CASSS Bioassays Workshop

Practical Applications for Method Design and Performance

April 15, 2024

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Interactive Workshop Agenda

1.	Introduction	1:30 to 1:40
2.	Study #1: Potency Strategy Design	1:40 to 3:00
3.	Break	3:00 to 3:20
4.	Study #2: The Problematic Method	3:20 to 5:00

Introduction

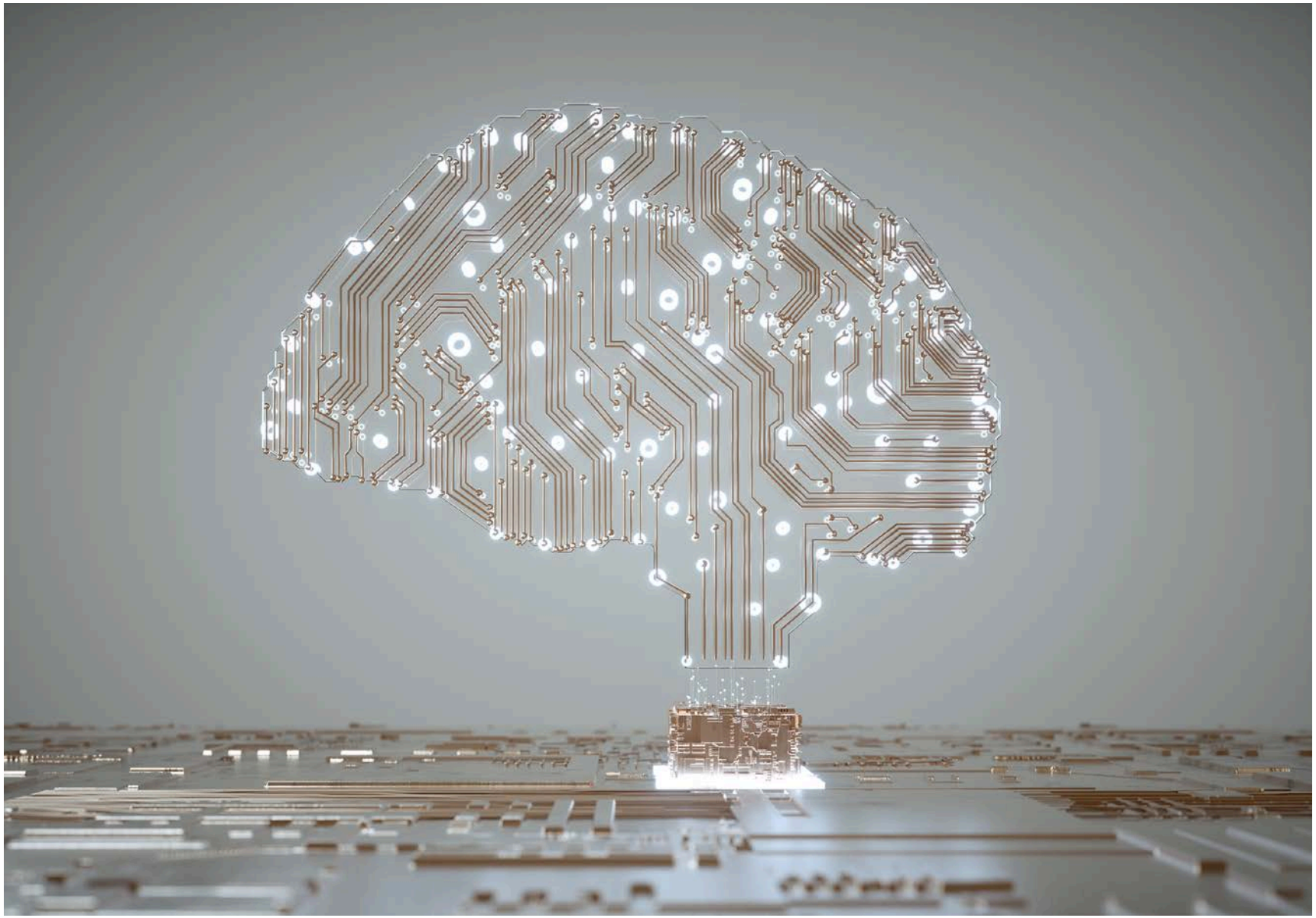
- Objective of today's workshop:
  - Exposure to key facets and considerations of potency strategy and method design
    - QC and characterization
  - Introduction to method performance troubleshooting
  - Important considerations in method design to support performance
  - Strategic phase-appropriate considerations

- Meet your instructors
  - Tara Stauffer, Director, Head of Biologics Bioassay Center of Excellence
  - Colleen Santoro, Principal Scientist, Biologics Bioassay Center of Excellence

Before we dive in….

- Please work in groups!
  - You will be asked to change groups for the second study
- Necessary information will be provided either in the workbook or handouts
- There are no “right” or “wrong” approaches - think outside the box!
- Open sharing of ideas in “safe space” mode

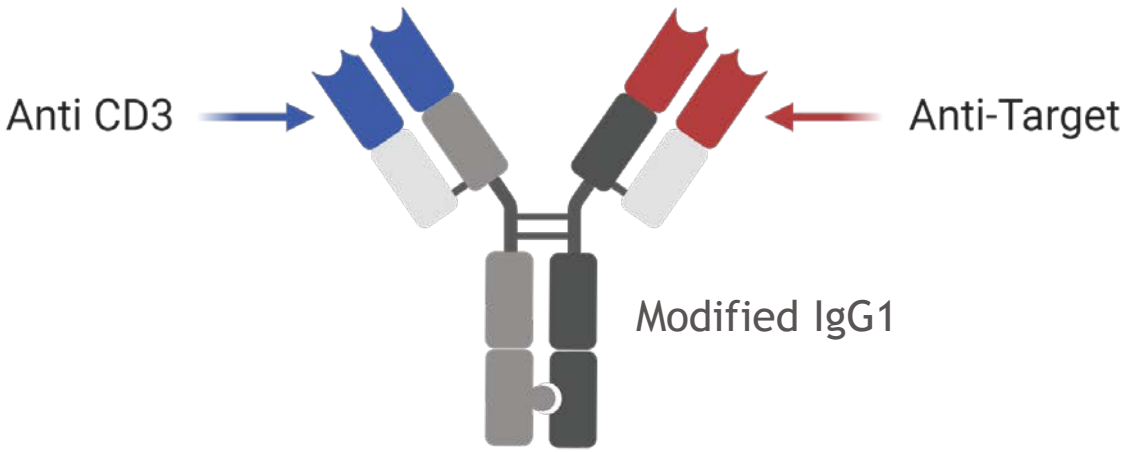
Study #1: Potency Strategy and Method Design



Study #1: Potency Strategy and Method Design

- Instructions:
  - Your group will be assigned New Blockbuster Drug (NBD) A or New Blockbuster Drug (NBD) B
  - Your workbook includes structure, MOA, and some relevant quality profile data
  - You are asked to design a potency strategy for your NBD
  - Your strategy should be inclusive of:
    - Proposal for FIH QC lot release and stability strategy (including analytical target profile)
    - Proposal for biological characterization
    - Method concepts
    - Considerations for late-stage (PPQ or BLA) lot release and stability strategy and associated analytical target profiles
  - You may use online resources if you wish

New Blockbuster Drug (NBD) A



- NBD A has two different Fab regions, one which is anti-CD3 and one which is anti-Target
- The backbone is a modified IgG1 that eliminates all effector function
- NBD A engages with a cancer cell through the anti-Target moiety, while simultaneously engaging T cells
- NBD A drives Target cell death by inducing T-cell activation and release of proinflammatory cytokines and cytolytic enzymes
- The clinical dose for this molecule has not yet been established
- Your first filing date is on an accelerated timeframe based on NBD status

Preliminary Quality Data for NBD A

Analytical Measurement	Result	Comment
Protein Concentration (mg/mL)	75	
Size Exclusion Chromatography		Preliminary lab stability did not show major changes to HMW or LMW upon stressed conditions
%Main Peak	95.4	
%HMW	2.5	
%LMW	2.1	
Charge Heterogeneity		No changes to main, acidic or basic isoforms observed in preliminary lab stability
% Main isoform	74.1	
% Acidic isoform	21.5	
% Basic isoform	4.4	
Purity	97.1	

New Blockbuster Drug (NBD) B

Wild-type IgG1

- NBD B has identical Fab regions, both of which are Anti-Target
- The backbone is a wild-type IgG1
- The molecule delivers a cytotoxic payload, with a target drug-antibody ratio (DAR) of 4
- NBD B has a remarkably stable linker
- Bystander effect is purported to be part of the MOA, however the small biotechnology company from which the molecule was acquired has not provided any data
- Your first filing date is on an accelerated timeframe based on NBD status

Preliminary Quality Data for NBD B

Analytical Measurement	Result	Comment
Protein Concentration (mg/mL)	50	
Size Exclusion Chromatography		Preliminary lab stability did not show major changes to HMW or LMW upon stressed conditions
%Main Peak	93.4	
%HMW	1.8	
%LMW	4.8	
Charge Heterogeneity		Increase in acidic isoforms observed in preliminary lab stability. Identified to be associated with N297
% Main isoform	68.3	
% Acidic isoform	22.5	
% Basic isoform	9.2	
Purity	94.6	
Drug-Antibody Ratio	3.5 – 4.3	

Study #2: Evaluation of a Method with Poor Performance

Study #2: Evaluation of a method with poor performance

- Instructions:
  - Please shuffle groups
  - You have encountered a method with a high failure rate
  - Your method was developed and validated externally, and was transferred to your laboratory for routine testing upon acquisition. The legacy method has been filed and is currently in Phase I.
  - The high failure rate now has quality events associated with it, and the issue has been escalated
  - The method validation summary is provided
  - You are asked to put together a plan for evaluating and correcting the high failure rate
    - You may ask for additional information
    - You may "consult" with other colleagues in your company for assistance (to propose theoretical options)
  - Your drug is a simple blocking monoclonal antibody (one hint: MOA is not implicated)

Method Details

Method Description

- ELISA that evaluates the binding of drug to Target.
- A microtiter plate is coated with the Target protein.
- Samples are added to the plate, followed by incubation with a secondary antibody conjugated to peroxidase.
- Signal is detected upon addition of a chemilumiscent substrate.
- RLU is proportional to the amount of bound Anti-Target monoclonal antibody drug.
- Results are reported relative to a reference standard.
- A quality control standard is included on each assay plate.

Method System Suitability

Test Article	Parameter	Acceptance Criteria
Reference Standard	EC50	≥0.7 and ≤1.4 µg/mL
	Range	≥38,000 RLU
	LOF P-value	≥0.01
QC Sample	LOF P-value	≥0.01
	Relative Potency	≥80% and ≤120% of COA value
Test Article	LOF P-value	≥0.01

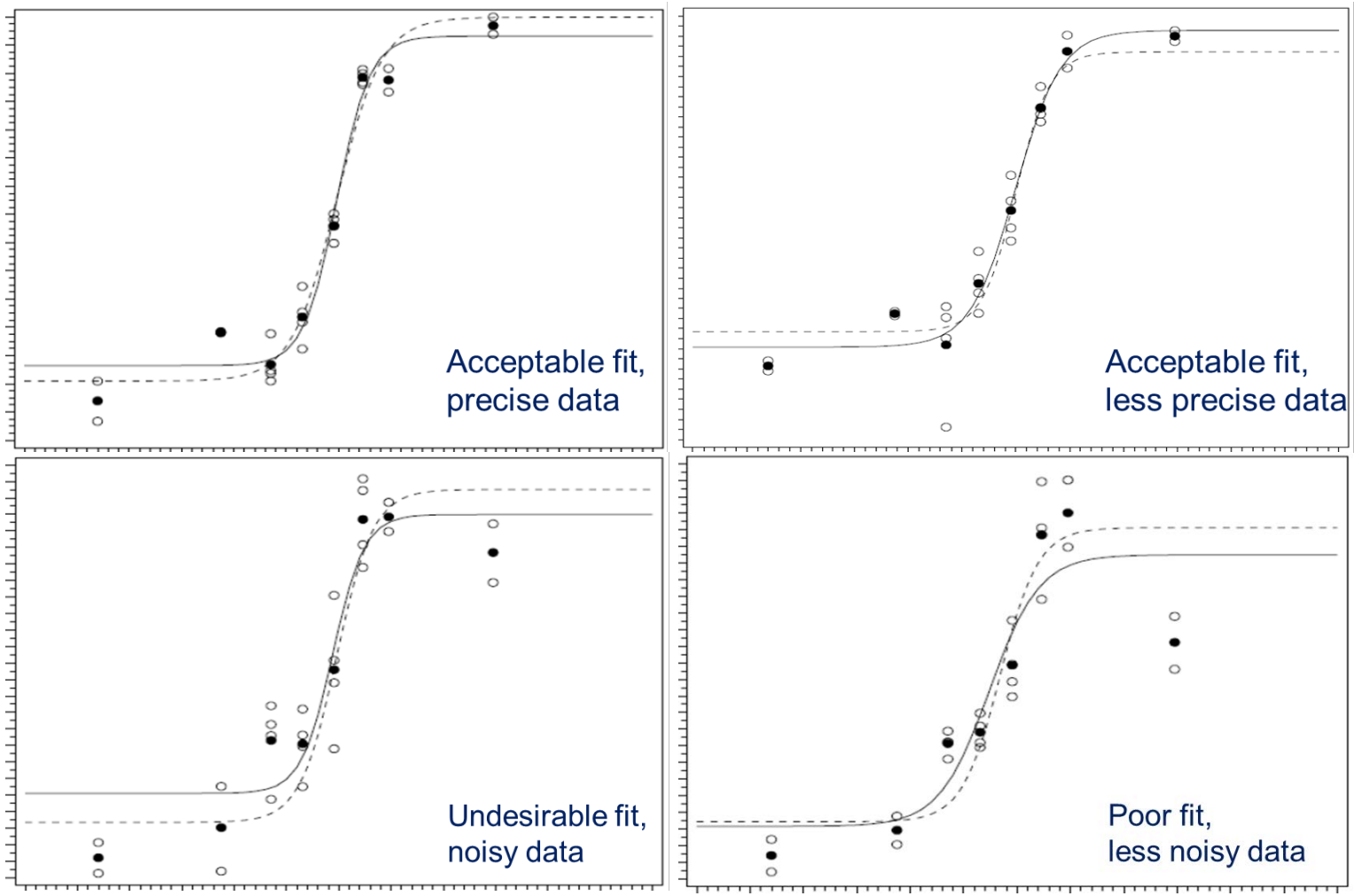
Method Validation Summary

Parameter		Result	Notes
Accuracy		95% - 115% across the method range	
Precision	Intermediate Precision	8.1%	6.5% (transfer RSD)
	Repeatability	5.4%	
Linearity		$R^2 = 0.99$	Linear regression analysis
Range		All validation acceptance criteria met across the range	
Specificity		Demonstrated	

Handout 1: Summary of Assay Failure (will not be a slide, printed separately)

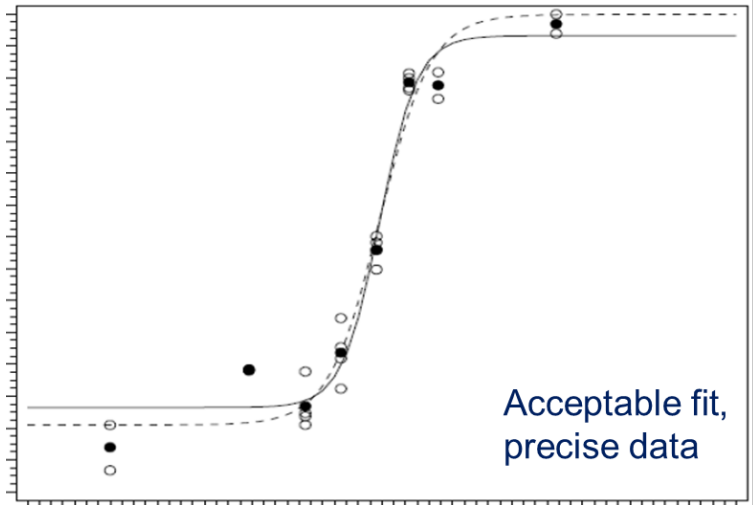
Test Article	Parameter	Acceptance Criteria	Individual Failure %	Overall Failure %	Total Assay Failure %
Reference	EC50	≥0.7 and ≤1.4 μg/mL	2.9	18.6	28.4 (n = 618)
	Range	≥38,000 RLU	5.5		
	LOF P-value	≥0.01	11.3		
QC Sample	LOF P-value	≥0.01	9.2	12.9	
	Relative Potency	≥80% and ≤120% of COA value	6.3		

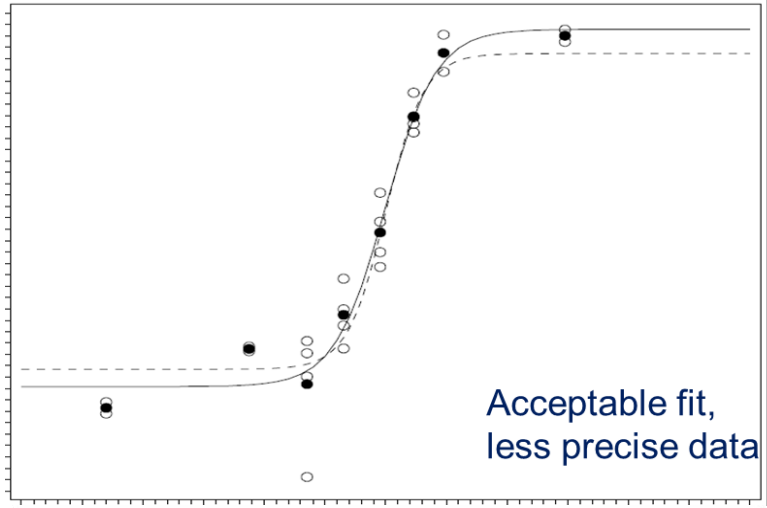
Handout 2: Selected Assay Graphs (will not be a slide, printed separately)



Handout 3: Selected Assay Graphs with LOF value example (will not be a slide, printed separately)

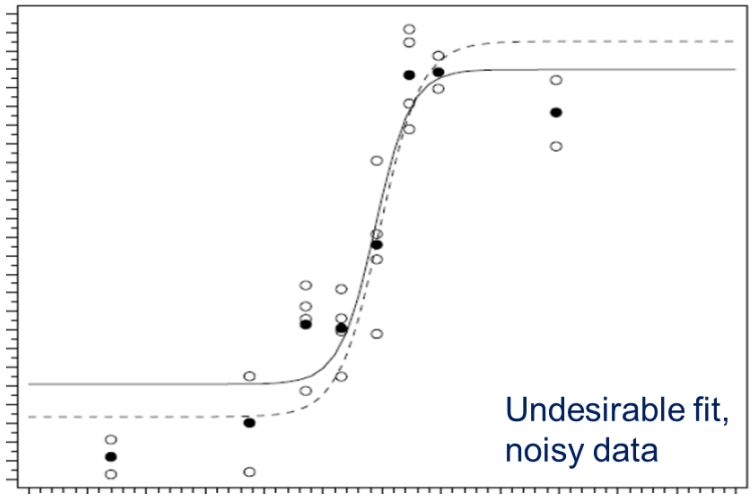
LOF p-value:
Failed

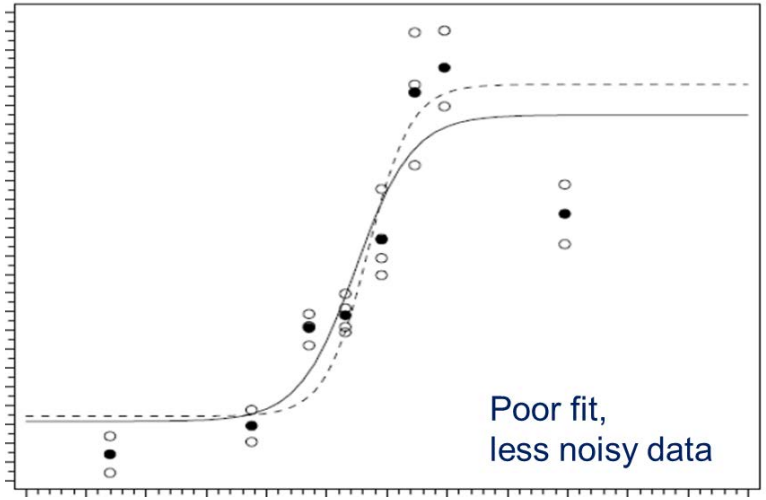




LOF p-value:
Passed

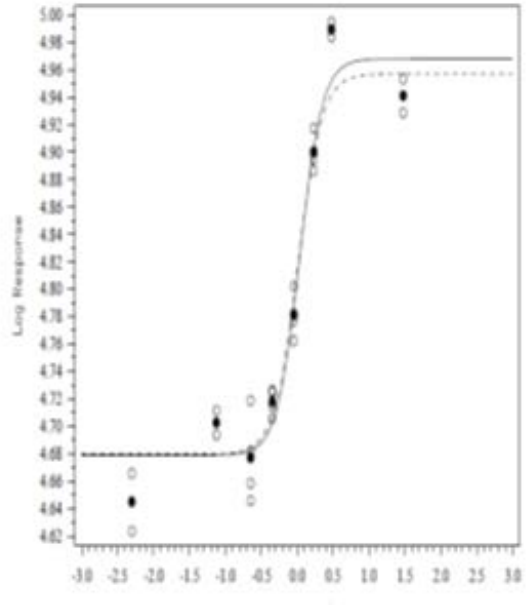
LOF p-value:
Passed



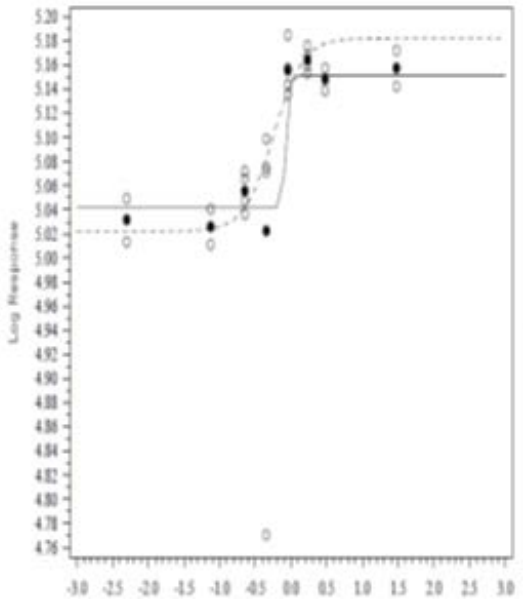


LOF p-value:
Failed

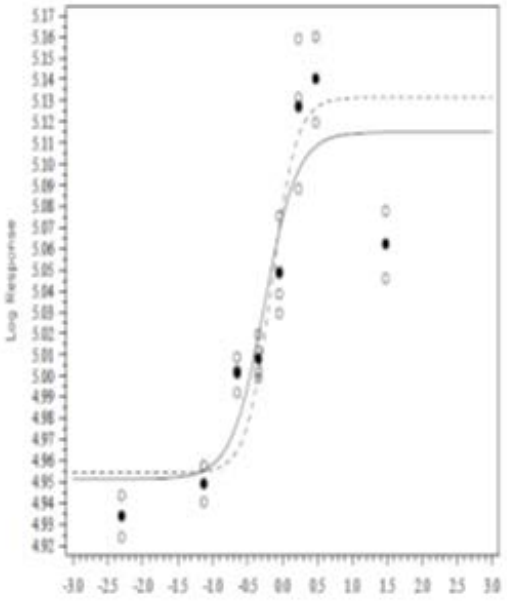
Handout 4: Selected Assay Graphs with Revised SST (will not be a slide, printed separately)



LOF p-value: Failed
LOF error: Passed



LOF p-value: Passed
LOF error: Failed



LOF p-value: Failed
LOF error: Failed

Handout 5: Possible Solution (will not be a slide, printed separately)

- Method validation was re-analyzed using adjusted SST parameters
  - Parallelism criteria were implemented
  - Criteria to assess quality of curve fit implemented
  - LOF p-value replaced with LOF error criteria
  - Criteria which did not add value (RLU range) removed
- Accuracy and precision remained the same compared to original validation

Test Article	Parameter	Acceptance Criteria	Individual Failure %	Overall Failure %	Total Assay Failure %
Reference	EC50	≥0.3 and ≤1.3 µg/mL	1.1	1.1	10.1 (n = 618)
	LOF relative error	≤10.0%	0.0		
QC Sample	LOF relative error	≤10.0%	3.1	9.4	
	Relative Potency	≥80% and ≤120% of COA value	2.9		
	Relative Potency Standard Error	≤25%	1.8		
	QC/Reference A ratio	0.99 – 1.01	0.5		
	QC/Reference B ratio	0.5 – 2.0	4.9		
	QC/Reference D ratio	0.99 – 1.01	1.1		

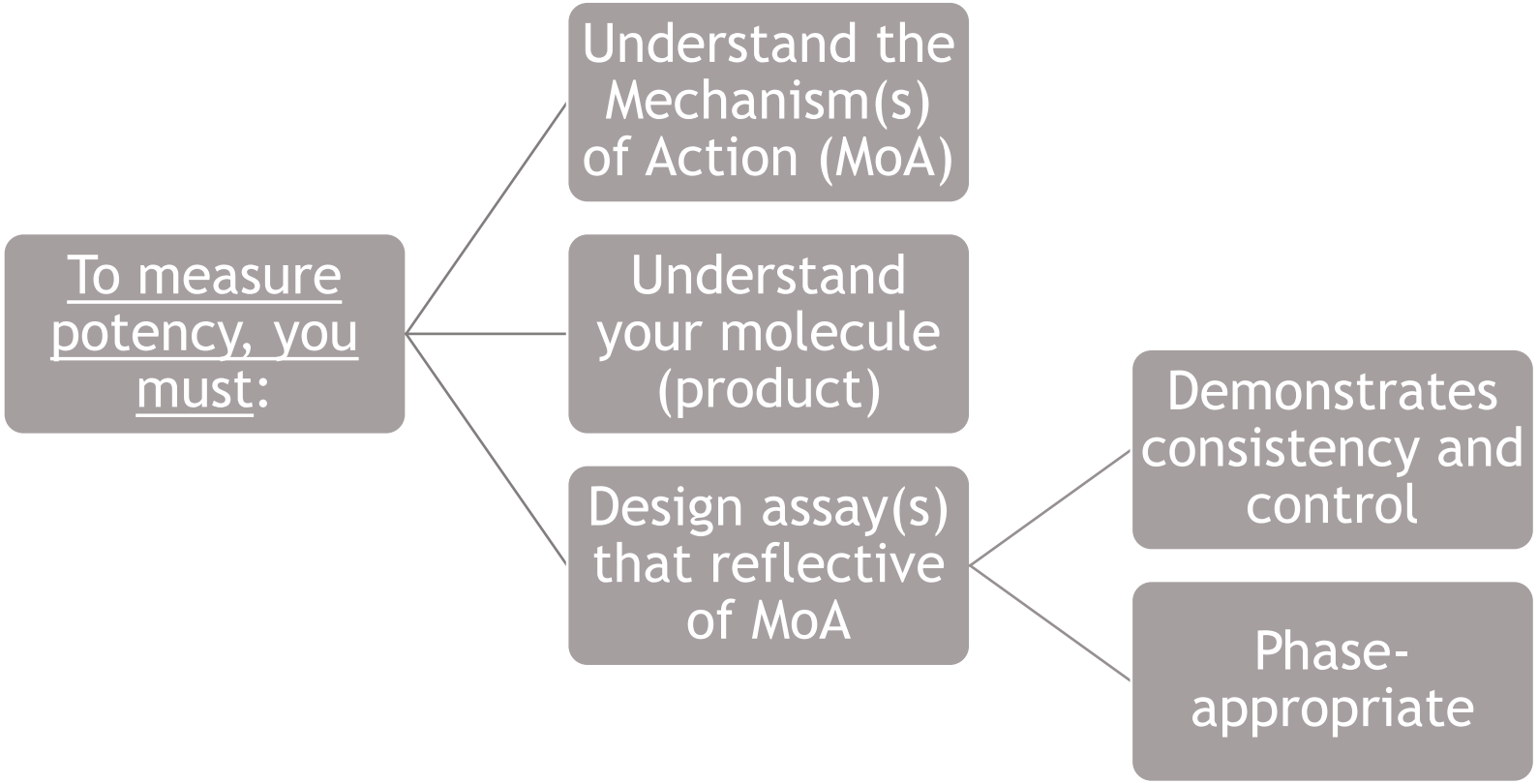
If time permits

- Now that you have identified the problem and proposed a solution:
- How would you implement this solution:
  - Pre-BLA?
  - Post-BLA?

Helpful Reminders

Potency Measurement

- **Potency is defined as a measure of biological activity using a suitably quantitative biological assay based on the attribute of the product which is linked to the *relative biological properties***



Progressive Potency Assay Implementation

Early Phase focus:

- Manufacturing consistency
- Stability
- Biological characterization

Late Phase focus:

- Functional assay (generally, cell-based bioassay) required for licensure
- PLUS all of the above

All potency assays used for release testing of biological drug products must…

- Comply with all applicable biologics and CGMP regulations including:
  - Indicate potency (biological activity/activities) specific to the product
  - Provide test results for release of the product
  - Provide quantitative data
  - Include appropriate reference materials, standards, and/or controls
  - Meet pre-defined acceptance and/or rejection criteria
  - Establish and document the accuracy, sensitivity, specificity and reproducibility of the test methods employed through validation
  - Provide data to establish dating periods

Structure-Function Role of Bioassay

A well-designed bioassay can inform on structure-function relationships

How degradation impacts the molecular mechanism

What is impactful to quality (critical quality attribute)

A high degree of precision and accuracy allows you to make decisions

This is a CQA, that is not a CQA…

Together, these can help you to understand appropriate storage conditions, what should be monitored during manufacture or on stability, what attributes may be critical for efficacy or safety…in short, how to best deliver a safe and effective medicine to patients

System Suitability = SST

Confirmatory procedures and/or parameters to ensure that the system will function correctly

Assesses the validity of an assay

For potency assays, system suitability generally applies to the reference standard and control sample

Why do we use system suitability?

Ensures the quality of potency assay results

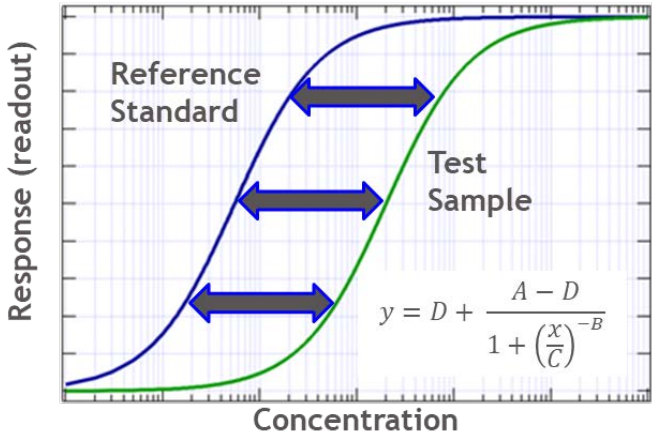
Ensures data are of sufficient quality to generate a reliable potency measurement

What happens if system suitability is not assessed properly or is insufficient?

Potency estimation is unreliable or meaningless

Examples of System Suitability (SST)

Parameter	Example Measurements	Acceptance Criteria
Assay Control	Potency of QC sample	Define potency range
Quality of Dose-Response Fit	Goodness of Fit Measurement	Defined limits or values for: Lack-of-fit (LOF) measurement, sum of squares, R^2 value, e.g.
	Precision	Residual mean squared error, Confidence intervals of potency estimate, inter-plate variability
Parallelism between Reference and Sample	Ratio or difference of curve fit parameters, non-parallelism sum of squares	Defined limits or ranges
Dynamic Range or Assay Window	Signal:Noise, Upper asymptote – lower asymptote	Defined limits or ranges



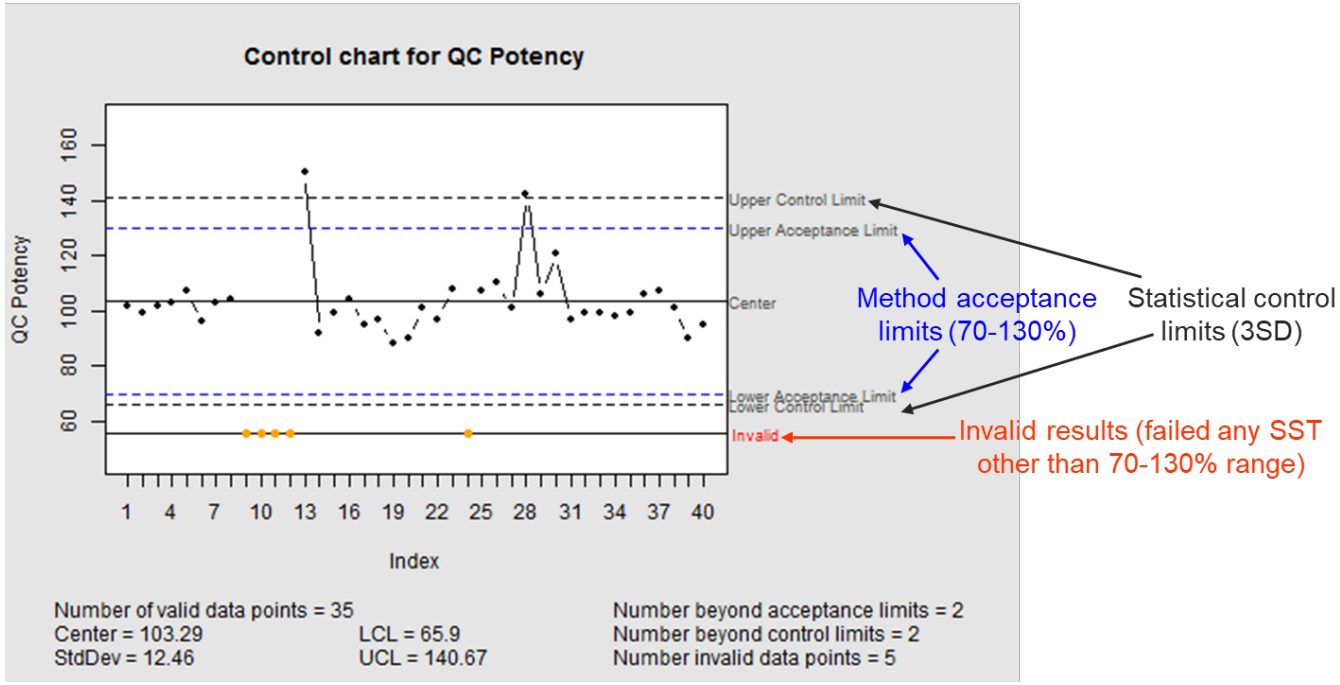
- System suitability will be unique to the method
- Method development will inform on the appropriate measures for control
- Both unrestricted and restricted potency curves are used to evaluate suitability of data

Evaluation of method performance over time

- Inclusion of a QC or assay control sample on each plate enables trending of method performance over time
- Routine monitoring of performance over time ensures that method remains suitable for use
  - Method remains in a state of control
  - Regulatory expectation
  - Ensures consistency across laboratories
  - Pro-actively identifies issue
  - Supports investigations

Illustrative example only

Plot of QC potency results



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Thank you

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