3.4	5.4	8.8	12.3	16.9	20.5	26.2	28.3	31.3
21	0.0	0.0	0.0	0.1	0.8	1.6	4.3	6.8	9.6	13.7	18.8	21.9	28.7	30.6	34.3
20	0.0	0.0	0.0	0.2	1.0	2.4	5.2	8.0	10.8	15.6	20.9	23.8	30.7	33.3	36.7
19	0.0	0.0	0.0	0.3	1.2	2.9	6.3	9.9	12.8	17.5	23.3	26.1	32.7	35.9	39.9
18	0.0	0.0	0.0	0.6	1.5	3.7	7.9	12.7	14.8	19.1	25.7	28.4	35.4	37.5	42.1
17	0.0	0.0	0.1	0.9	2.7	4.6	9.7	15.0	17.7	23.3	27.8	30.5	37.8	39.3	44.9
16	0.0	0.0	0.3	1.2	3.3	6.1	12.7	17.4	20.0	26.1	31.3	33.3	40.2	42.7	47.3
15	0.0	**0.0**	0.4	1.5	4.9	7.3	**15.4**	19.4	22.5	29.4	34.3	36.5	44.8	44.8	50.2
14	0.0	0.0	1.2	2.6	6.7	9.0	18.6	23.0	25.1	32.7	37.4	39.7	48.1	47.7	52.5
13	0.0	0.1	1.7	3.4	9.2	11.7	21.7	26.6	29.1	35.5	40.2	43.9	52.3	50.8	55.9
12	0.0	0.2	2.2	4.9	11.9	15.3	24.1	30.9	32.4	39.1	44.4	48.2	54.5	55.4	59.3
11	0.0	0.3	3.6	7.6	16.1	18.8	28.2	34.2	36.4	43.1	48.3	51.9	58.0	59.5	62.6
10	0.0	1.2	4.9	10.7	20.4	22.6	32.6	39.5	41.0	47.3	52.9	55.7	60.8	63.4	66.0
9	0.6	2.5	7.9	15.8	25.4	27.8	37.2	43.8	46.7	52.1	57.7	59.9	64.4	66.5	68.9
8	1.4	4.3	10.8	20.2	29.9	34.4	41.6	48.8	53.0	58.6	61.6	64.3	68.2	70.9	72.4
7	3.5	7.2	15.1	26.9	35.0	40.6	48.7	53.1	57.2	63.0	66.0	69.5	71.5	73.5	75.5
6	5.6	12.8	21.5	35.7	42.3	49.1	55.4	60.3	63.0	67.6	70.7	73.5	75.5	77.2	78.4
5	11.5	20.6	30.5	42.5	50.6	56.1	62.3	66.5	69.6	72.0	75.8	77.3	79.9	81.3	82.0
4	19.6	30.2	42.8	51.6	60.1	65.3	69.6	72.9	77.0	76.9	80.7	81.3	84.7	86.0	85.0
3	34.6	43.0	56.3	64.1	70.1	73.4	77.2	78.9	81.8	81.5	84.9	85.7	88.1	89.6	88.0
2	54.5	59.7	70.5	75.7	80.4	82.0	85.0	86.9	87.6	87.7	90.0	90.8	93.0	93.0	91.8
1	76.7	78.8	84.1	87.7	89.4	89.8	92.6	93.4	94.0	94.2	95.3	95.0	96.2	96.8	96.3

- Improved procedure should dramatically reduce batch failure
- Reduced patient risk by minimizing Type 2 error
- For nominal dosing, a superior titer assay ensures more consistency in patient dosing

Procedure RSD	+/- 15% Acceptance Criterion	
	Type 1 Errors (%)	Type 2 Errors (%)
2% RSD	0.0	0.0
7% RSD	11.8	1.5

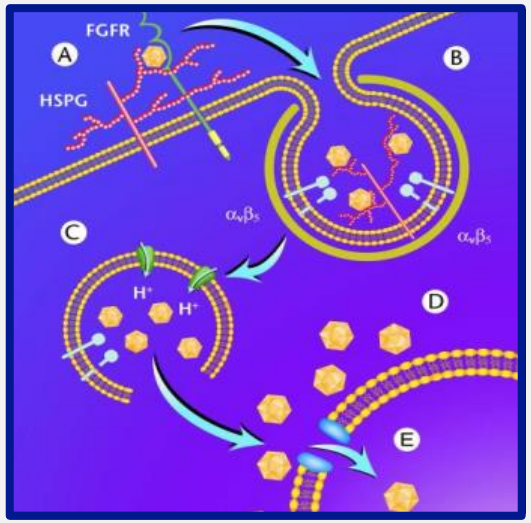
Control of Potency- Example of Selected Control Strategy Elements

AAV Elements Involved in Transduction	AAV Transduction Process	Control
• **Capsid** ✓ Cellular attachment and internalization ✓ Endosomal escape ✓ Nuclear pore entry ✓ Second strand synthesis ✓ Expression of transgene • **Genome** ✓ Uncoating ✓ Second strand synthesis ✓ Second strand annealing ✓ Expression of the transgene	• Correct transgene sequence confirmed prior to generation of AAV • Correct sequence for elements like promoter, ITRs for packaging etc. • Sequence elements key to transduction and expression of therapeutic transgene • Transduction of AAV • **Expression** of target protein • Activity of the expressed target protein (complexity)	• **Capsid** ✓ Capsid Identity ✓ Capsid Purity ✓ Capsid Impurities ✓ Capsid Modifications ✓ Plasmid Control (Sequence) • **Genome** ✓ Genome Identity ✓ Genome Integrity ✓ Genome Purity ✓ Particle Content

Expert Opinion on Biological Therapy 21, 749-766 (2021)

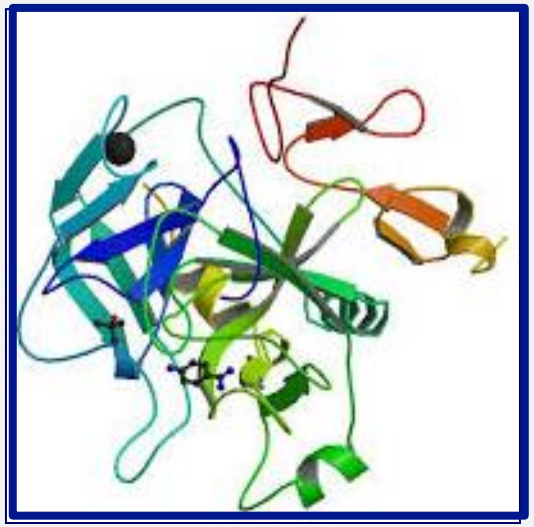
GTx Potency Assay Options

Infectivity



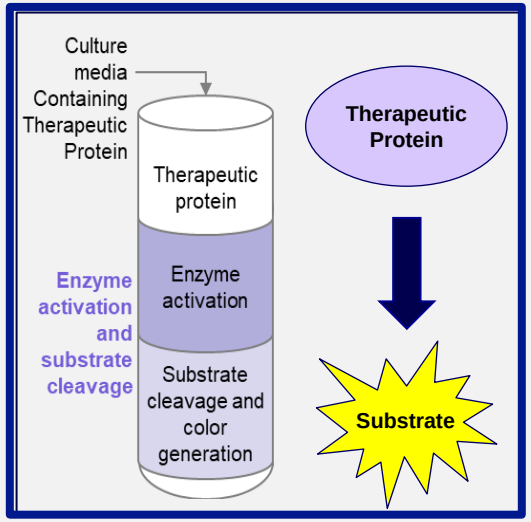
- **Optimal cell line & selectivity conditions**
- **Readout: PCR**

Infectivity → Expression



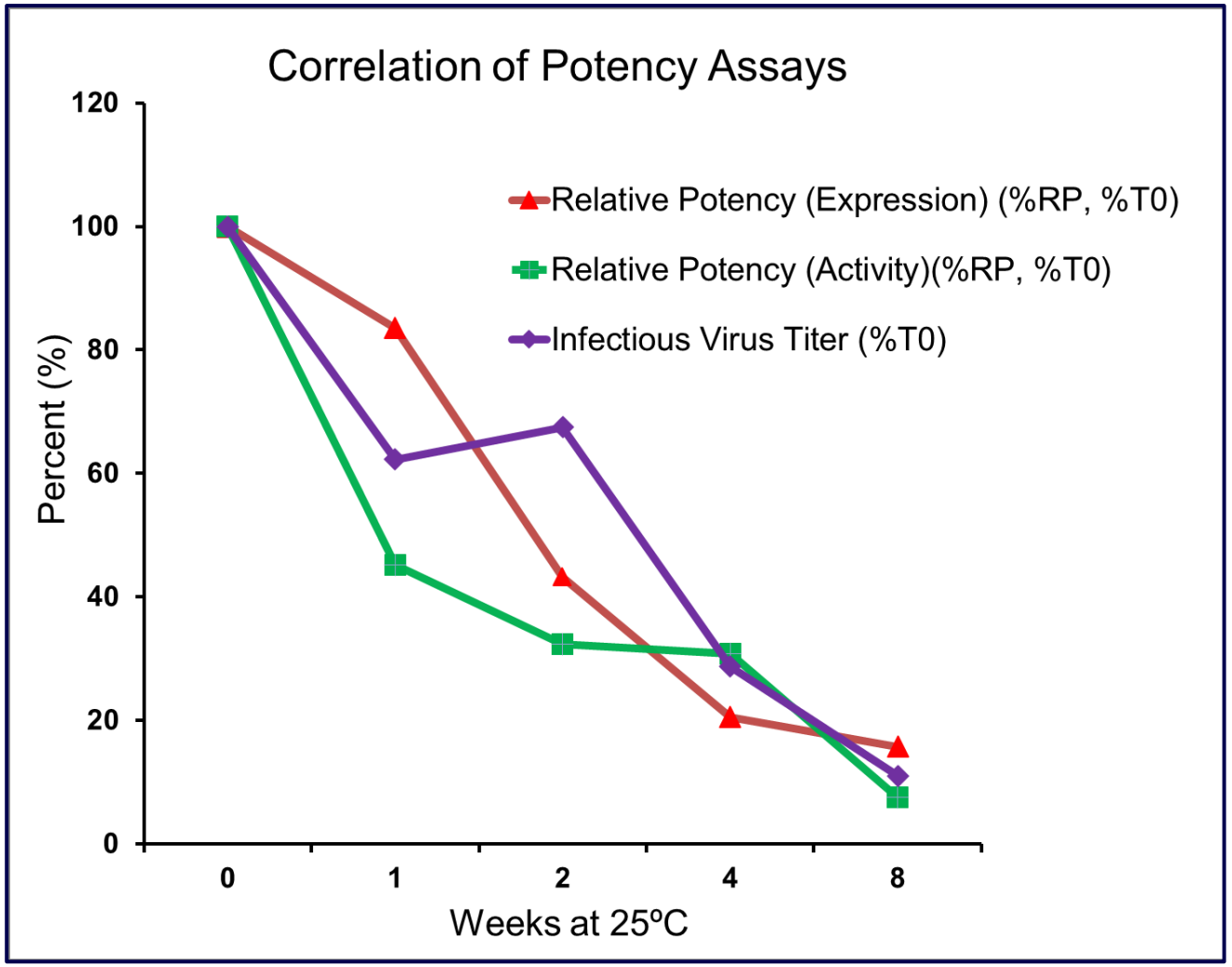
- **Optimal cell line & selectivity conditions & tissue-specific promoter**
- **Readout: ELISA, western blot, RT qPCR**

Infectivity → Expression → Activity



- **Optimal cell line & selectivity conditions & tissue-specific promoter & activity assessment**
- **Readout: activity**

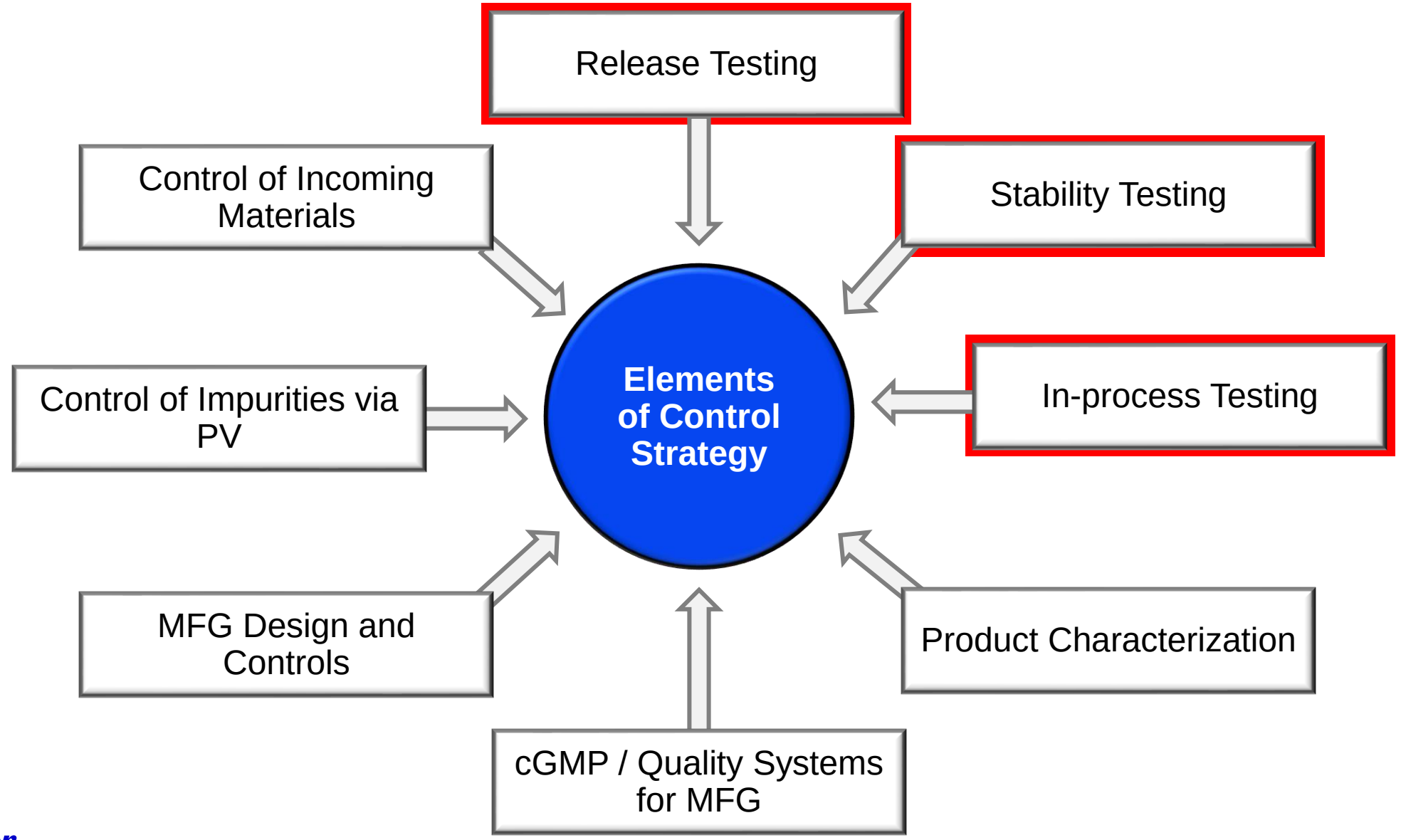
Potency Assays are Stability Indicating- Expression and Activity Assays Correlate



Correlation of Assays

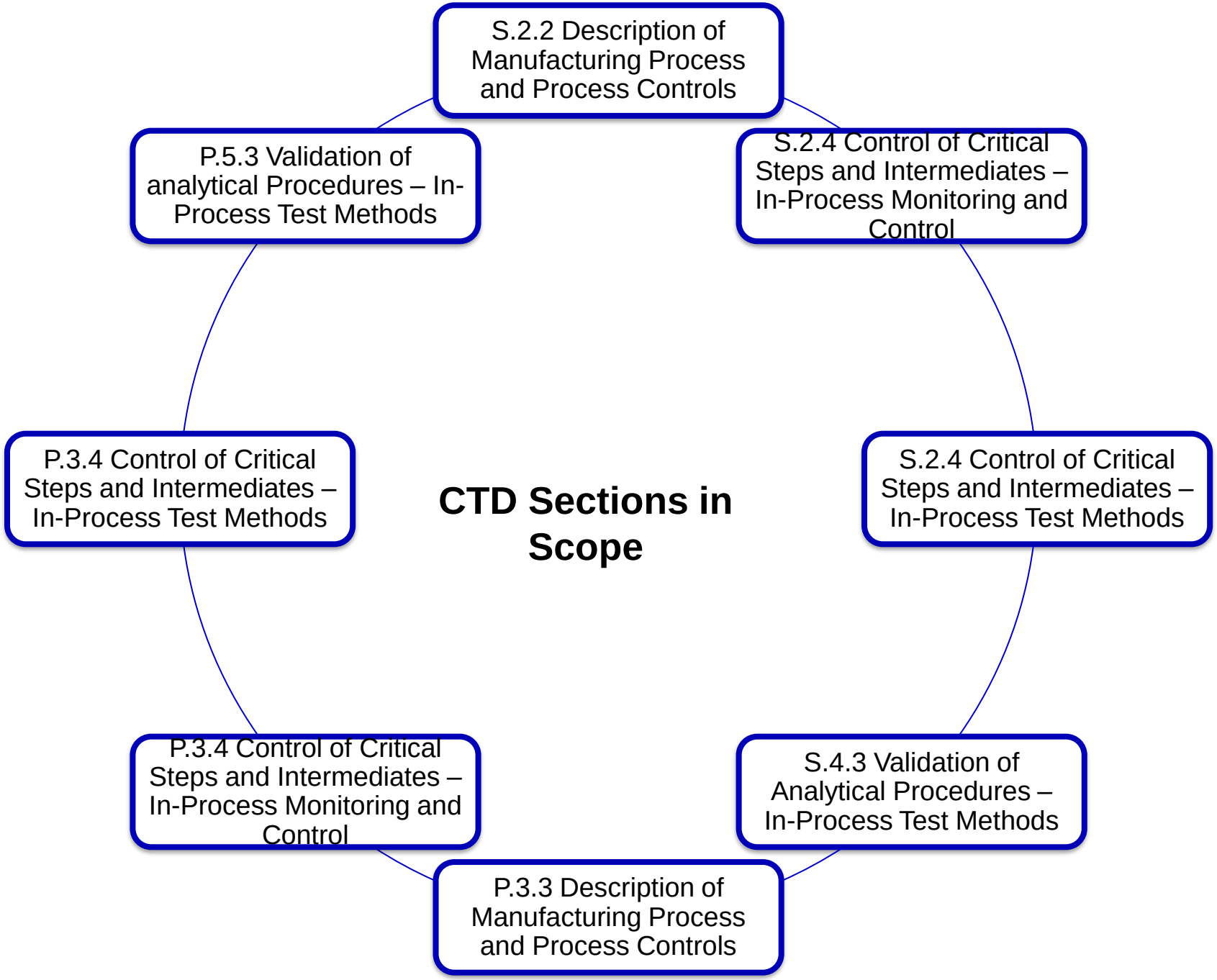
- ✓ Potency measured by expression (red), activity (green), and infectivity (purple)
- ✓ Activity is impacted only by expression
- ✓ Expression demonstrates control of potency
- ✓ Challenging to develop an activity assay depending on MOA
- ✓ Attributes do not change at intended storage (data not shown)

Elements of the Comprehensive Control Strategy

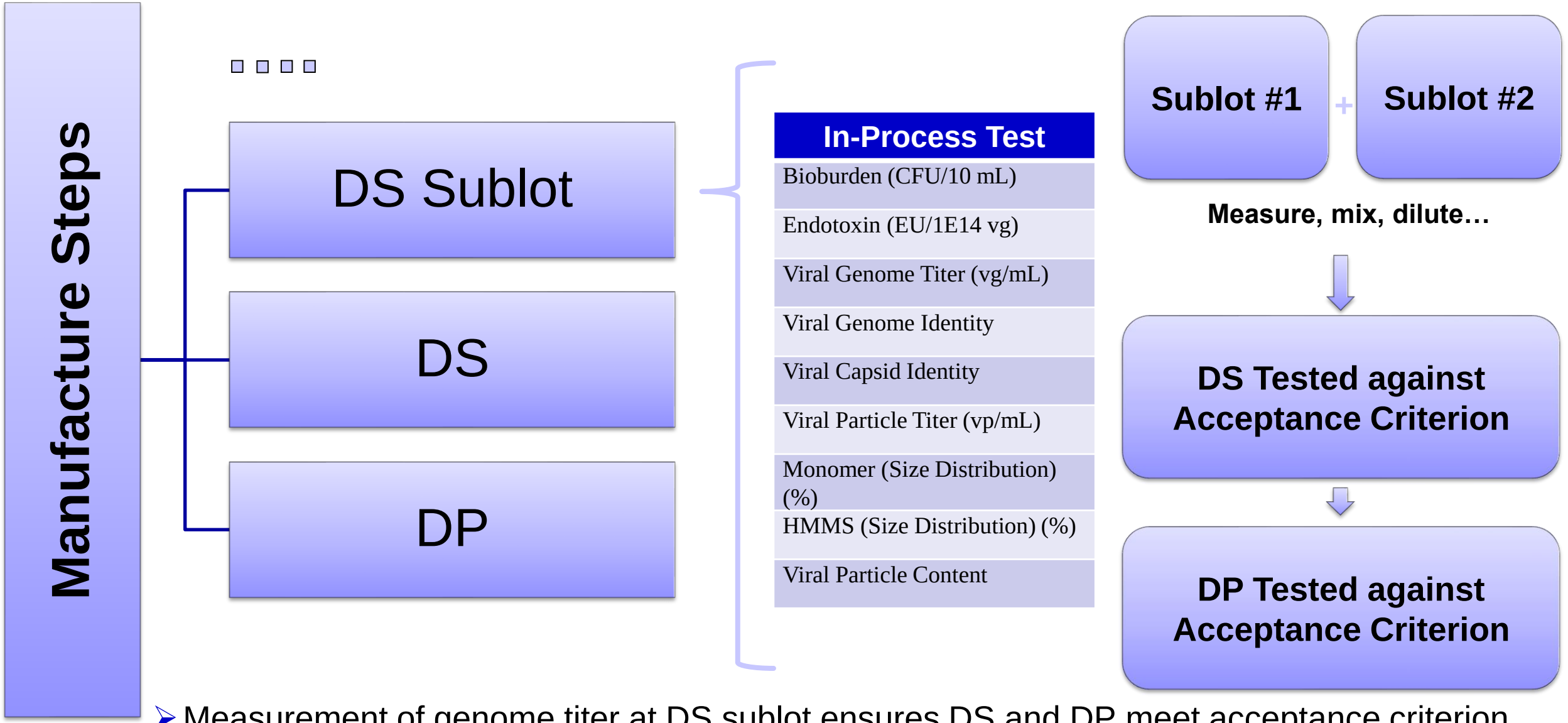


In-Process Testing

- Ensures the quality attributes are maintained within the acceptable limits or ranges
- Procedures used for in-process testing are validated

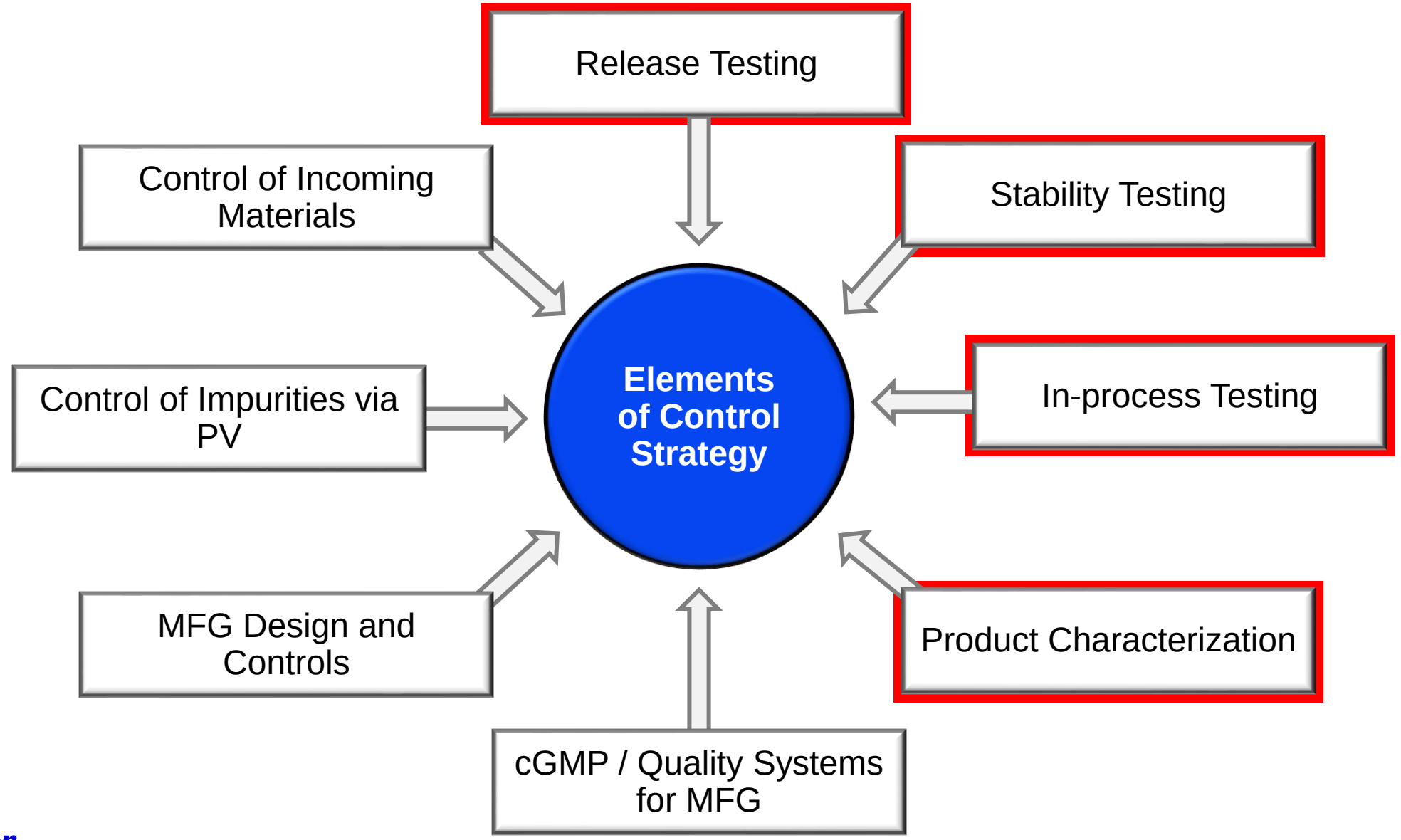


Example of DS Sublot In-Process Tests



- Measurement of genome titer at DS sublot ensures DS and DP meet acceptance criterion

Elements of the Comprehensive Control Strategy



Analytical Control Strategy for AAV GTx Programs

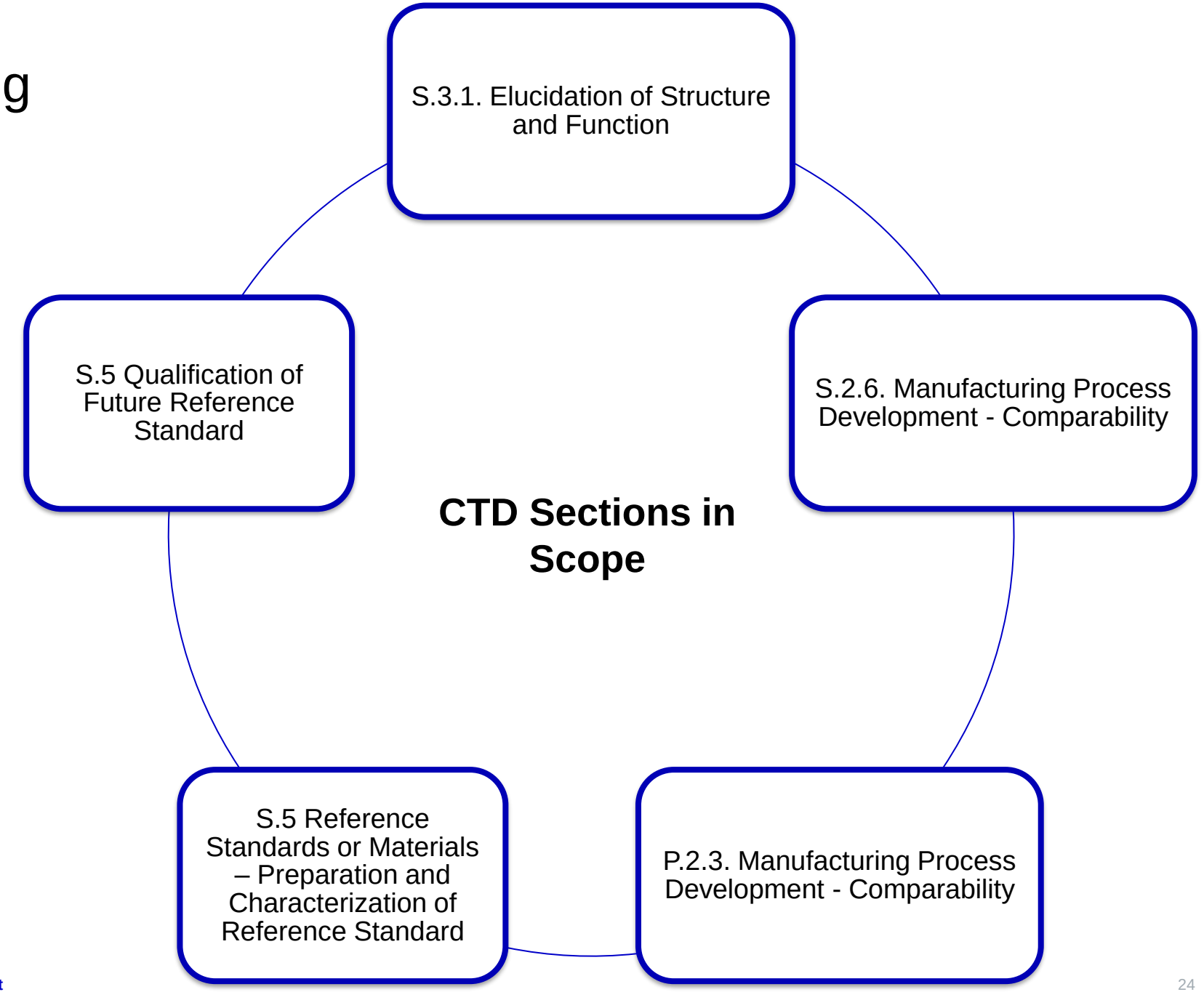
Release/Stability Tests Cover These Attributes

Supporting Characterization Tests

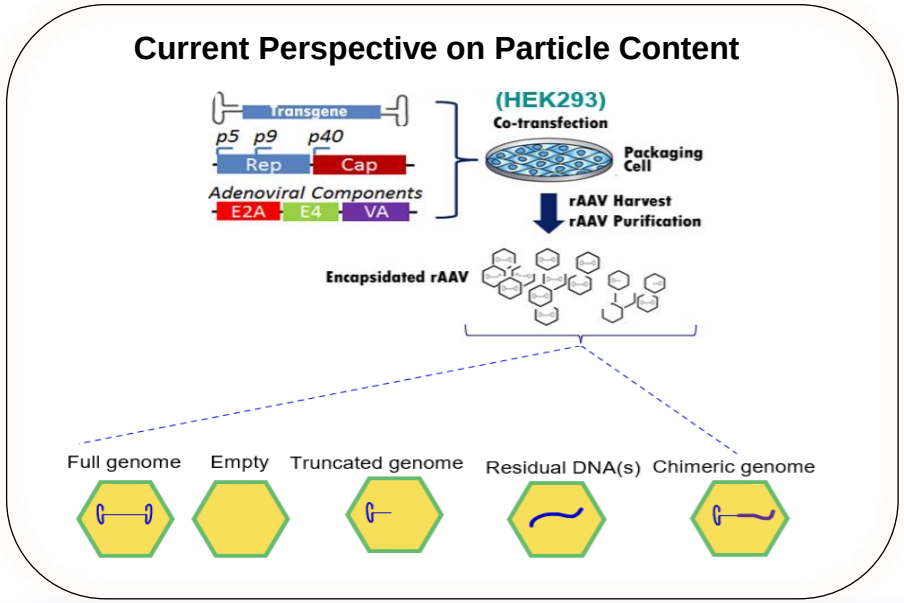
Identity, Strength, Potency	Quality and Purity	Compendial Requirements	Safety	Characterization
• Capsid Identity • Genome Identity • Genome Titer • Particle Titer • Potency	• Capsid Purity and Impurities • Genome Purity and Impurities • Genome Integrity • Size Distribution	• Appearance • pH • Osmolality • Volume in Container • Subvisible Particle	• rcAAV • Host Cell DNA • Host Cell Protein • Plasmid DNA • Other process-related impurities • Sterility • Endotoxin • Bioburden	• VP Ratio (CGE) • Capsid Modifications (MAM, LC-MS/MS) • Particle Content (AUC, CDMS) • Capsid Assembly (TEM, QELS, MALS) • Packaged Genome (NGS)

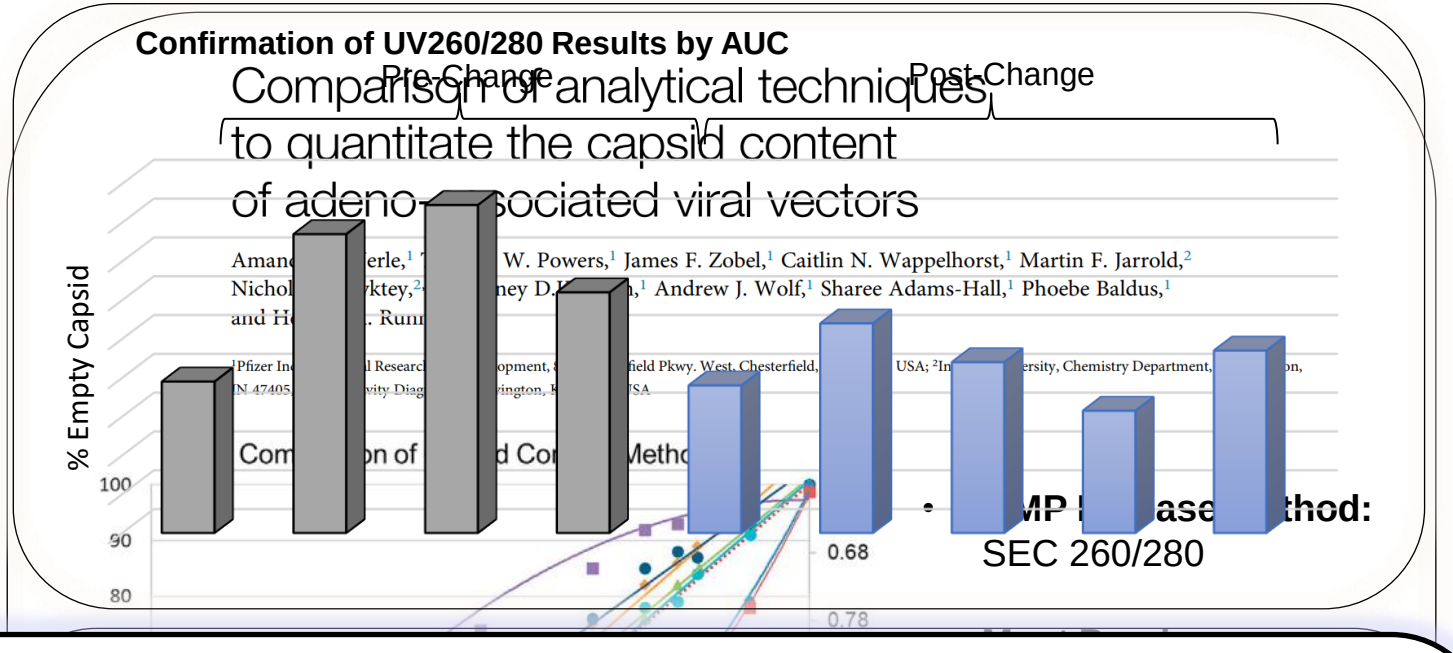
Characterization Testing

- Results in superior understanding of the molecule and elucidation of structure and other characteristics
- Includes both release procedures (when applied to non-routine release and stability testing) and characterization methods



Characterization Testing Supports Particle Content Understanding





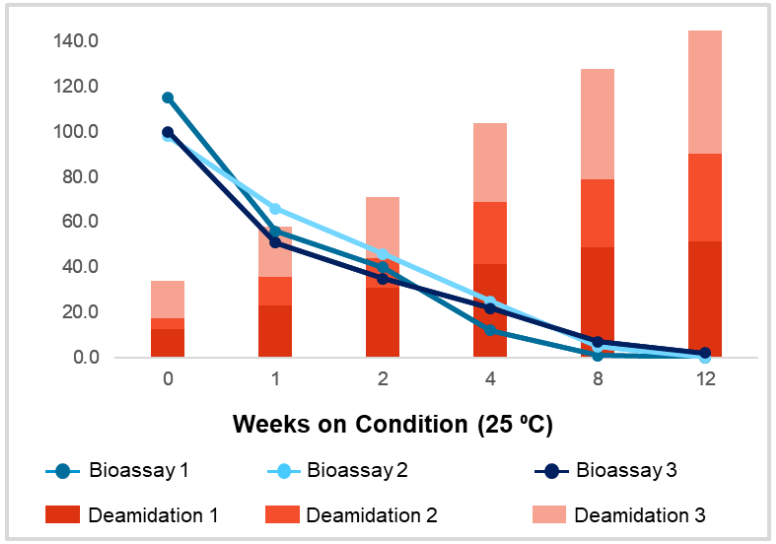
A precise SEC 260/280 method is used as the release method for particle content and is a surrogate of % empty/full capsid

A more specific characterization assay, AUC, supports the 260/280 results and provides complementary information

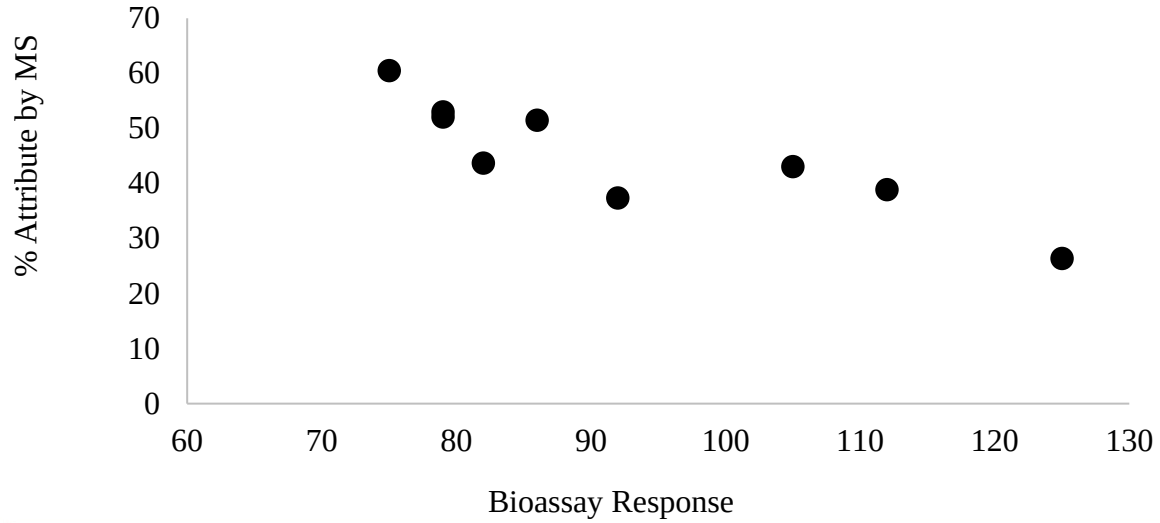
Additionally, NGS can be used to support both the 260/280 ratio and AUC to confirm the presence of the intended genome

MAM Supports GTx Comparability Assessments

Linking Deamidation Data to Function



Linking Additional Attributes to Function

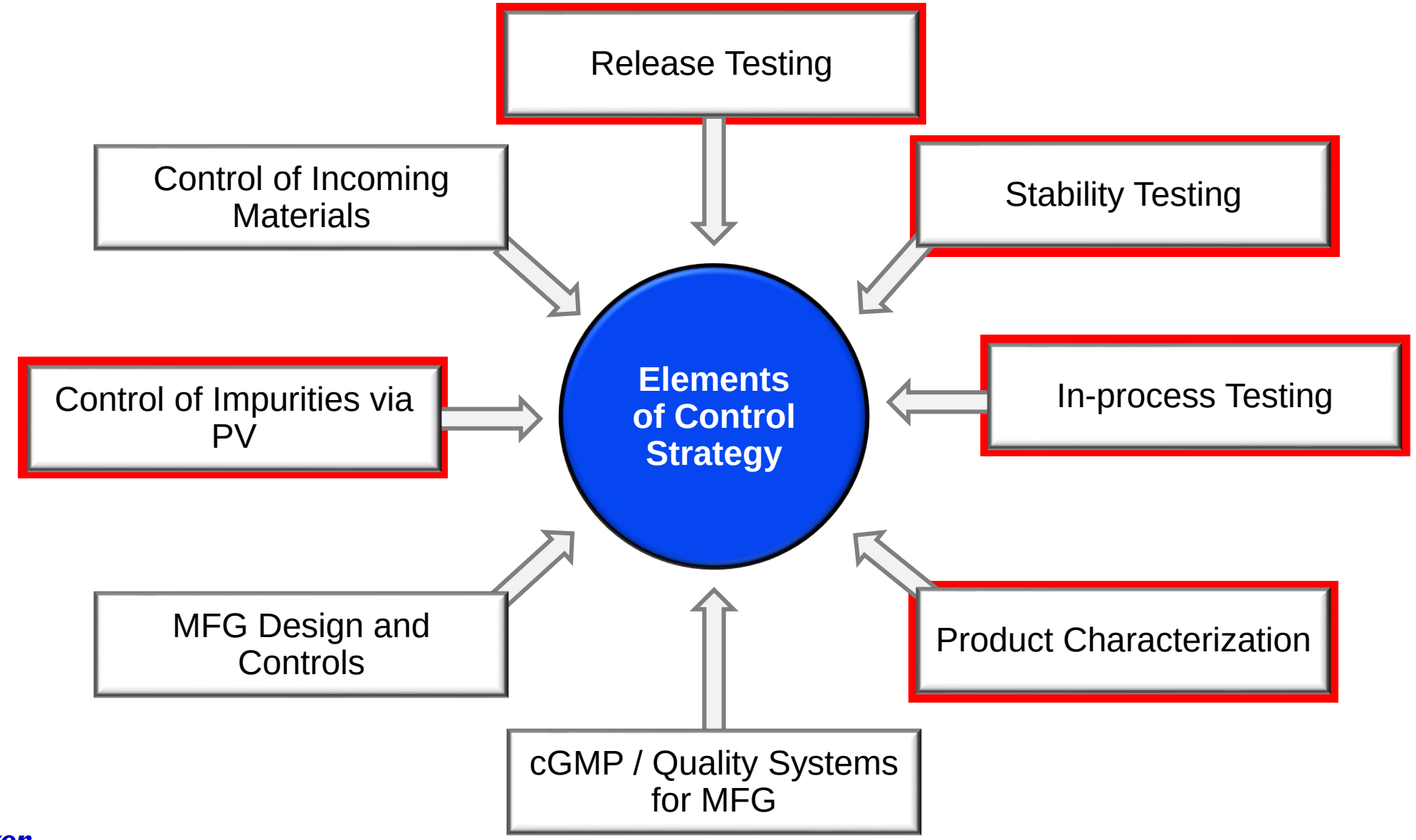


MS characterization identified several modifications on the protein moiety that can impact relative potency measurements

MAM can be leveraged for targeted quantitation of the protein modifications using a peptide map LC-MS method with automated data processing

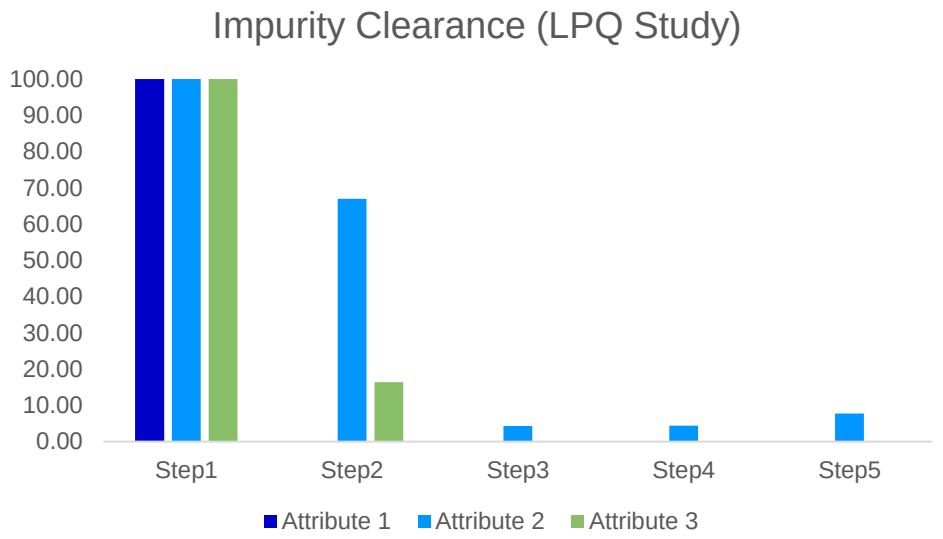
MAM can be implemented to demonstrate comparability of modifications across processes

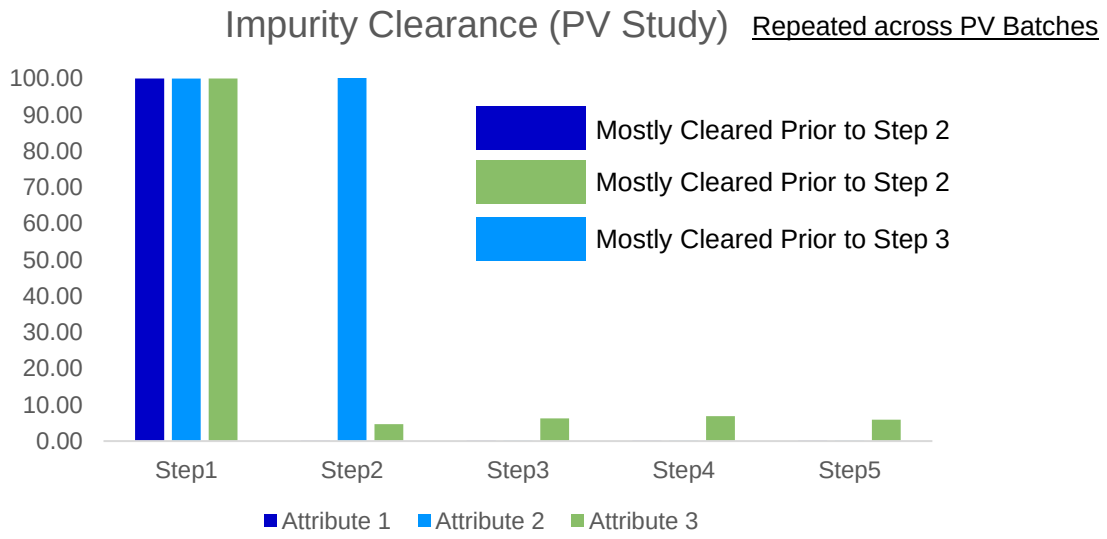
Elements of the Comprehensive Control Strategy



Control of Impurities Through PV

- Demonstration of process capability for consistently meeting product quality based on process validation data
- Supported by process characterization and manufacturing history
- Documents to consider
  - S.2.5 Process Validation and / or Evaluation
  - S.2.6. and P.2.3 Manufacturing Process Development
  - S.3.2. Impurities
  - Residual Impurity Risk Assessments
  - Prior release and additional testing results
  - LPQ studies





Process capability and robustness was generated but procedures kept on release testing

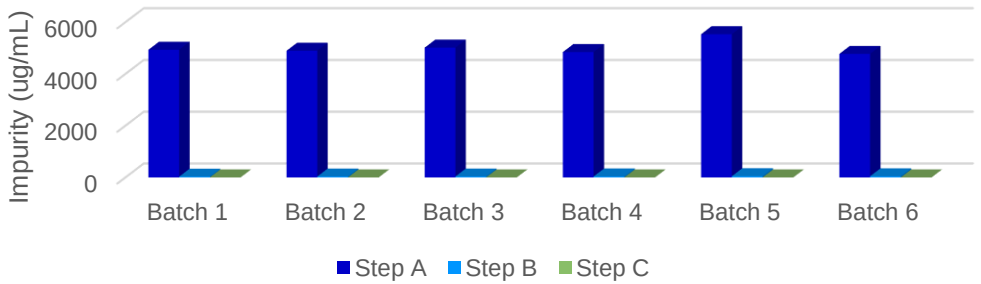
Control of Impurities Through PV

- Demonstration of process capability for consistently meeting product quality based on process validation data
- Supported by process characterization and manufacturing history
- Documents to consider
  - S.2.5 Process Validation and / or Evaluation
  - S.2.6. and P.2.3 Manufacturing Process Development
  - S.3.2. Impurities
  - Residual Impurity Risk Assessments
  - Prior release and additional testing results
  - LPQ studies

Impurity Risk Assessment

Worst case µg/dose assuming no clearance	**Worst case µg/dose with expected clearance**
Analytical capabilities (µg/dose)	**Known safety information**

Testing During PV



Clearance demonstrated and removal of test supported by PV study and impurity assessment

Documenting Analytical Control – P.2.3. Manufacturing Process Development

Quality Attributes	In-process Testing	Process Design and Controls	Control of Process Yield	Non-routine Characterization Testing	Release Testing	Stability Testing	Control of Materials	In-process Testing	Process Design and Controls	Non-routine Characterization Testing	Release Testing	Stability Testing	Control of Materials
	Drug Substance Section 3.2.S.2.6 Control Strategy							Drug Product This Section					
Characteristics													
Attribute 1					X	X					X	X	
Attribute 2					X	X					X	X	
Attribute 3									X		X	X	X
Attribute 4		X			X	X					X	X	
Attribute 5		X			X						X		
Attribute 6								X	X		X		
Attribute 7											X	X	
Attribute 8									X			X	X
Attribute 9	X	X		X	X	X			X	X	X	X	X
Attribute 10	X	X		X	X	X			X	X	X	X	X
Attribute 11				X	X		X			X	X		
Attribute 12		X											X
Identity													
Attribute 13	X			X	X		X			X	X		
Attribute 14	X			X	X		X			X	X		
Purity and Product-Related Impurities													
Attribute 15	X	X		X	X	X				X	X	X	
Attribute 16		X		X	X	X				X	X	X	
Attribute 17		X		X	X	X				X	X	X	
Attribute 18		X		X	X	X			X	X	X	X	
Attribute 19		X		X	X	X			X	X	X	X	

- All CQAs are controlled by **at least one** element of the control strategy
- The quality attribute summary documents elements of control for each attribute from DS to DP
- In addition to the high level-summary, each attribute is discussed in the document and control of that attribute is described with references to applicable sections in the BLA

Conclusions

- A comprehensive control strategy is critical to ensure proper controls are in place, maintain ensure quality material, and minimize risk to patients
  - Analytics play a key element in many aspects of the control strategy
- Knowledge gained throughout the program lifecycle is essential to refining CQAs and establishing a suitable control strategy
- Release/stability, in-process, and characterization tests are all impactful for ensuring control
- The comprehensive control strategy leaflet provides an excellent summary of how each CQA is controlled and applicable references to data demonstrating each element of control

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