Potency Method Development, Bridging and Control Strategies for AAV Gene Therapy

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Life-transforming therapies

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REDEFINING THE COURSE OF NEURODEGENERATIVE CONDITIONS

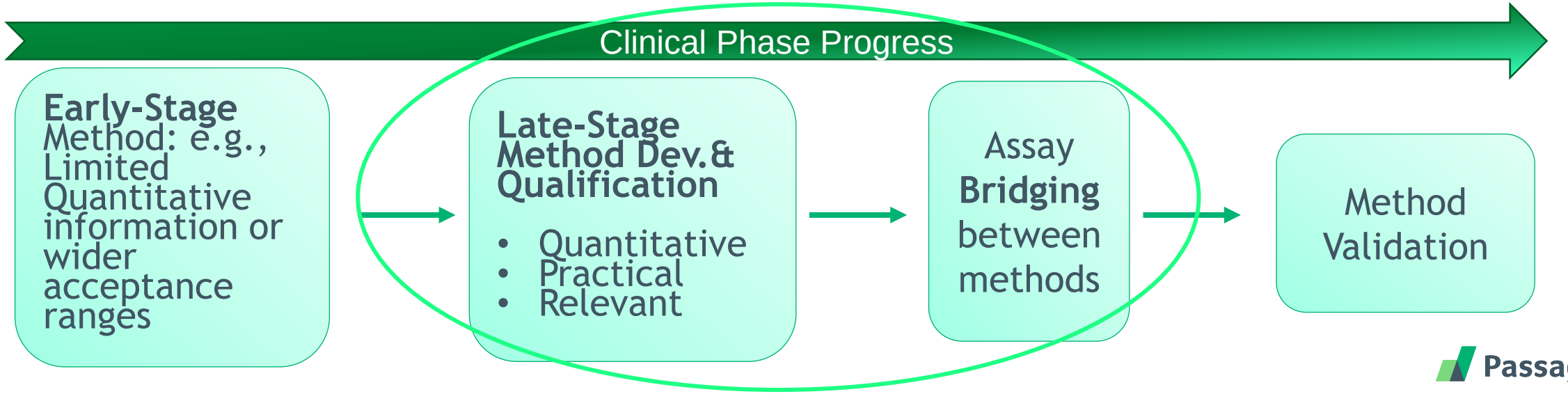
- Advancing potential best-in-class, one-time AAV gene therapy
- Exploring benefits of elevated target proteins/enzymes in multiple adult neurodegenerative diseases
- In-house manufacturing and process analytics to support program execution

Outline

- Potency Assay Strategies for AAV Gene Therapy Supporting Clinical Phases
  - Development & Optimization
  - Qualification & Bridging
  - Potency Method Control Strategies

In-vitro Potency Assay Strategies

- An in-vitro potency assay is established at preclinical stage to support IND applications based on gene expression.
- A new or revised potency assay is developed to support late phase clinical release
- A phase appropriate potency method validation under cGMP guidance is conducted to meet regulatory requirements (Q2R2)
- Method bridging study is conducted to bridge the late-phase method with the early phase potency method (Q14)
- Method validation and life cycle management



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Considerations for Designing Potency Assays

Transduction

- ❑ Mechanism of Action (MoA)
- ❑ Selection of cell lines, growth and stability
- ❑ Permissiveness of viral infection
- ❑ Infection and cell lysis conditions

Expression

- ❑ Transgene expression
- ❑ Lysis buffer and matrix interference
- ❑ Detection and quantitation Method

Function

- ❑ Transgene Function (Reflective of MOA)
- ❑ Measure CQAs
- ❑ Detection and quantitation Method

Robustness

- ❑ Sufficient # of replicates, randomization
- ❑ Appropriate assay controls
- ❑ Reduce variability: qualified reagents, equipment, operators

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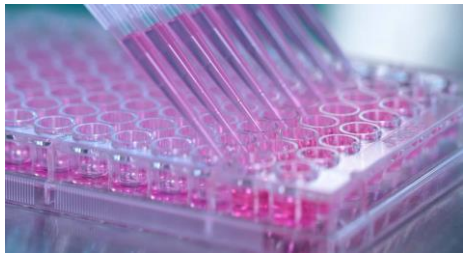
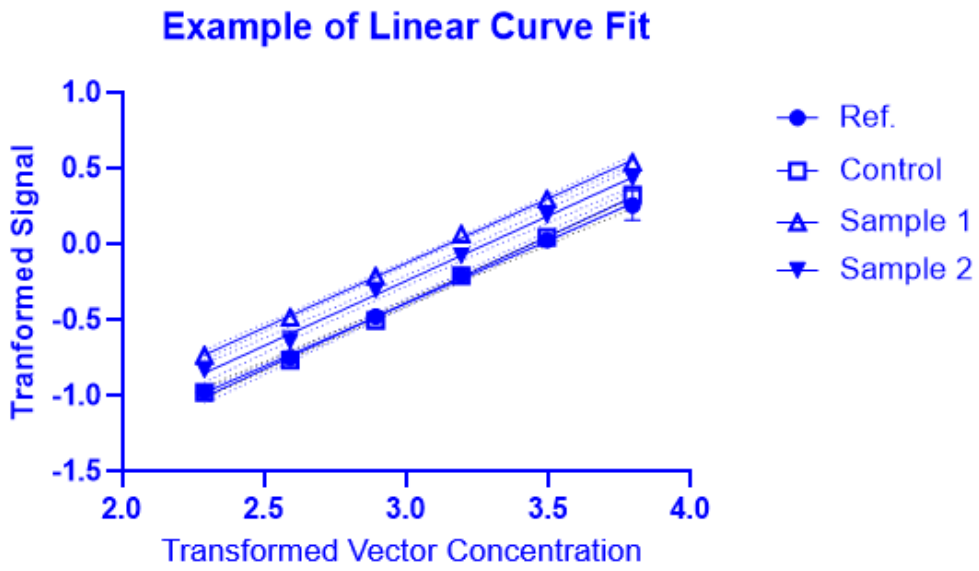
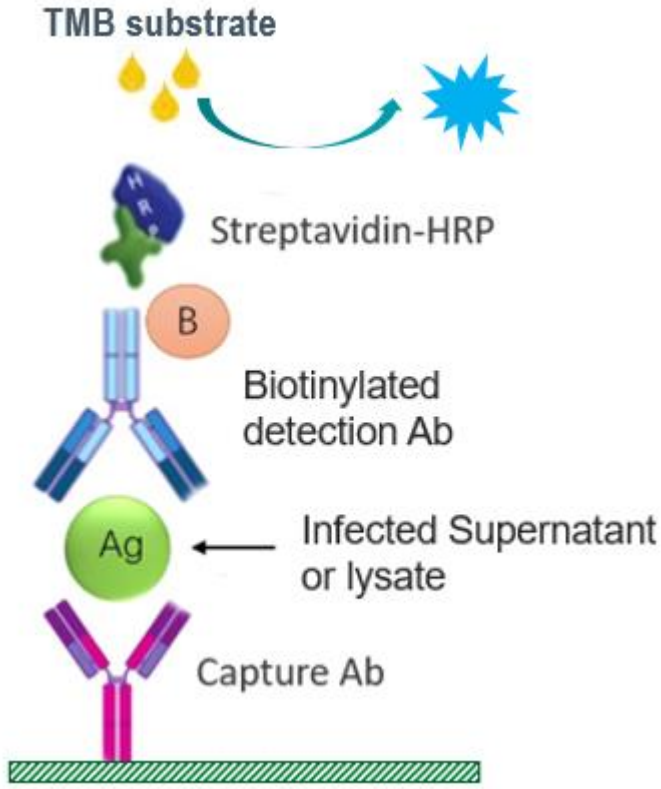
Potency Method Overview

Infection



Detection for protein expression





- Apply curve fitting and parallelism acceptance criteria for each plate;
- Reportable mean potency from multiple independent plates

Considerations for Data Analysis

Procedure	Details	Justification
Data analysis software	SoftMax Pro built-in analysis	All-in-one system from data collection to analysis; Eliminate data integrity review step
Parameters for similarity	Use Ratios and Differences between Sample and RS curve parameters	- Set acceptance limits for confidence interval
Acceptance criteria for curve fitting	- Add stringent model/curve fitting parameters - Add system suitability for control sample	- Reduced data manipulation in GMP Environment - Tightened Acceptance criteria

Pre-qualification & Robustness DOE Study

# of Runs	Sample Type	Plate type	Incubation time	Reagent Lot#	Lysis buffer	Analyst
1	Frozen	Pre-coated	90	1001	Vendor A	A1
2	Frozen	Self-coated	30	2001	Vendor B	A1
3	Frozen	Self-coated	90	1001	Vendor B	A2
4	Fresh	Self-coated	90	2001	Vendor A	A2
5	Frozen	Self-coated	30	2001	Vendor A	A1
6	Fresh	Pre-coated	90	2001	Vendor B	A1
7	Fresh	Pre-coated	30	1001	Vendor A	A1
8	Fresh	Pre-coated	30	2001	Vendor B	A2
9	Frozen	Pre-coated	30	1001	Vendor B	A2
10	Fresh	Self-coated	90	1001	Vendor B	A1
11	Fresh	Self-coated	30	1001	Vendor A	A2
12	Frozen	Pre-coated	90	2001	Vendor A	A2

Execution:

- Cover the whole intended potency range i.e., 50-200%
- Different equipment/days/labs, if available
- At least two trained analysts

Data Analysis:

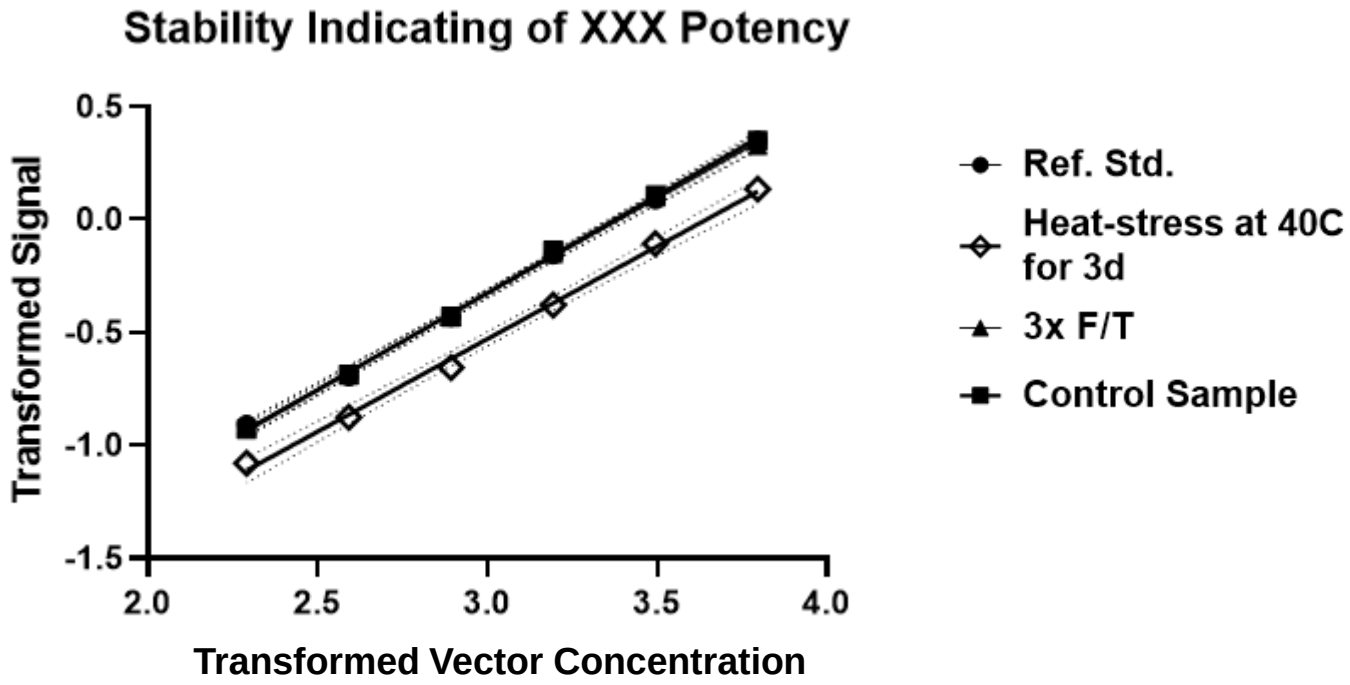
- Trend the assay parameters and establish preliminary acceptance criteria

Phase-Appropriate Validation (PAV) Under a Protocol

Parameters per ICH Guideline	Target Limit	PAV Result Example
Accuracy	80-120%	99-104%
Intermediate precision	≤20%	6%
Repeatability	≤20%	5%
Specificity	Product-Specific	FB and unrelated products showed no measurable potency
Linearity (R^2)	≥0.95	1.00
Qualified range	50-200%	50-200%
System Suitability	Met preliminary system suitability	Established final system suitability
Robustness (during development)	No significant impact (accurate/precise)	Parameters built into PAV assay design; small variations had no impact on assay performance

- Combined data: Pre-qual + Robust + PAV
- Assay Acceptance Criteria (AAC) for RS and Assay control
- Sample Acceptance Criteria (SAC) for samples
- Precision limit of multiplate replicates

Potency Method Demonstrates Stability-Indicating



- ✓ ~50% potency loss by heat-stress
- ✓ No potency loss by 3x F/T
- ✓ Potency loss relates to ~20% DNA leakage in heat-stress but may also relates to other attributes i.e., aggregation or post-translational modification (PTM) changes.

Method Bridging

- A secondary method with improved robustness, sensitivity or accuracy and operational simplicity is developed to support clinical lot release and stability.
- However, an existing method is tied to the historical release and stability. Introducing the new method requires a bridging study with the existing method.
- Per ICH guideline Q14 on analytical procedure development (Mar2024), an appropriate bridging strategies is used to demonstrate that the late-stage method is fit for purpose.
- Risk-assessment is used to support the extent of the study design; and the selected bridging strategies should be dependent on the extent of the change, the availability of the retention of the clinical batches etc.

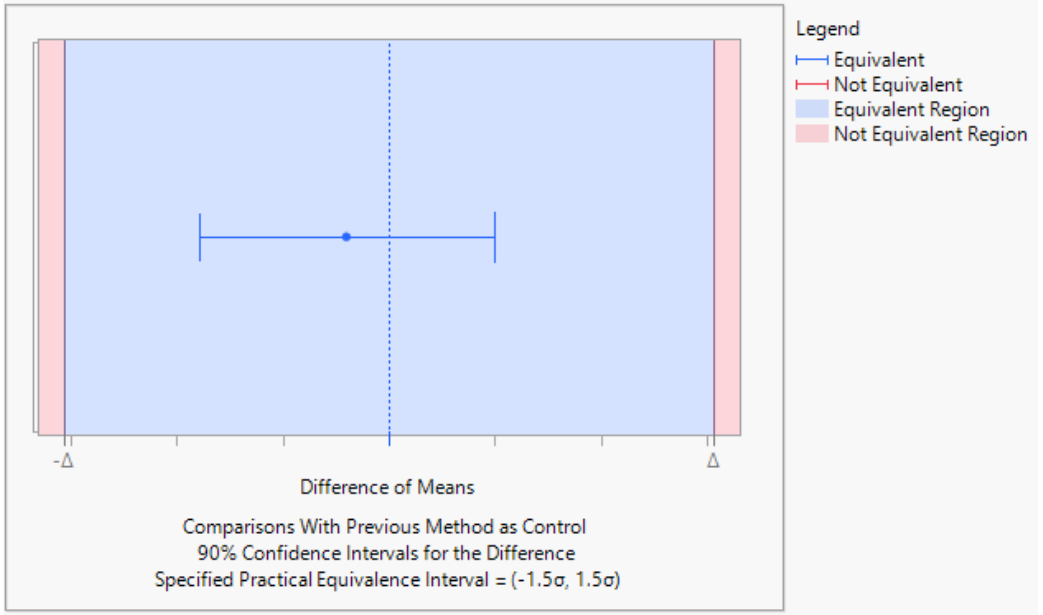
Method Bridging Example with Multiple Retention Lots

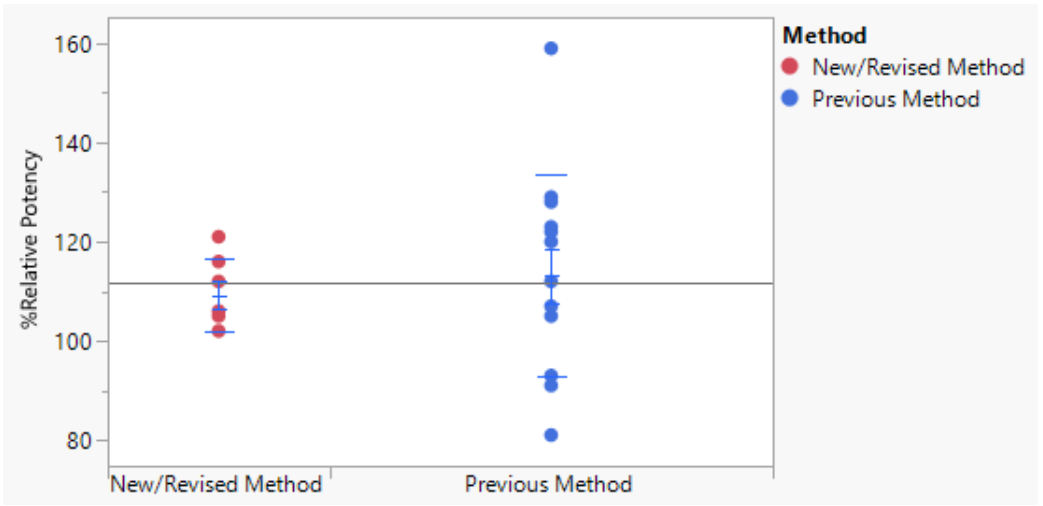
Equivalence test per USP guideline:

- Statistical Equivalency testing of the new method with the previous Method as Control
  - Equivalence Acceptance Criteria = $\pm$ 1.5σ
  - σ = Standard deviation of Previous Method
  - α = 0.05

$$H_0: \text{Mean Diff.} \leq -1.5\sigma \text{ OR } \geq 1.5\sigma$$
$$H_1: -1.5\sigma < \text{Mean Diff.} < 1.5\sigma$$

- Methods shown to be statistically equivalent
  - Maximum p-value < 0.01



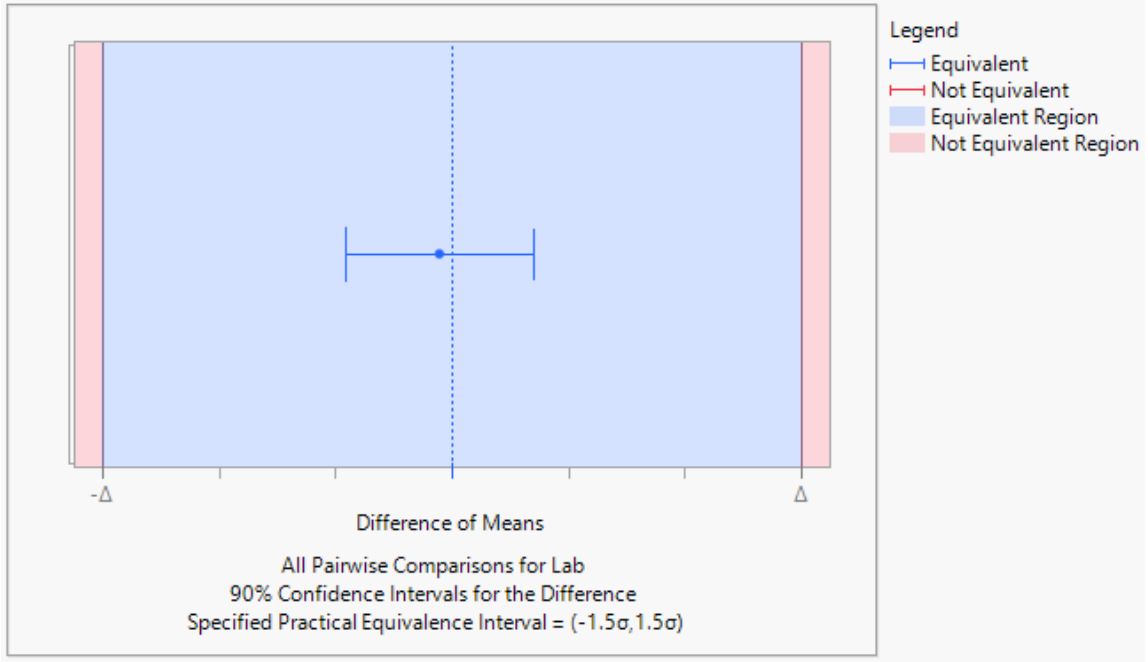


Method Bridging Example with Single Lot

Sample ID	Potency Level
S-0001	70%
S-0002	100%
S-0003	130%
S-0004	100%
S-0005	100%
S-0006	130%
S-0007	70%
S-0008	100%
S-0009	100%
S-0010	130%
S-0011	100%
S-0012	70%

The two one-sided tests (TOST) method is used to test for practical equivalence by the mean differences of %Recovery (n=12):

- *H0: Mean diff.< -1.5σ OR > 1.5σ*
- *H1: - 1.5σ < Mean diff.< 1.5σ*
- *Alpha = 0.05*
- *Methods shown to be statistically equivalent, p-value < 0.0001*



Purpose	Acceptance Criteria	Result
Accuracy	The new/revised method must demonstrate **comparable or better accuracy/precision** results than the previous method	The accuracy was 103% and 104%, respectively; Mean Recovery Difference was 1%
Precision		The precision of both methods was 9%.

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Potency Method Control Strategies

Critical Reagents Management:

- GMP Cell Banking Inventory
- Cell passage limit
- Cell growth trending
- Establish other critical reagent qualification procedures, e.g.,
  - Antibodies and Std for ELISA use;
  - Fetal bovine Serum
  - Ligand or recombinant proteins
  - Assay Control

Reference Standard Management:

- Establish RS qualification Procedure
- Manage RS inventory
- Monitor RS stability
- Assay Parameter trending

Assay Control Management:

- Establish assay control qualification Procedure
- Manage assay control inventory
- Assay Parameter trending

✓ **Annual assay trending report for RS and assay control**
✓ **Annual cell growth trending report**

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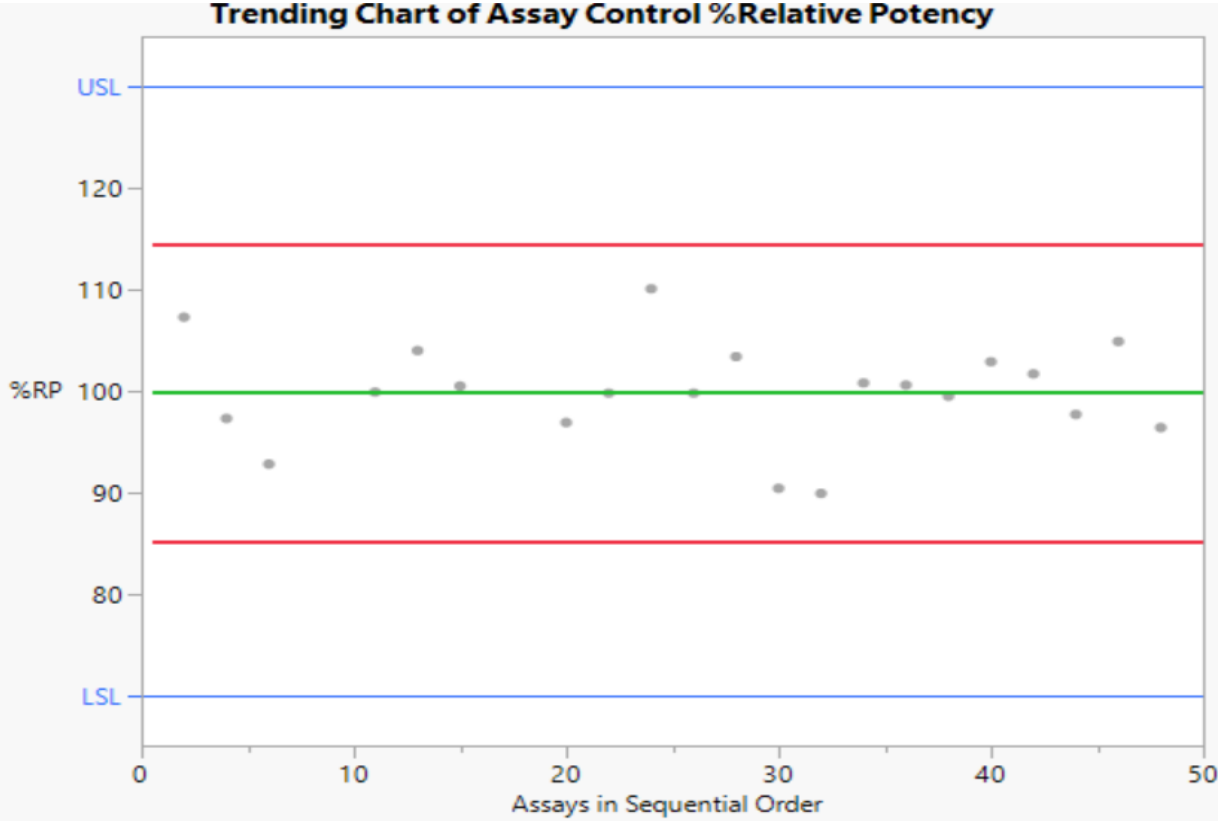
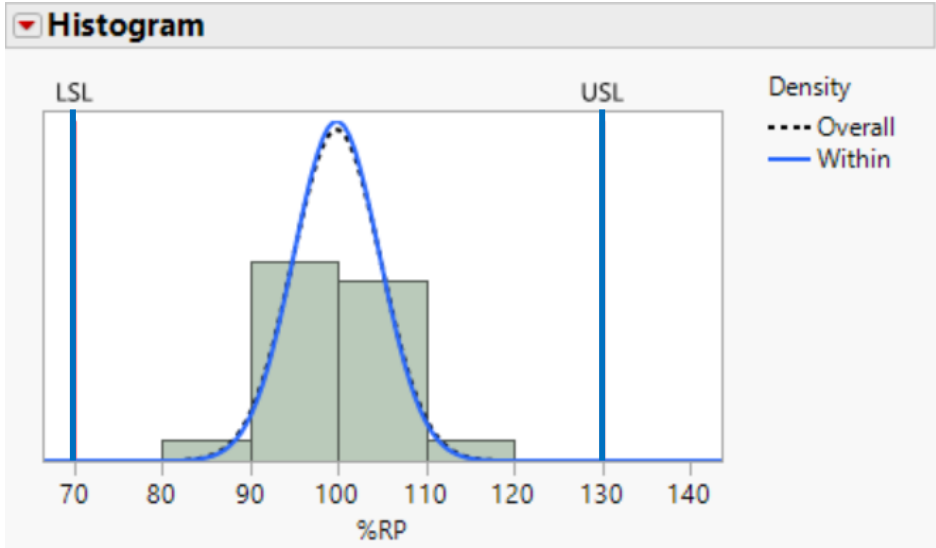
Assay Control and Method Trending

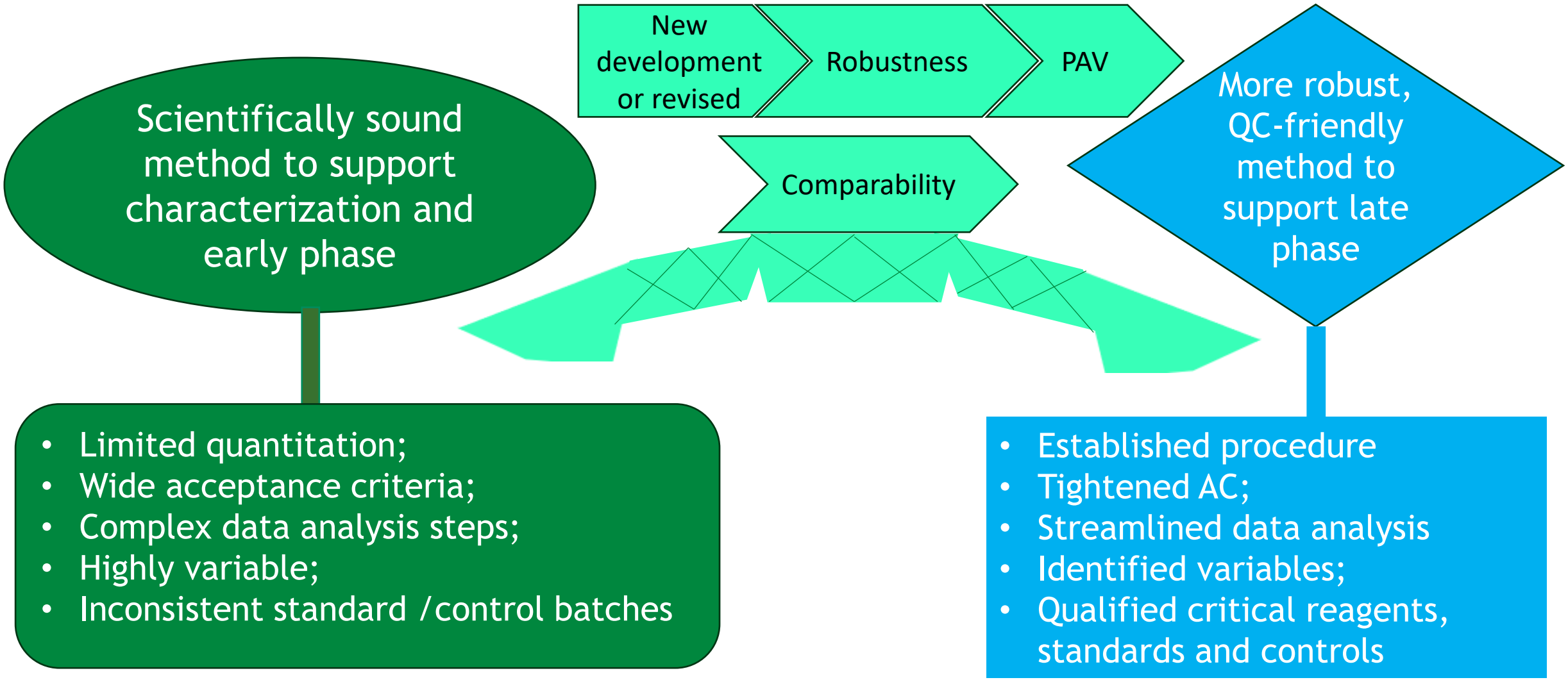


%RP Limit Summaries

Points plotted	LCL	Avg	UCL
Individual	85.22864	99.8381	114.4475



Summary of Bioassay Strategies for CMC Development



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Acknowledgement

- Bioassay Team
- Analytical Development Teams
- Process Development Teams

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