System and Sample Suitability Assessment for Potency Methods

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Outline

- Overview of system and sample suitability assessment for potency methods
- Suitability parameters and acceptance criteria considerations
- Examples

Introduction

System suitability

- Apply to reference standard and control sample
- Assess the validity of an assay

Sample suitability

- Apply to test sample
- Assess the validity of the sample potency estimate

Why Do We Need System and Sample Suitability Assessment?

Ensure the quality of potency assay results

- Biologically meaningful
- Good data quality (acceptable dose-response curve fit, similarity) which ensure reliable potency estimation

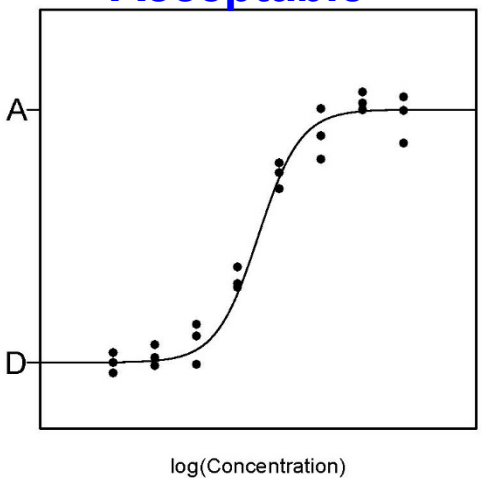
What could happen when the suitability was not assessed properly

- Unreliable or meaningless potency estimation
  - Due to poor curve fit
  - Due to violation of inherent assumption for potency calculation (similarity between reference and sample)
- Unreasonable high assay/sample failures

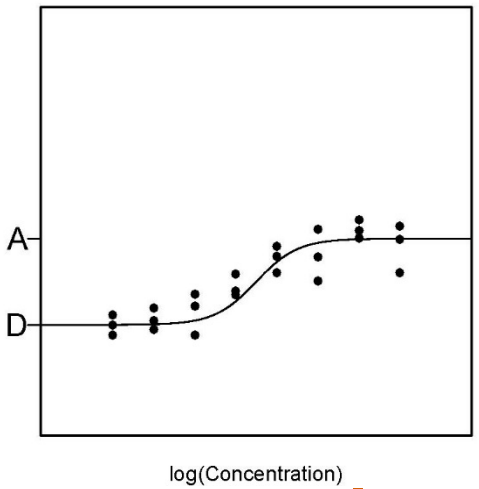
Examples of Suitability Parameters

- **Parameters to ensure acceptable signal and noise**
  - **Sufficient signal: A > xx**
  - **Under controlled noise: D < xx**
  - **Sufficient signal to noise separation: A/D, A-D**

Acceptable



Undesirable



- **Parameter to ensure proper control sample result**
  - **Potency of control falls within expected range**

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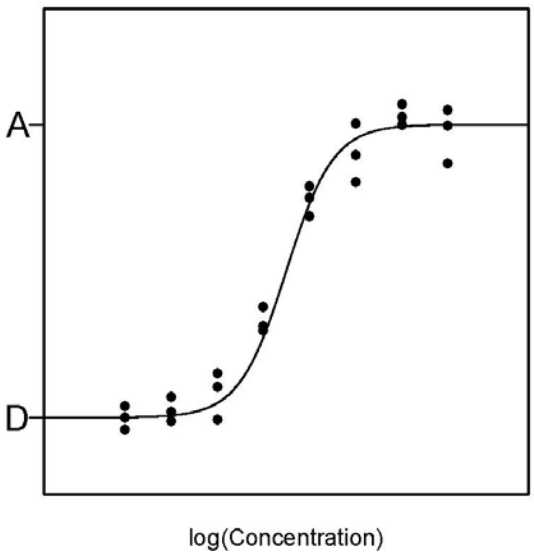
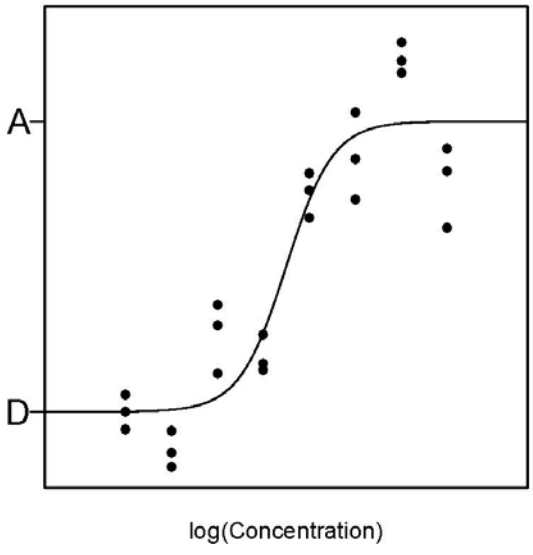
Examples of Suitability Parameters

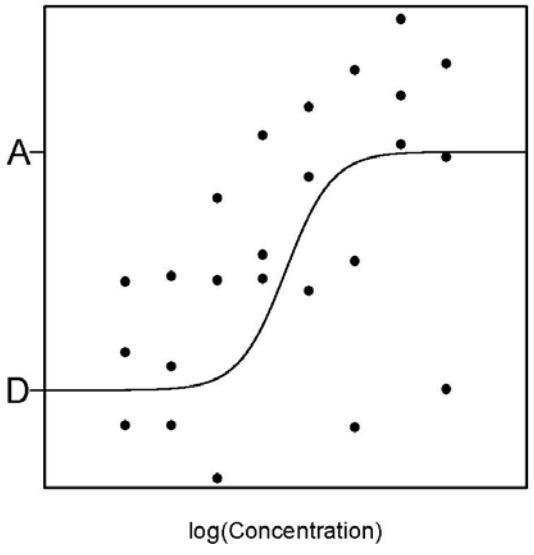
- **Parameters for quality of dose-response curve fit**
  - **Goodness of fit: Lack-of-fit (LOF) measurements (sum of squares, P-value, etc.), R^2**
  - **Precision: Residual mean squared error, %CV of replicated response, confidence interval for potency estimate**



Acceptable



Undesirable (Lack of fit)



Undesirable (Poor precision)

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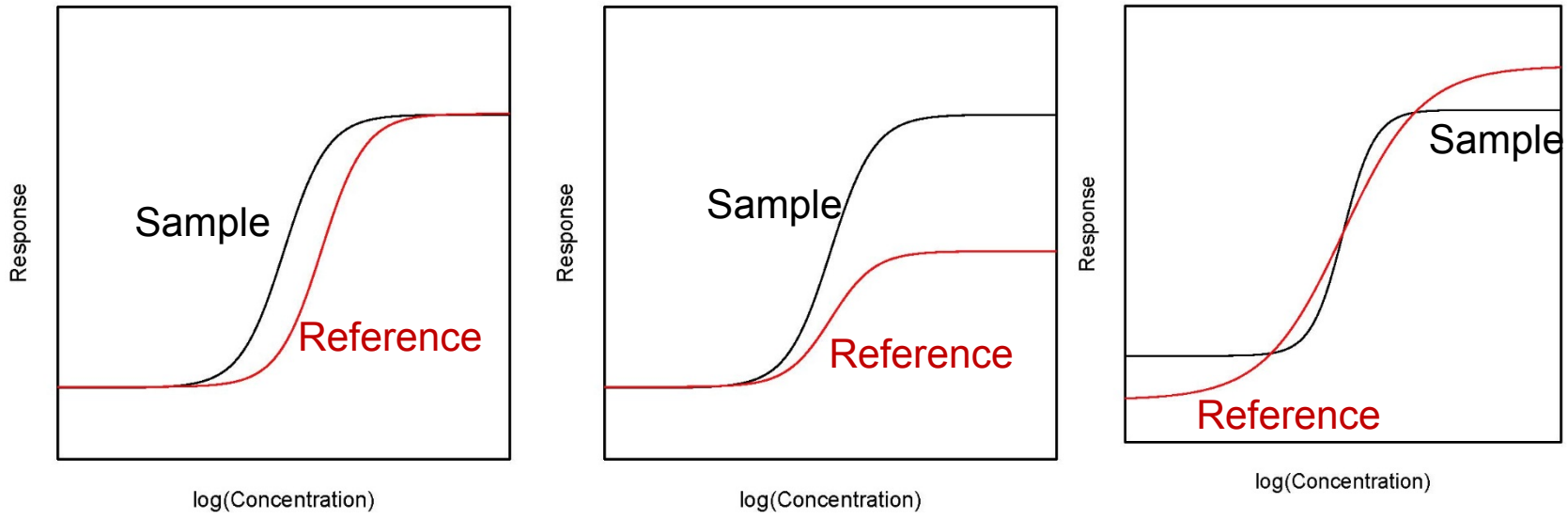
Examples of Suitability Parameters

- **Parameters for similarity between reference and sample curves**
  - **Ratio or difference between reference and sample curve parameters, non-parallelism sum of squares**



Acceptable **Undesirable** **Undesirable**

Selection of Suitability Parameters

General principle

Use parameters with low correlations to provide meaningful assessment for quality of assay results

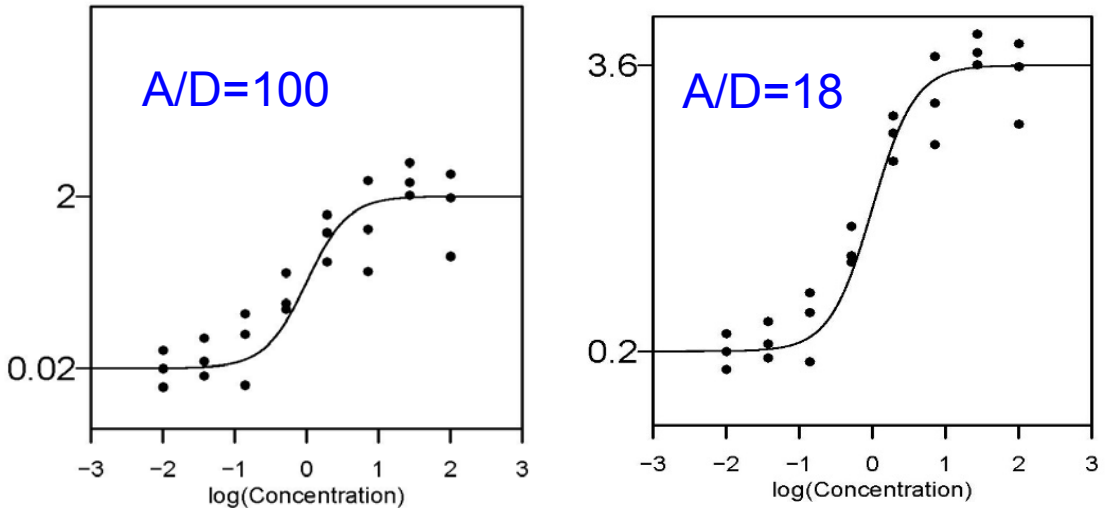
Considerations for suitability parameter selection

- **What is the intended use of the parameter? Is it directly related to the quality of assay and/or potency estimation?**
  - **Many curve fitting parameters may be routinely monitored. However, not all should be applied for suitability assessment**
- **Does the parameter provide meaningful assessment for the intended purpose?**
  - **Suitable for the type of assay**
  - **Effectively reject undesirable assay/sample and retain acceptable assay/sample**
- **Do other parameters provide similar assessment?**
  - **Avoid redundancy**

Example: Does signal to noise ratio (A/D) properly ensure meaningful dose-response curve?

- Reference curve signal to noise ratio (A/D) is a commonly used system suitability parameter
- A/D provides meaningful control for many methods
- However, caution should be taken to avoid arbitrary or insufficient assessment.

Example 1: D values (noise) are expected to be nearly 0



- Small changes in D have big impact on A/D.
- Alternative parameters (e.g., A-D) should be considered.

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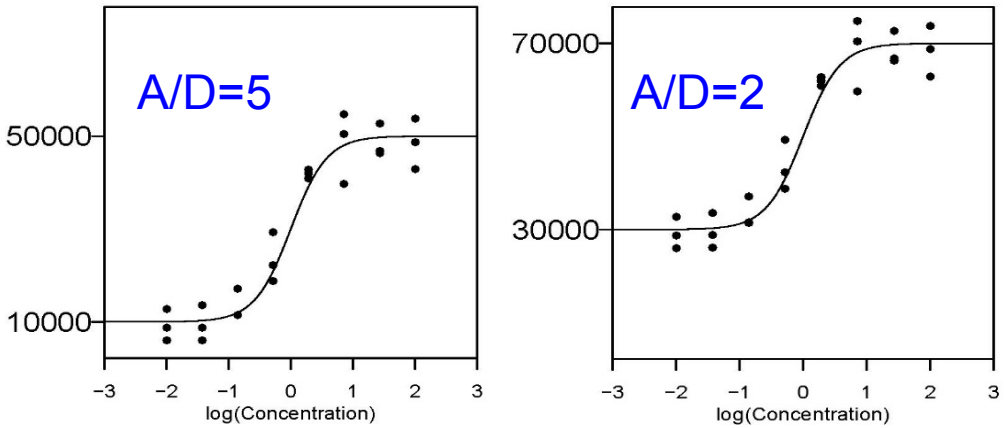
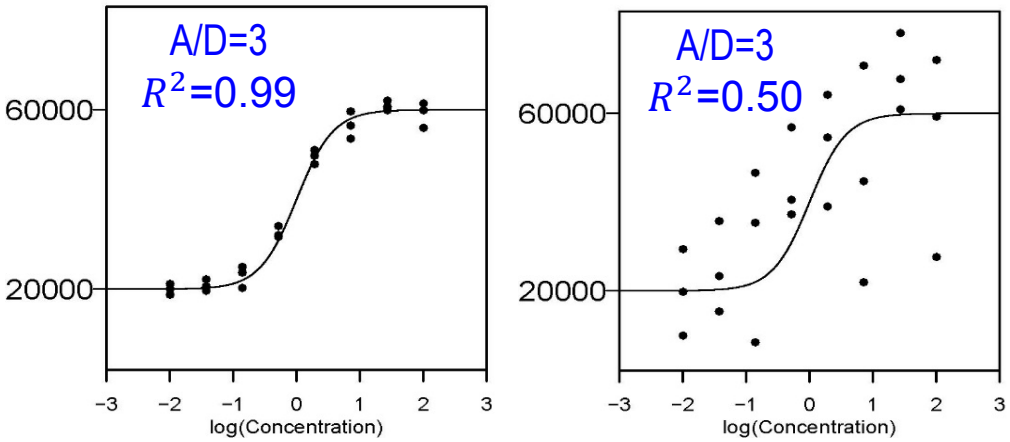
Example: Does signal to noise ratio (A/D) properly ensure meaningful dose-response curve?

Example 2: A and D values vary significantly from assay to assay



- A/D heavily rely on the absolute readouts and may become an arbitrary measurement.
- Alternative parameters (e.g., A-D) should be considered.

Example 3: Assays with same A/D but different variability



- Same A/D doesn't mean same quality of curve fit.
- A/D should be coupled with other parameters (e.g., R^2) to provide meaningful control)

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Determination of Acceptable Range for the Selected Suitability Parameters

General principle:

Use representative data and proper evaluation to determine the suitability acceptance criteria

Considerations for suitability criteria determination

- Representative data set
  - Data generated under final assay condition
  - Consider typical sources of variation
- Distribution of the suitability results
- Examination of extreme results
- Impact on potency estimation
- Intended purpose of the method / Phase of study / Method knowledge and experience

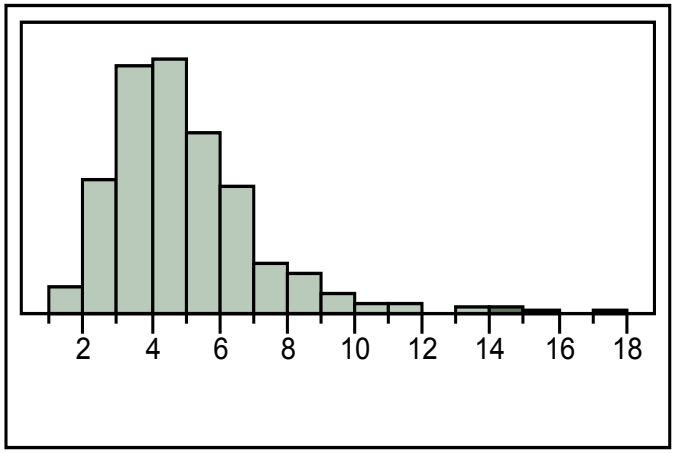
Example: Determination of Suitability Parameter Acceptable Range

Parameter: Relative LOF error

- A measure of lack-of-fit
- High value indicate inadequate model fit

Evaluation of data distribution

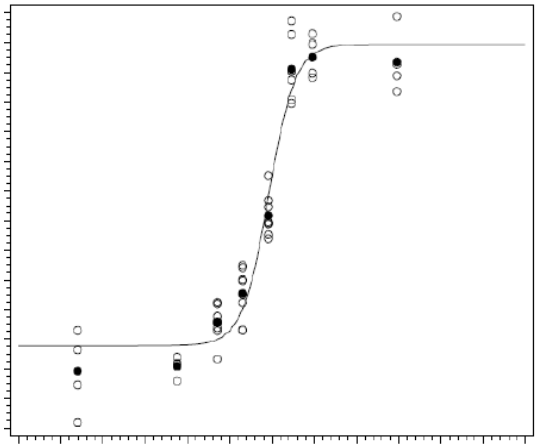
- Histogram of relative LOF results generated during method validation



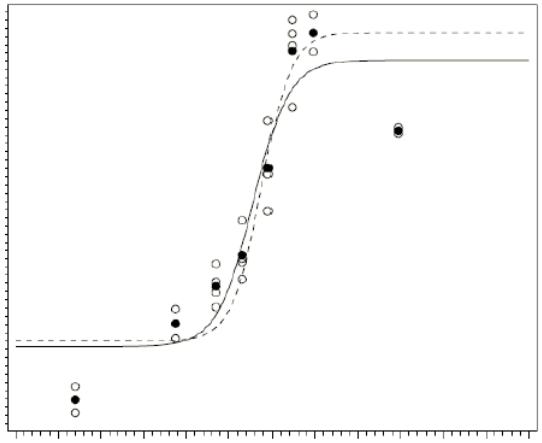
Example: Determination of Suitability Parameter Acceptable Range

Examination of extreme results

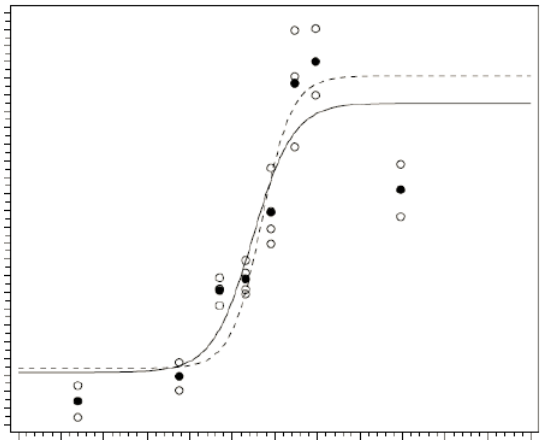
Examples:



Acceptable fit
(Relative LOF error =6.0%)



undesirable fit
(Relative LOF error =11.5%)



Poor fit
(Relative LOF error =14.6%)

Determined suitability criteria:

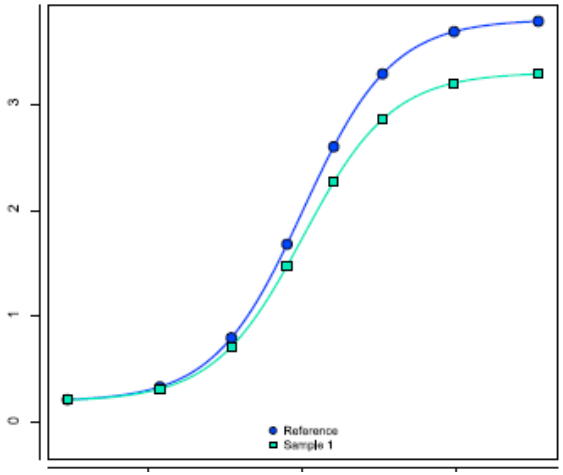
Relative LOF error ≤ 10.0%

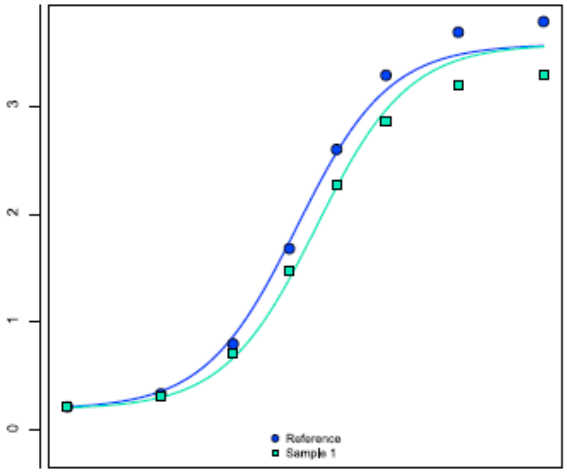
Example: Consideration of Impact on Potency Estimation

The acceptance range of the suitability parameter may also be informed by the impact on potency estimation

Example: Impact of non-parallelism

Assay 1





Unrestricted curves

Upper asymptote: A_{ref}=3.8, A_{sample}=3.3

Lower asymptote: D_{ref}=D_{sample}=0.2

Inflection point: C_{ref}=C_{sample}=1

Hill's slope factor: B_{ref}=B_{sample}=1

Restricted curves

Estimated sample potency =65% (significantly impacted by the deviation at upper asymptote)

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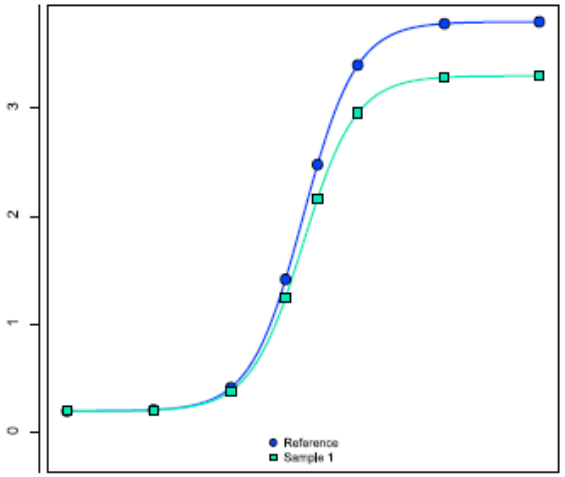
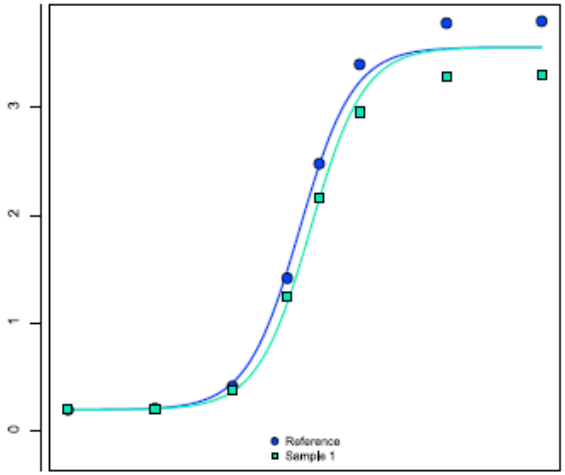
Example: Consideration of Impact on Potency Estimation

Example: Impact of non-parallelism

Assay 2





Unrestricted curves

Upper asymptote: A_{ref}=3.8, A_{sample}=3.3

Lower asymptote: D_{ref}=D_{sample}=0.2

Inflection point: C_{ref}=C_{sample}=1

Hill's slope factor: B_{ref}=B_{sample}=3

Restricted curves

Estimated sample potency =87%

(same level of the deviation at upper asymptote has less impact on potency estimation due to steeper slope)

All the curve parameters are the same as assay 1 except for B factor

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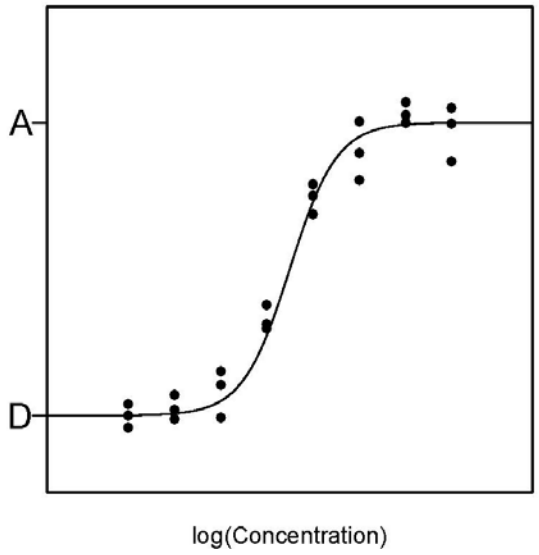
Example: Consideration of Impact on Potency Estimation

- Same level of non-parallelism have different impact on potency estimation for assays with different B factor
  - Assays with smaller B are more sensitive to non-parallelism and require tighter control of the non-parallelism parameters
  - Assays with steeper slope are less sensitive and can tolerate higher level of non-parallelism
- The impact on potency estimation should be taken into consideration when setting acceptance range for suitability parameter

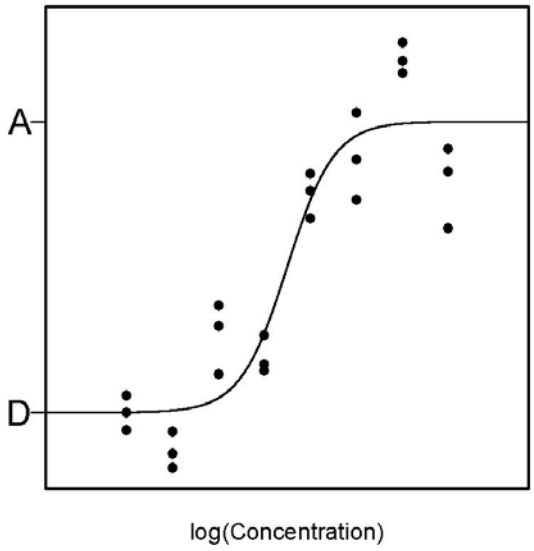
Example: Different Suitability Parameters for Lack-of-Fit Assessment

Lack-of-Fit (LOF) assessment:

Assess the adequacy of the dose-response model. Measure the closeness of the fitted curve to the observed data.



Acceptable fit



Undesirable fit

Example: Different Suitability Parameters for Lack-of-Fit Assessment

LOF P-value

$$F\ ratio = \frac{SS_{LOF}/DF_{LOF}}{SS_{PE}/DF_{PE}} = \frac{\sum_{i,j}(\bar{y}_i - \hat{y}_i)^2/DF_{LOF}}{\sum_{i,j}(y_{i,j} - \bar{y}_i)^2/DF_{PE}}$$

- Based on ANOVA F test
- Conclude lack of fit if P-value is significant (e.g., < 0.05)
- Compare LOF error to pure error
  - SS_{LOF}: Measures overall LOF error (difference between local average $\bar{y}_i$ and fitted value $\hat{y}_i$)
  - SS_{PE}: Measures overall pure error (difference between individual observation $y_{i,j}$ and local average $\bar{y}_i$)

LOF sum of squares

$$SS_{LOF} = \sum_{i,j}(\bar{y}_i - \hat{y}_i)^2$$

- Directly measures LOF error without comparing to pure error
- Conclude lack of fit if SS_{LOF} is large

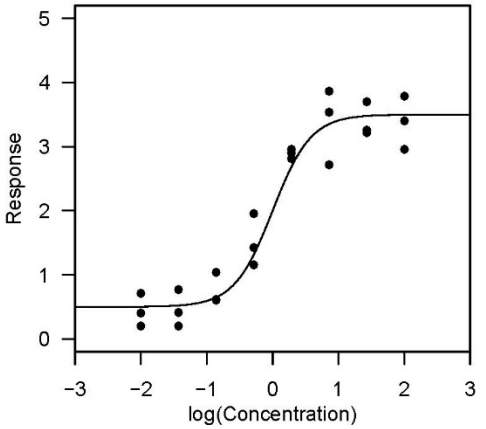
Relative LOF error

$$\frac{\sqrt{SS_{LOF}/N}}{\boldsymbol{A}_{ref} - \boldsymbol{D}_{ref}} \times 100\%$$

- LOF error normalized against reference A-D

Example: Different Suitability Parameters for Lack-of-Fit Assessment – LOF P-value vs. SS_{LOF}

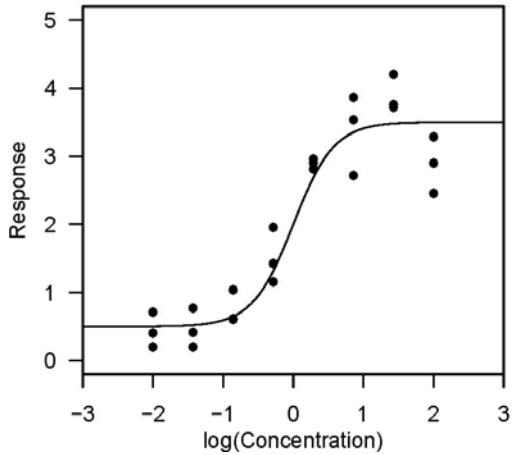
Example 1: LOF P-value successfully conclude good vs. poor fit



SS_{LOF}=0.04 (pass)

SS_{PE}=1.9

LOF P-value = 0.99 (pass)

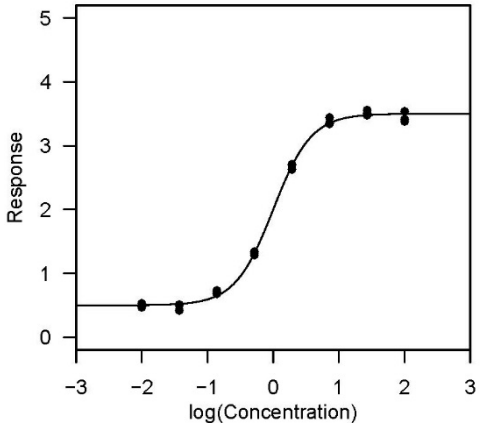


SS_{LOF}=1.6 (fail)

SS_{PE}=1.9

LOF P-value = 0.04 (fail)

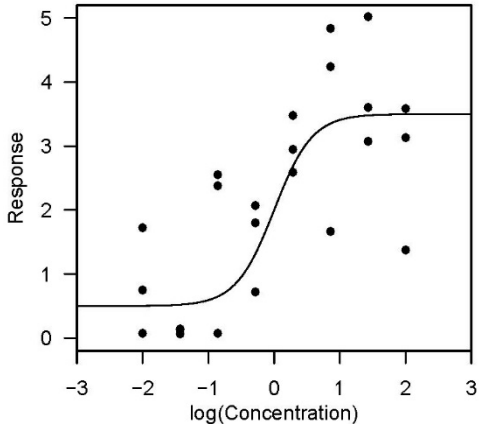
Example 2: LOF P-value unable to properly conclude good vs. poor fit



SS_{LOF}=0.03 (pass)

SS_{PE}=0.03

LOF P-value = 0.03 (fail)



SS_{LOF}=5.5 (fail)

SS_{PE}=17.0

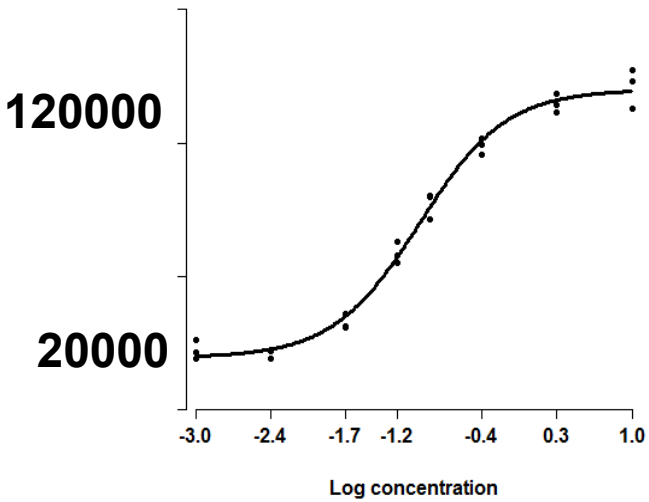
LOF P-value = 0.32 (pass)

In both examples, SS_{LOF} works properly

Example: Different Suitability Parameters for Lack-of-Fit Assessment - SS_{LOF} vs. Relative LOF Error

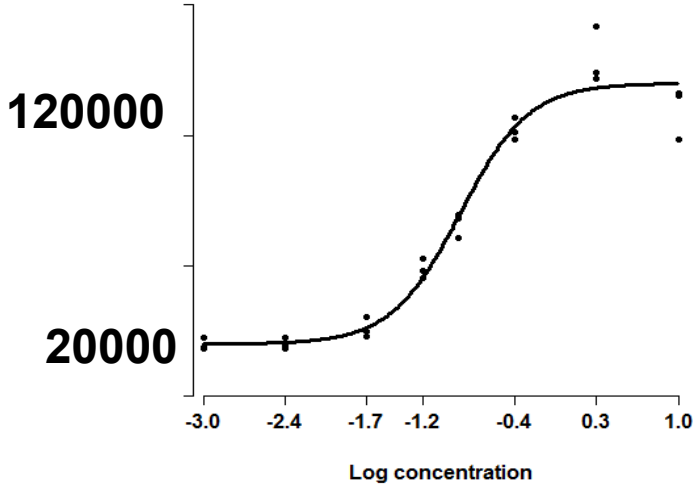
Example 3: LOF sum of squares successfully conclude good vs. poor fit when comparing curves from same instrument with high readouts

Curve 1
Acceptable fit
Instrument A (High readouts)



$SS_{LOF} = 7.2 \times 10^7$ (low)
Relative LOF error = 1.7% (low)

Curve 2
Undesirable fit
Instrument A (High readouts)

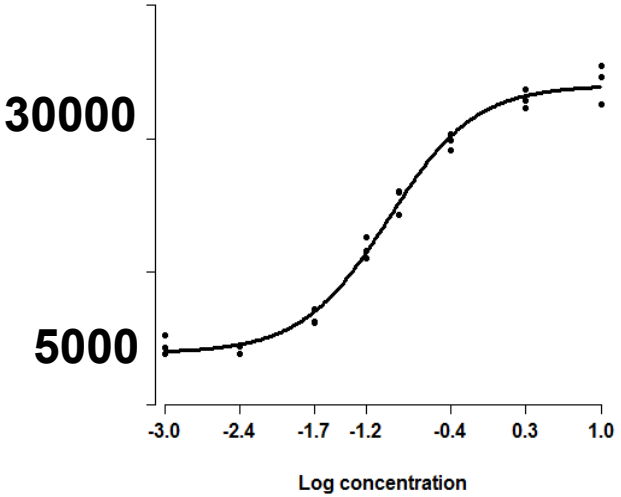


$SS_{LOF} = 7.0 \times 10^8$ (high)
Relative LOF error = 5.4% (high)

Example: Different Suitability Parameters for Lack-of-Fit Assessment - SS_{LOF} vs. Relative LOF Error

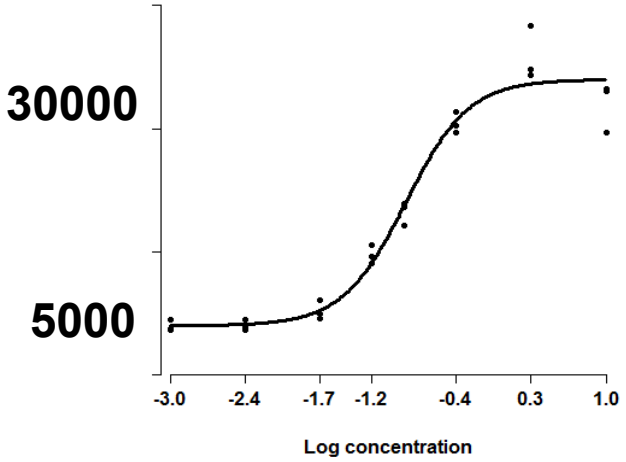
Example 4: LOF sum of squares successfully conclude good vs. poor fit when comparing curves from same instrument with low readouts

Curve 3
Acceptable fit
Instrument B (low readouts)



$SS_{LOF} = 4.5 \times 10^6$ (low)
Relative LOF error = 1.7% (low)

Curve 4
Undesirable fit
Instrument B (low readouts)

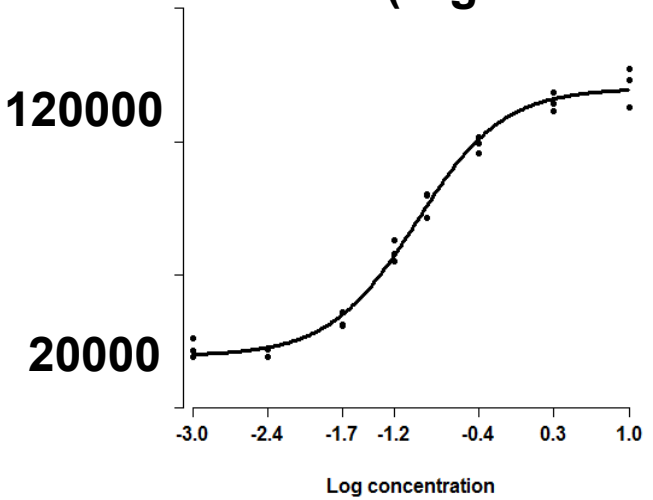


$SS_{LOF} = 4.4 \times 10^7$ (high)
Relative LOF error = 5.4% (high)

Example: Different Suitability Parameters for Lack-of-Fit Assessment - SS_{LOF} vs. Relative LOF Error

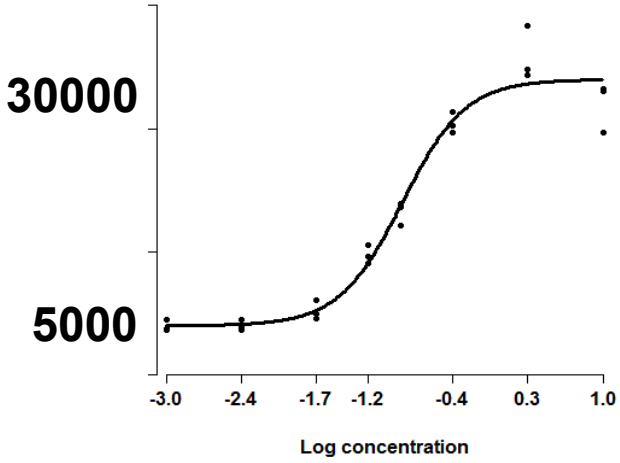
Example 5: LOF sum of squares doesn't work properly when comparing curves between instruments with different readouts

Curve 1
Acceptable fit
Instrument A (High readouts)



$SS_{LOF} = 7.2 \times 10^7$ (high)
Relative LOF error = 1.7% (low)

Curve 4
Undesirable fit
Instrument B (low readouts)



$SS_{LOF} = 4.4 \times 10^7$ (low)
Relative LOF error = 5.4% (high)

Relative LOF error still works properly

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Example: Different Suitability Parameters for Lack-of-Fit Assessment

LOF P-value

- Works properly when the level of pure error are consistent from assay to assay
- May over-sensitively reject precise data and propensity to retain undesirable noisy data

LOF sum of squares

- Overcomes the limitation of LOF P-value since not impacted by the pure error
- Requires A-D to be consistent across labs /instruments /analysts in order to provide meaningful assessment

Relative LOF error

- Independent of the magnitude of the response readings. Therefore more robust than LOF sum of squares when A-D vary from assay to assay

Summary: Common Types of Parameters for Suitability Assessment

- Signal to noise
  - E.g., A, D, A/D, A-D
- Potency of the control sample
- Quality of dose-response curve fit
  - Goodness of fit
  - Precision
- Similarity between reference and sample

Summary: Considerations for Suitability Parameter and Criteria Determination

- Be aware of intended use and limitations of the parameters. Carefully select suitable parameters.
- Set acceptance range based on representative data set and thorough evaluation
  - Data distribution / examination of extreme results
  - Impact on potency estimation
  - Phase of study / experience

General Conclusion

- Suitability assessment is a integral part of the potency methods
- Proper suitability assessment ensure scientifical meaningfulness and good data quality which produce reliable potency results
- It is critical to carefully determine system and sample suitability parameters and acceptance criteria that are suitable for intended purpose

Acknowledgement

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Isam Qahwash

Scott Umlauf

Back-Up Slides

Example: Different Suitability Parameters for Lack-of-Fit Assessment

Case study

- The LOF P-value criterion of a ELISA method was replaced by LOF sum of squares criterion
- Summary of retrospective analysis results demonstrate that the new criterion (LOF sum of squares) more efficiently reject undesirable results and retain acceptable results

Case Study: Method Performance Comparison: LOF P-Value vs. LOF Sum of Squares

Data Set		Mean of LOF Sum of Squares	Mean of pure Error Sum of Squares	Mean of QC Potency (%)	SD of QC Potency (%)
Overall (N=321)*		0.11	0.52	101.1	7.2
LOF P-value (Old)	Pass (N=268)	0.10	0.59	101.3	7.5
	Fail (N=53)	0.19	0.15	100.4	5.4
LOF sum of squares (New)	Pass (N=303)	0.09	0.46	100.9	7.1
	Assays that pass LOF sum of squares & failed P-value (N=46)	0.12	0.11	100.2	5.5
	Fail (N=18)	0.57	1.46	104.8	8.0

* Assays failed other suitability criteria (e.g., non-parallelism) were excluded

Summary of Case Study

- The LOF P-value criterion causes higher failure rate.
- The assays that failed the LOF P-value criterion have better accuracy and precision than the assays that passed the LOF P-value.
- The LOF sum of squares criterion can more effectively invalidate assays with poor fit and retain assays with precise fit.

Simulation Study: Impact of Non-Parallelism on Potency Estimation

Objective: study the impacts of non-parallelism on potency estimation.

- **Fix the reference curve**
- **The test curve varies by different combinations of lower and upper asymptote ratios, and slope ratios.**
- **Calculate potency based on restricted model**

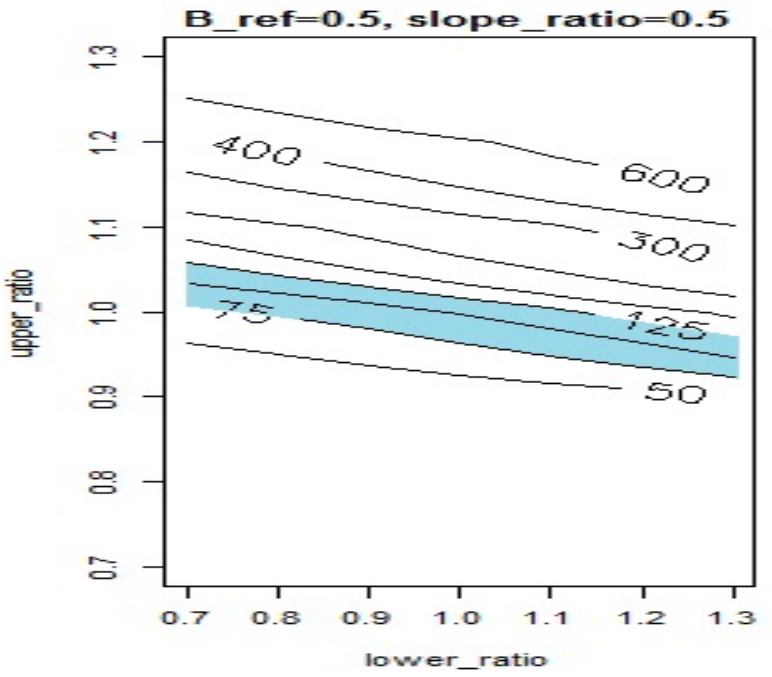
Parameter	Setup
Articles	Reference and test sample
A, C, D values (reference)	A = 0.5, C = 1, D = 3.5
B value (reference)	0.5, 1, 2, 3
Low asymptote ratio (test over reference)	0.7 – 1.3 by 0.1
Upper asymptote ratio (test over reference)	0.7 – 1.3 by 0.1
Slope ratio (test over reference)	0.5 – 2.0 by 0.1

Simulation Study: Impact of Non-Parallelism on Potency Estimation – Contour Graph

Contour graph of potency given reference B and slope ratio

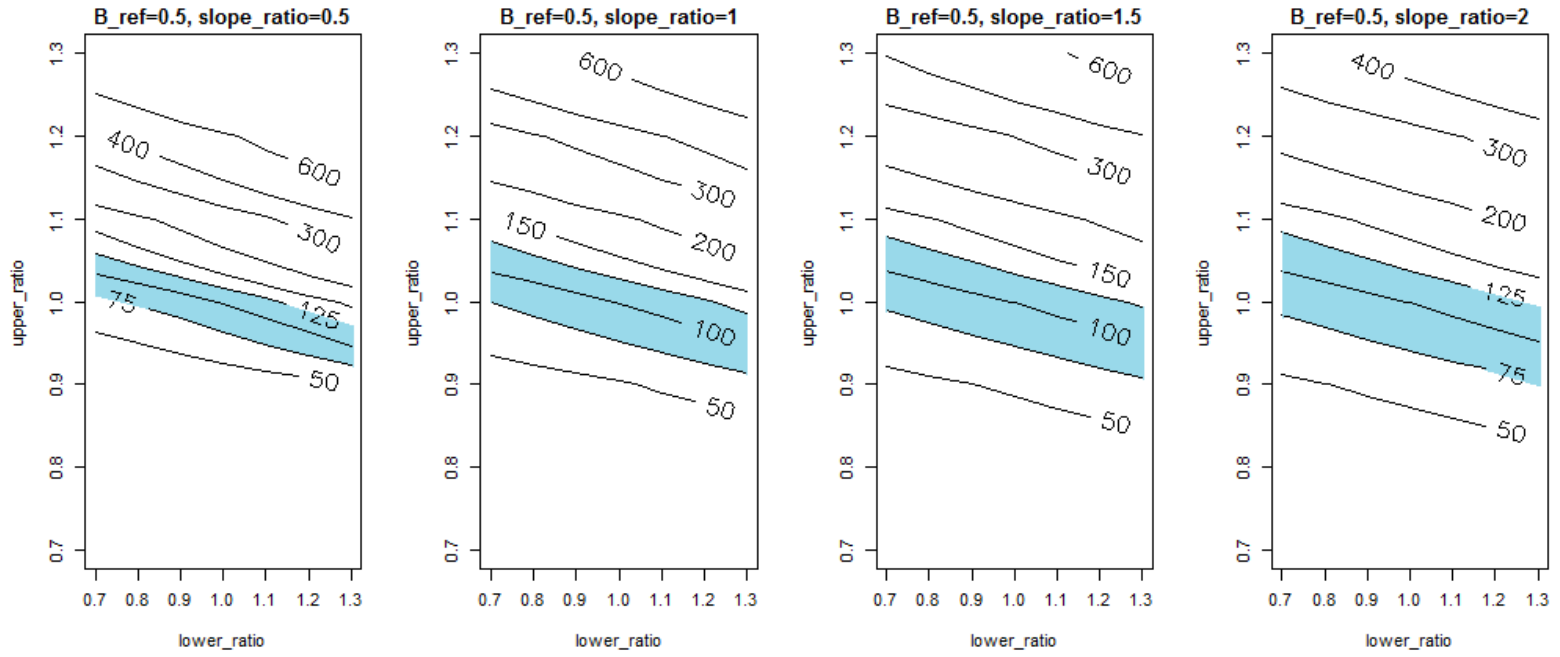
- **Show how the estimates of relative potency changes along the lower and upper asymptote ratios at a given slope ratio.**

- **Highlight the contours of potency between 75% and 125% (expected potency is 100%).**



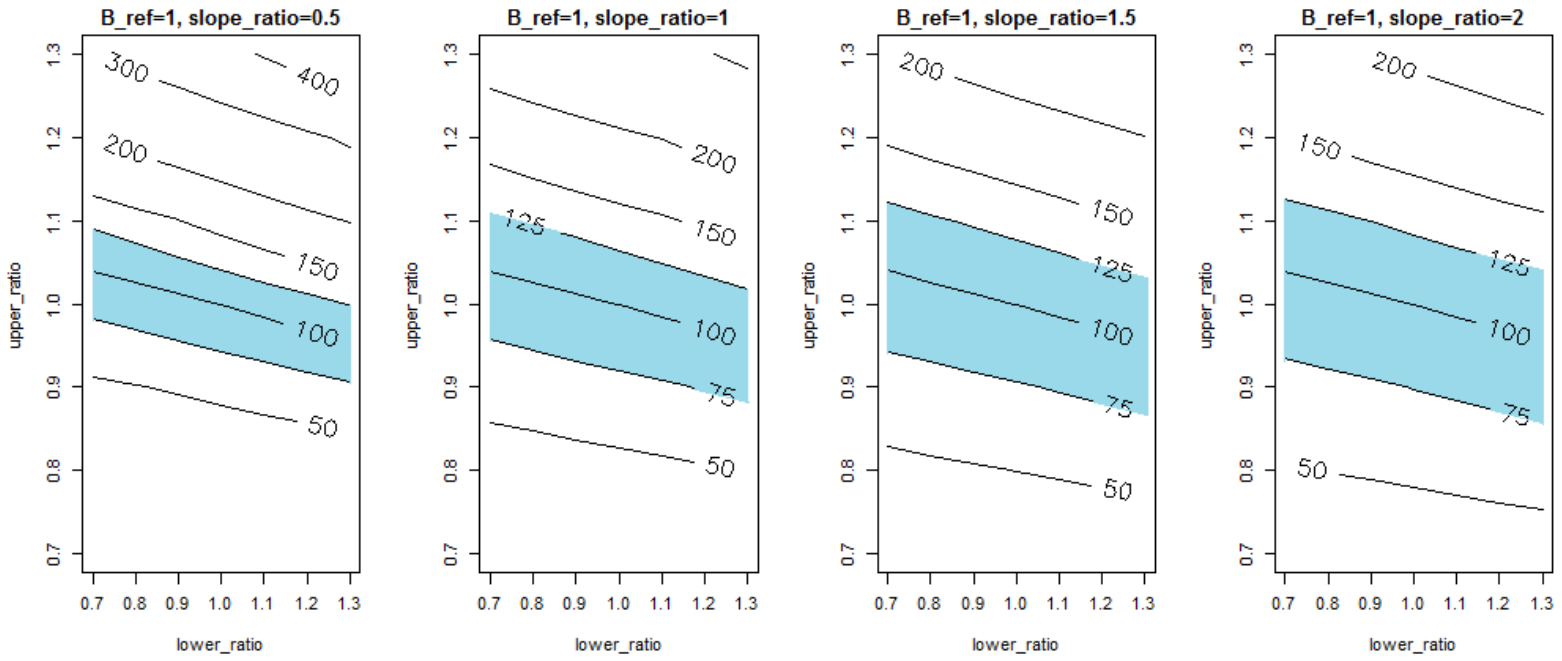
Simulation Study: Impact of Non-Parallelism on Potency Estimation – Results (B=0.5)

Slope ratio is 0.5, 1.0, 1.5, and 2.0



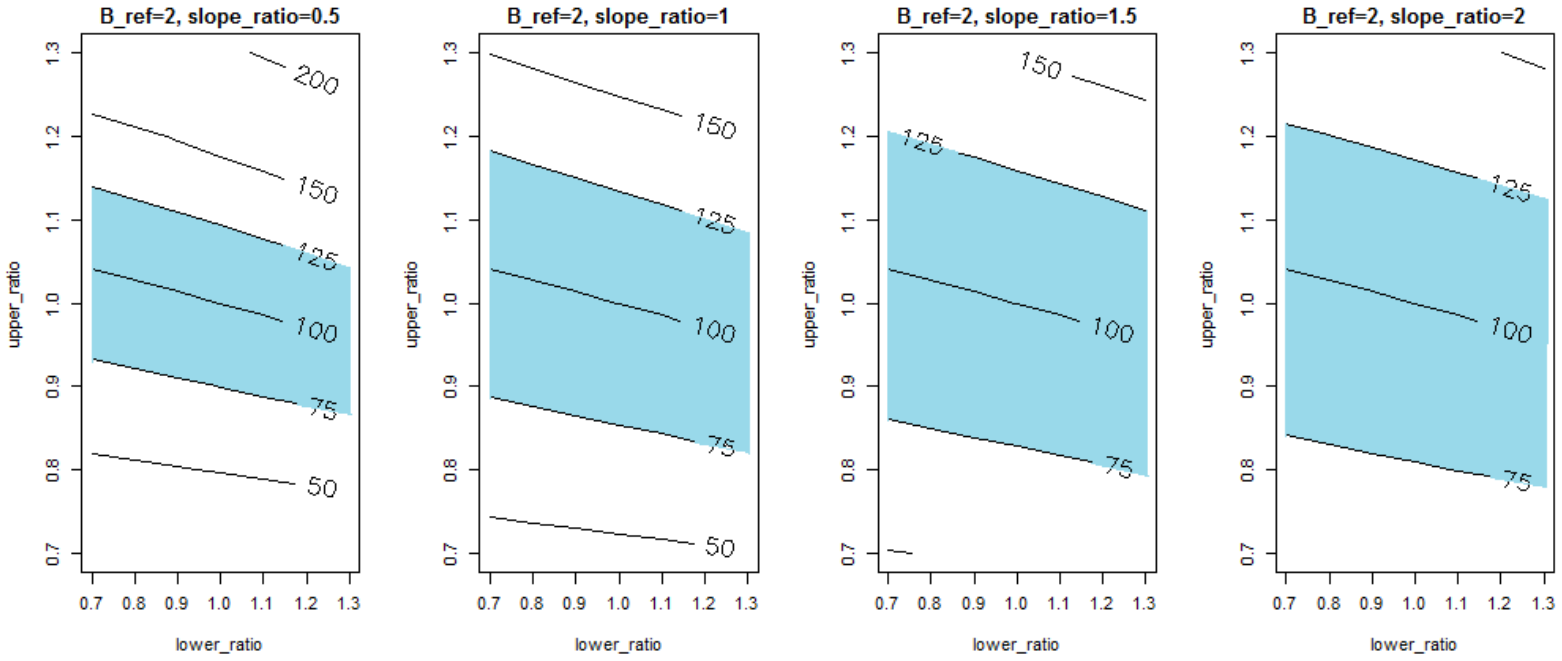
Simulation Study: Impact of Non-Parallelism on Potency Estimation – Results (B=1)

Slope ratio is 0.5, 1.0, 1.5, and 2.0



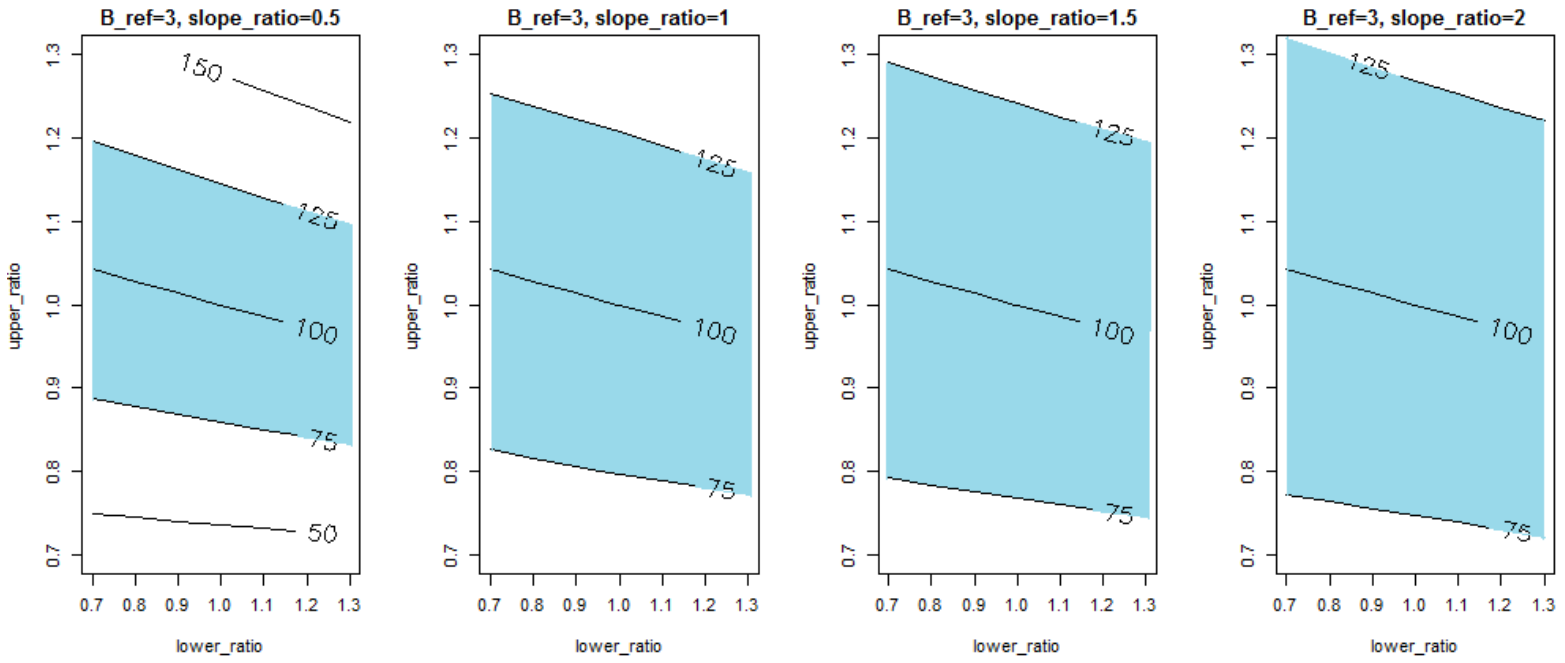
Simulation Study: Impact of Non-Parallelism on Potency Estimation – Results (B=2)

Slope ratio is 0.5, 1.0, 1.5, and 2.0



Simulation Study: Impact of Non-Parallelism on Potency Estimation– Results (B=3)

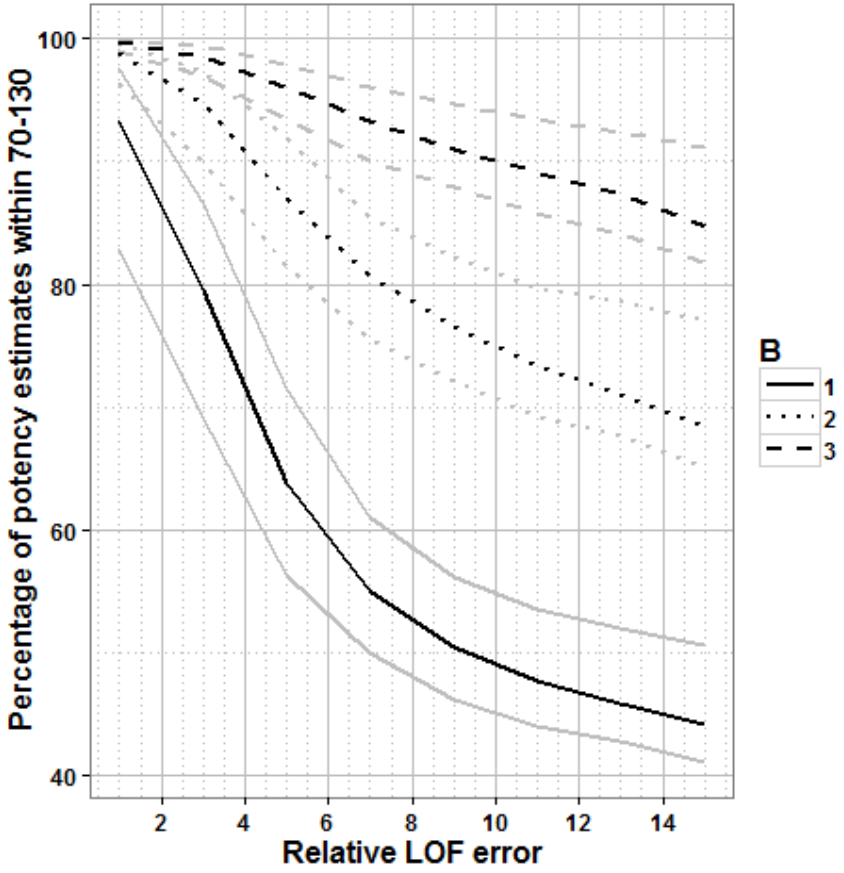
Slope ratio is 0.5, 1.0, 1.5, and 2.0



Simulation Study: Impact of Non-Parallelism on Potency Estimation – Summary

- The range of 75%-125% contours of potency
  - wider as the reference curve gets steeper.
- Upper ratio has more significant effects on potency estimation than lower ratio and slope ratio, thus needs to be more tightly controlled.
- Similar results were obtained for nominal potency 70% and 130%.

Simulation Study: Impact of Relative LOF Error on Potency Estimation



Simulation study results:

The median (black lines) and 5%, 95% percentiles (surrounding grey lines) of the probability of having relative potency within 70-130% are plotted separately for dose-response curve slope factor B of 1, 2 and 3.