

Curve Similarity Index for testing Parallelism in Bioassay

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CASSS Bioassays 2020

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Agenda

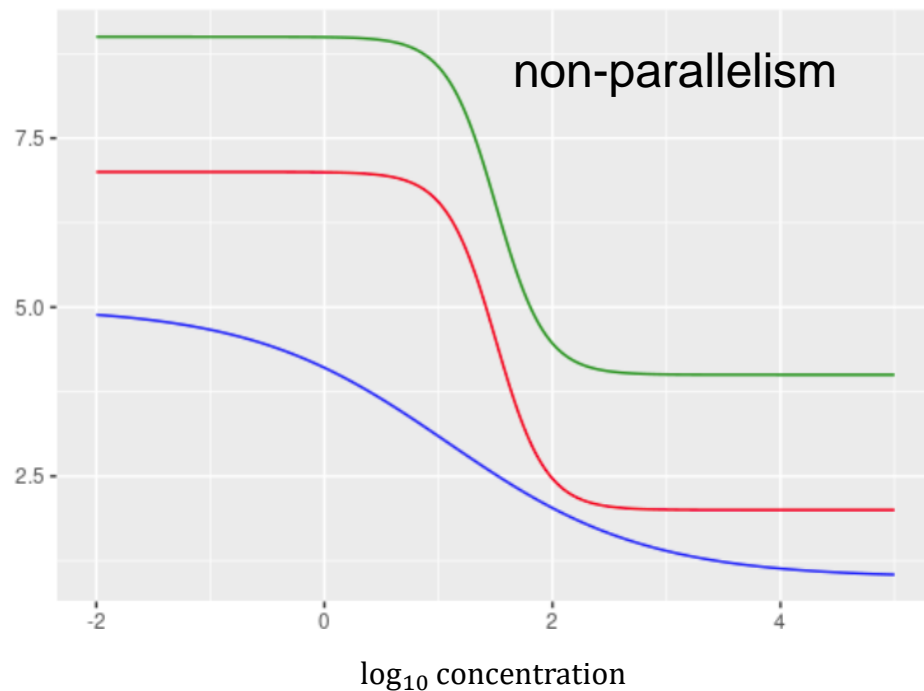
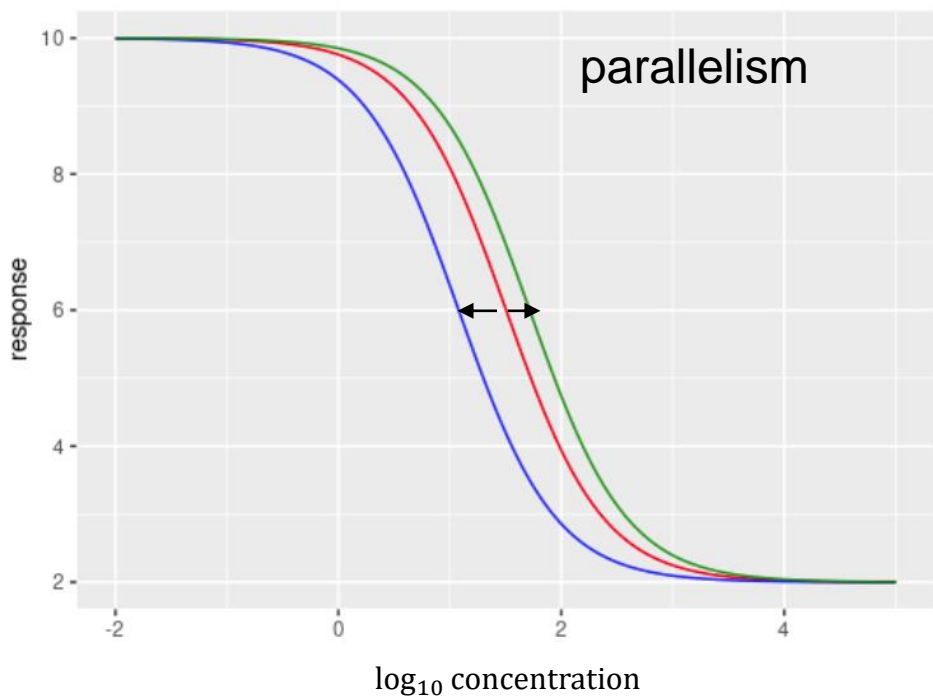
- Background
- Parallelism
- Traditional Approaches
- Curve Similarity Index
- Acceptance Limit
- Simulation Study

Background

- United States Pharmacopeia (USP)<1032>
 - “Because of the inherent variability in biological test systems, *an absolute measure of potency is more variable than a measure of activity relative to a Standard*”
 - “Assuming that the **Standard** and **Test** materials are *biologically similar, statistical similarity should be present*, and the Test sample can be expected to behave like a *concentration or dilution* of the Standard.”

Parallelism

- Parallelism a.k.a. “equivalence” or “similarity”
- Is the TS a dilution or concentration of the RS?
 - i.e.: the only difference between the two curves is the EC50



Tests for Parallelism

$$y = D_i + \frac{A_i - D_i}{1 + \left(\frac{x}{C_i}\right)^{B_i}}, \quad i = 1, 2$$

- Difference Test (F-Test)
 - $H_0: A_1 = A_2$ and $B_1 = B_2$ and $D_1 = D_2$
 - $H_1: A_1 \neq A_2$ or $B_1 \neq B_2$ or $D_1 \neq D_2$

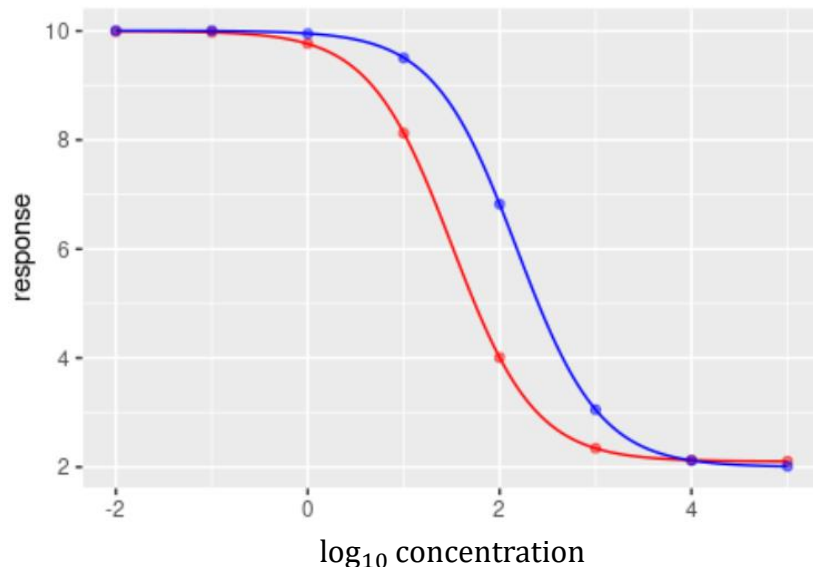
$$F = \frac{\text{signal}}{\text{noise}}$$

Tests for Parallelism

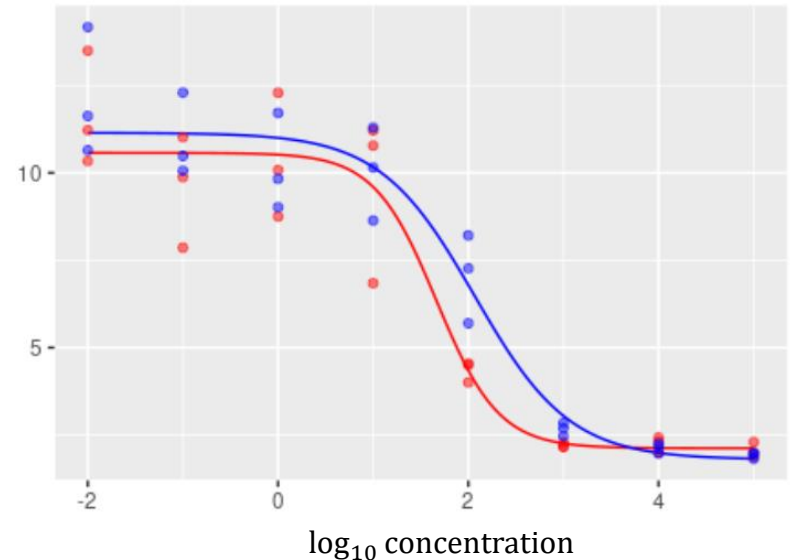
- F-Test

- Often rejects H_0 when the curves appear to be parallel in the presence of good assay precision
- Often fails to reject H_0 in the case of non-parallelism due to poor assay data precision

p-value < 0.000, rejects parallelism



p-value = 0.8, fails to reject parallelism



Tests for Parallelism

- Equivalence Test
 - Equivalence margins, D_L, D_U

$$H_0: \frac{A_1}{A_2} \leq D_L \text{ or } \frac{A_1}{A_2} \geq D_U$$

$$\text{or } \frac{B_1}{B_2} \leq D_L \text{ or } \frac{B_1}{B_2} \geq D_U$$

$$\text{or } \frac{D_1}{D_2} \leq D_L \text{ or } \frac{D_1}{D_2} \geq D_U$$

$$H_1: D_L < \frac{A_1}{A_2} < D_U$$

$$\text{and } D_L < \frac{B_1}{B_2} < D_U$$

$$\text{and } D_L < \frac{D_1}{D_2} < D_U$$

Tests for Parallelism

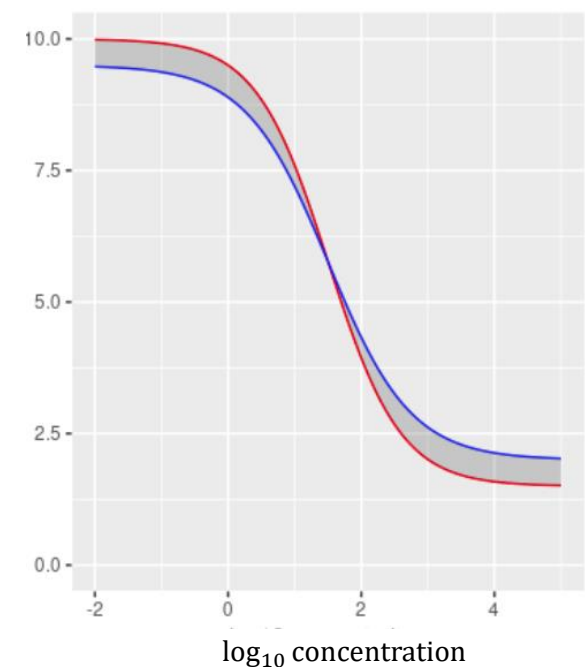
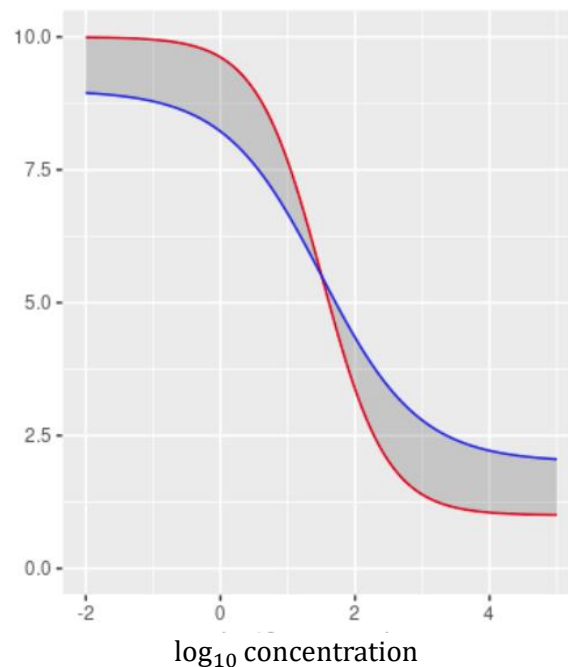
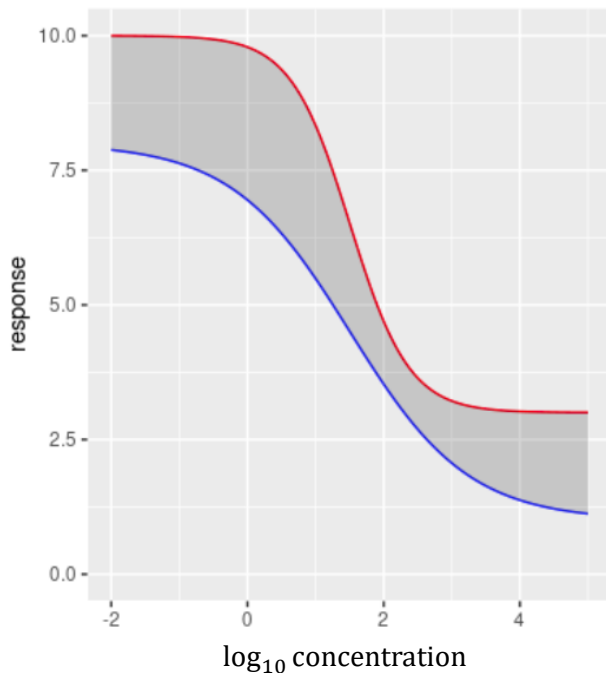
- Equivalence Test Challenges
 - Slope challenge, B is not the slope (Stegmann, 2017)
 - Parameters are correlated
 - Establishing equivalence margins is challenging
 - It's a multivariate problem
 - False-positive rate is difficult

Guidelines

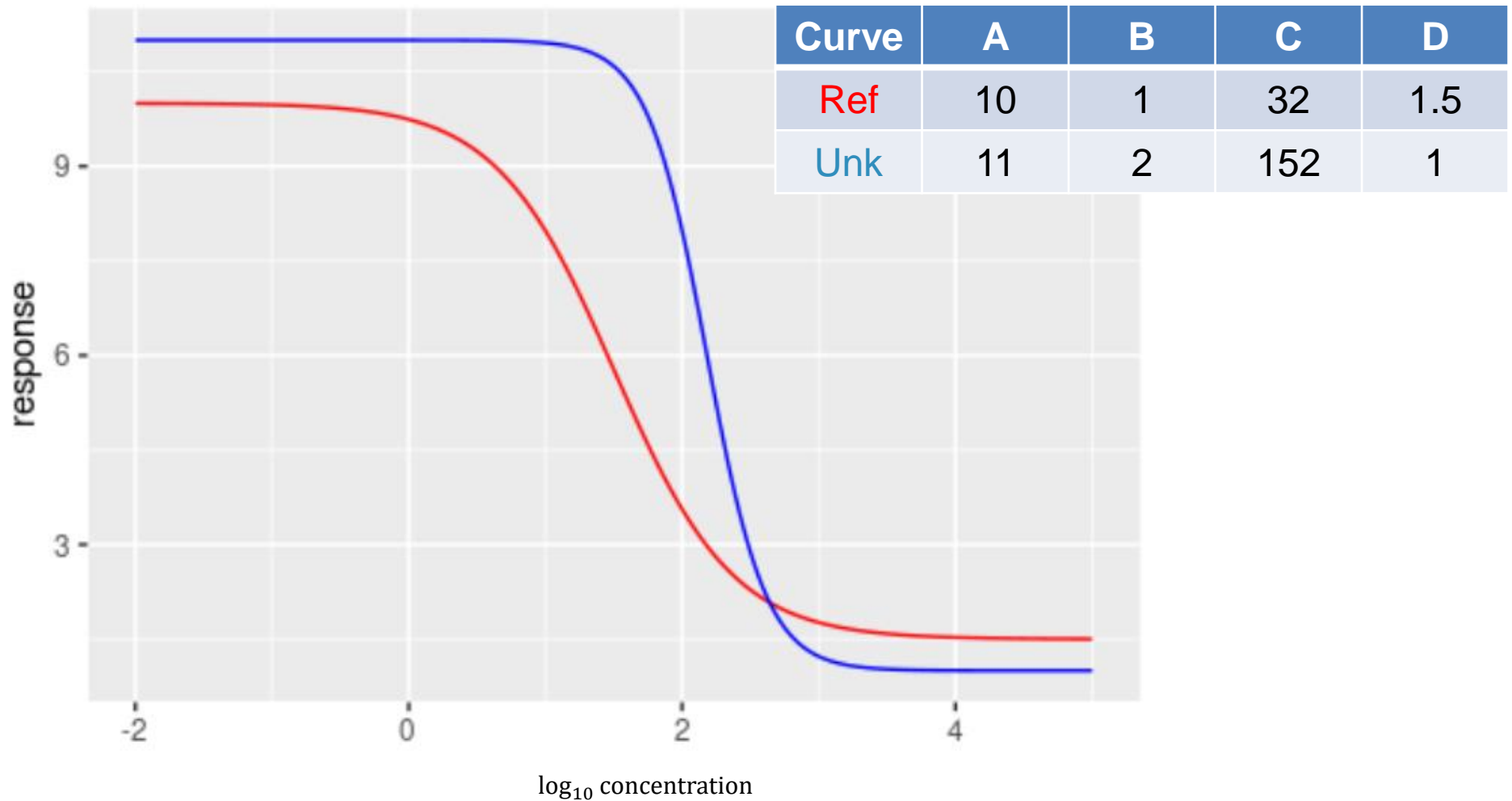
- United States Pharmacopeia (USP)<1032>
 - Section 4.7: “The determination of similarity could be based on the *individual parameters*... Alternatively, evaluation of similarity can be based on a *single composite measure of nonparallelism*...”
- European Pharmacopoeia (Chapter 5.3)
 - Section 1: “*Alternative methods can be used and may be accepted by the competent authorities, provided that they are supported by relevant data and justified during the assay validation process*”

Curve Similarity Index (CSI)

- Can we reduce the test to a single measure that is intuitive?
- Consider area between curves as an indicator of curve similarity

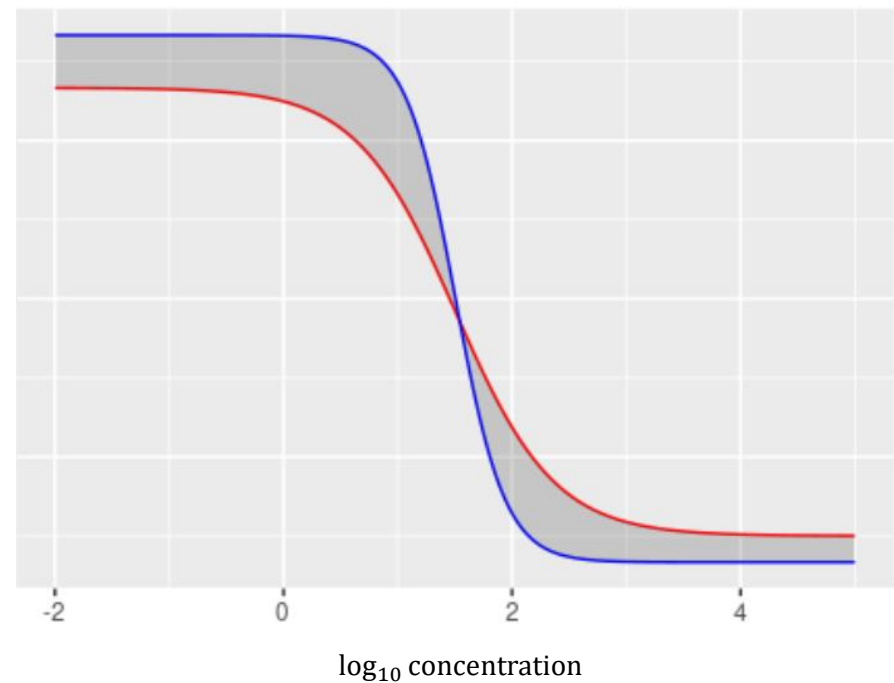
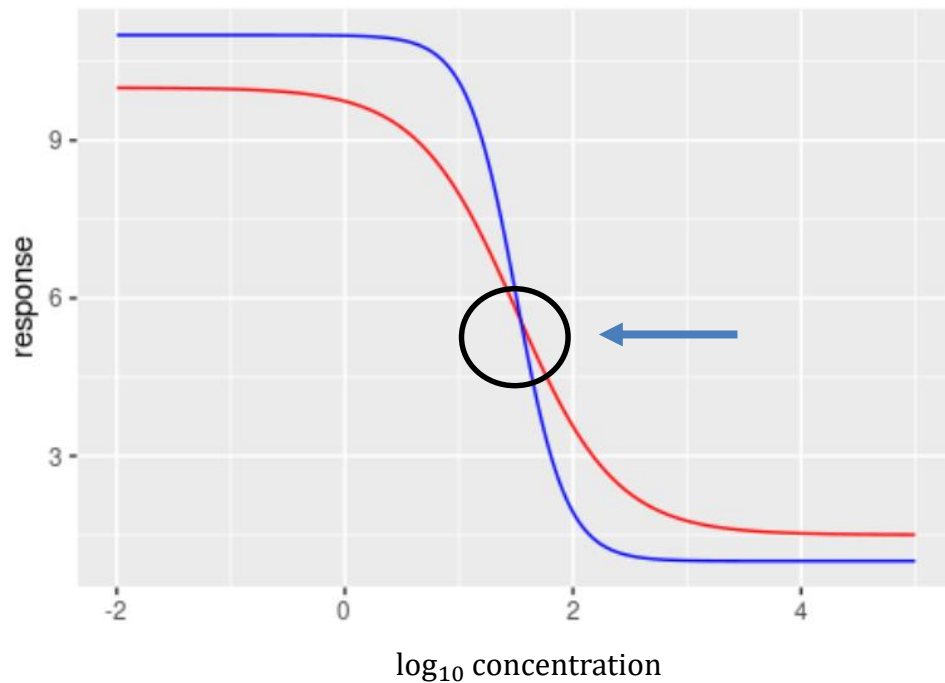


Step 1: Obtain independent fits

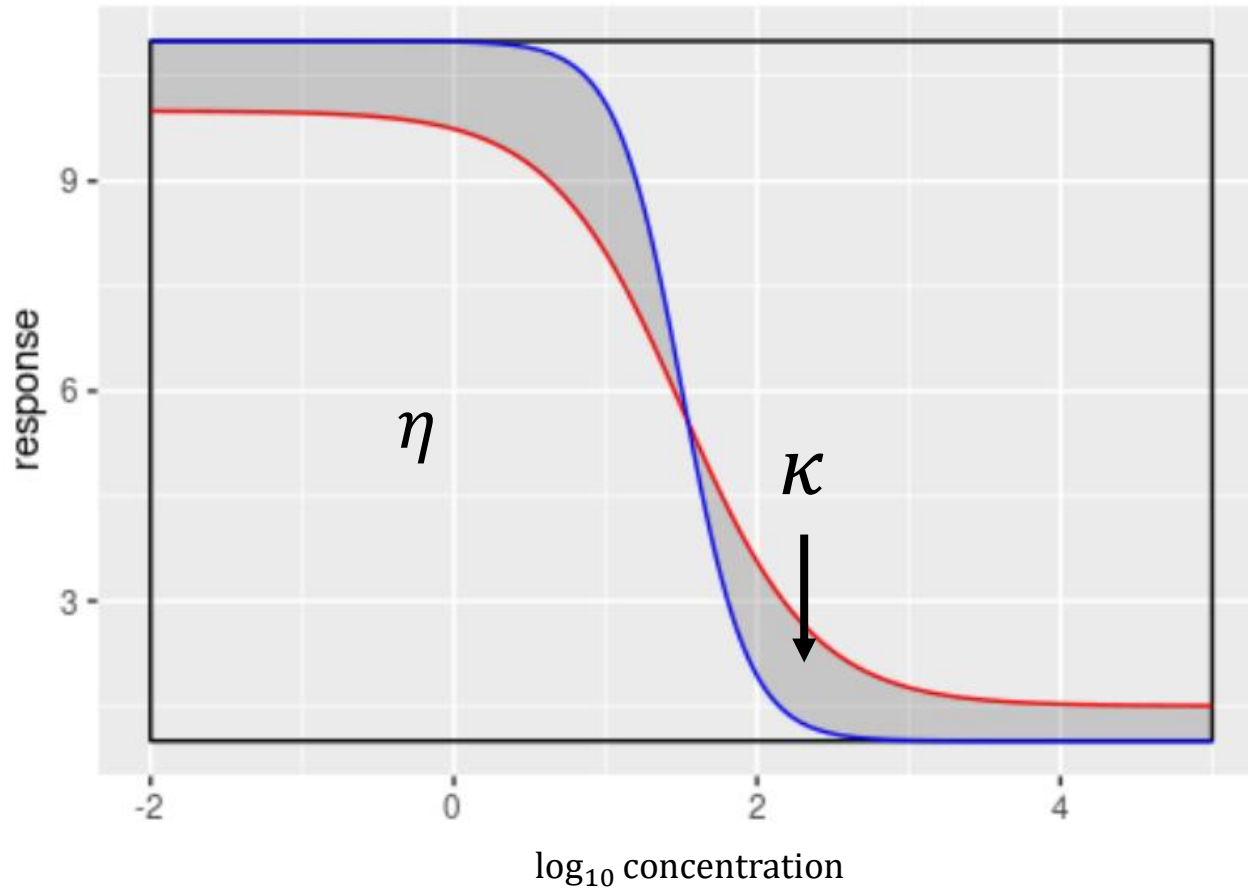


Step 2: Align curves at EC50

Curve	A	B	C	D
Ref	10	1	32	1.5
Unk	11	2	32	1



Step 3: Compute CSI Metric



$$CSI = 1 - \frac{\kappa}{\eta}$$

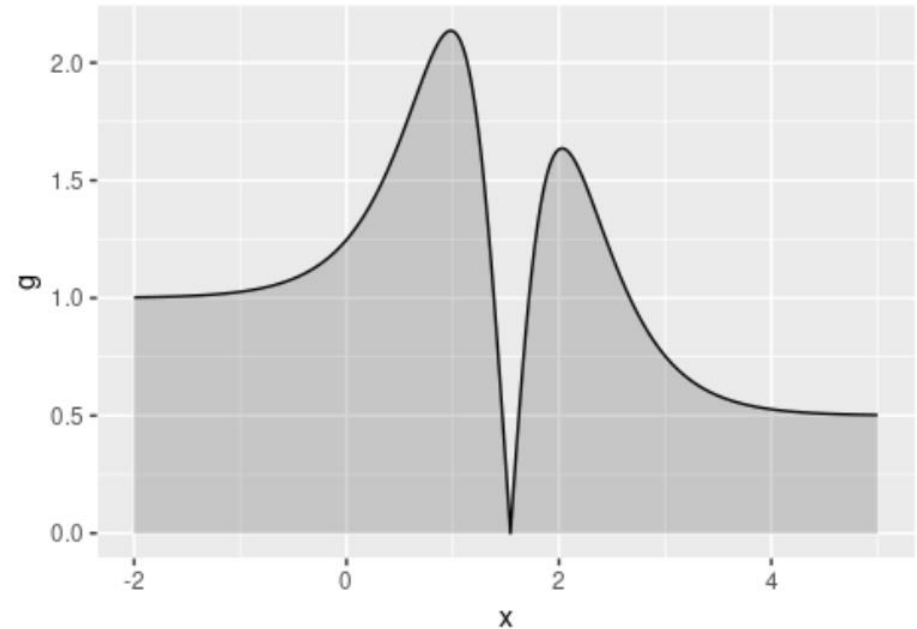
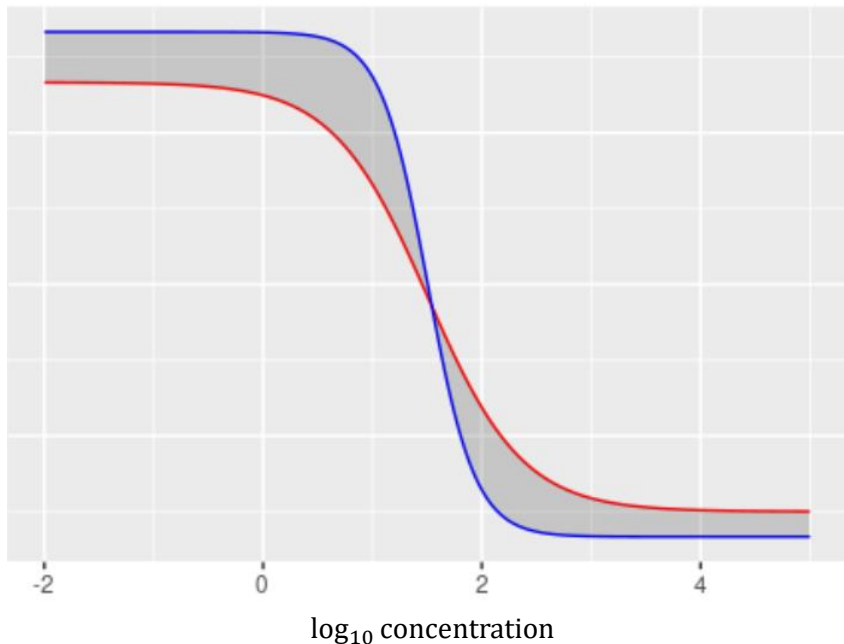
$$= 0.89$$

Note:

$$0 \leq CSI \leq 1$$

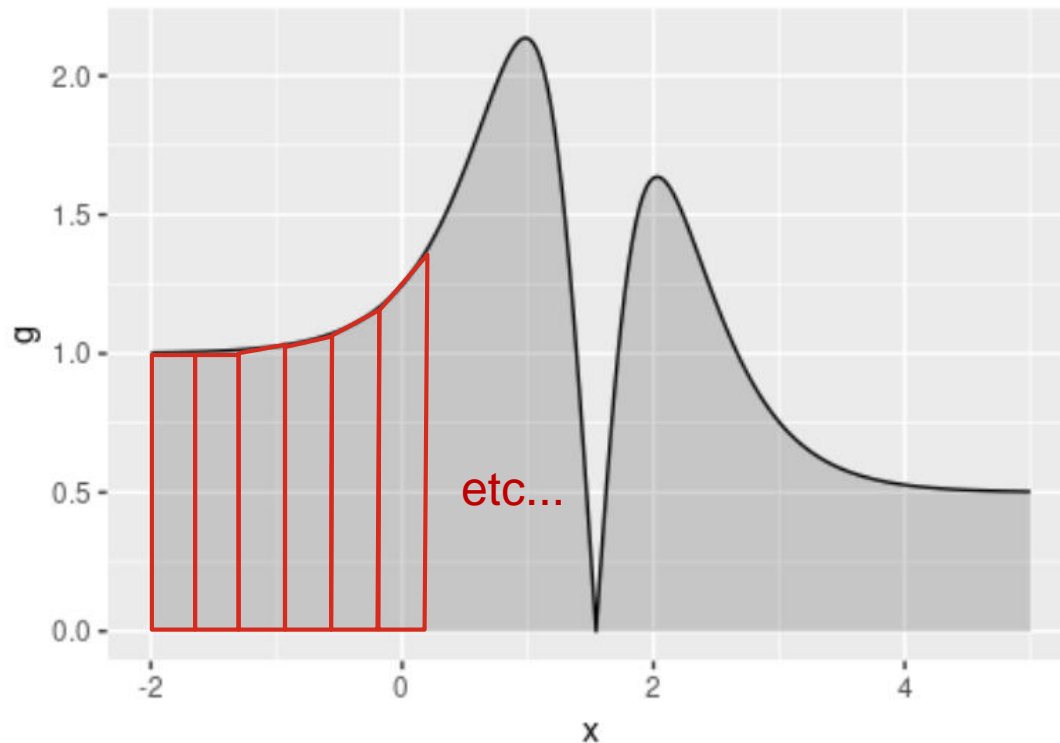
Step 3: Compute CSI Metric

- Parallel maximum – parallel minimum



Step 3: Compute CSI Metric

- Make use of trapezoidal rule to compute area under the curve
- κ = Area Under Curve = sum of area of trapezoids
- $\eta = (\text{max conc} - \text{min conc}) \times (\text{max asymptote} - \text{min asymptote})$



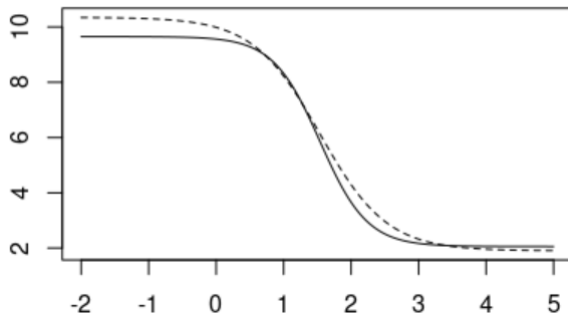
Step 4: Compare CSI Result to Limit

- Could take hypothesis test approach (Faya et al., 2020)
 - $H_0: CSI < \delta$ vs. $H_1: CSI \geq \delta$
 - p-value decision based on t-test
 - Computation of standard error for t-test is complex
- Probabilistic approach
 - Compare point estimate of CSI to limit δ
 - CSI can be computed using built-in functions in SoftMax Pro, for example
 - The limit δ is chosen based on simulation studies, historical data, and SME input

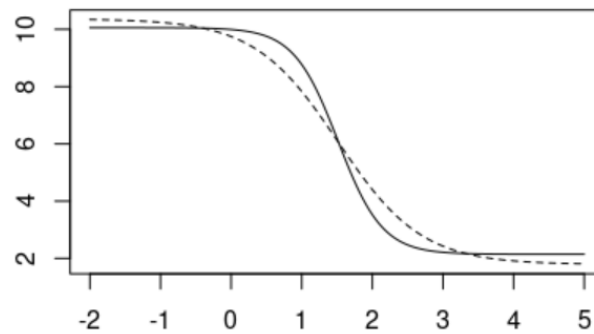
CSI Acceptance Limit

- What is considered practically parallel?
- Look at unconstrained fits vs. CSI with SME
- For example, SME decides $CSI \geq 0.95$

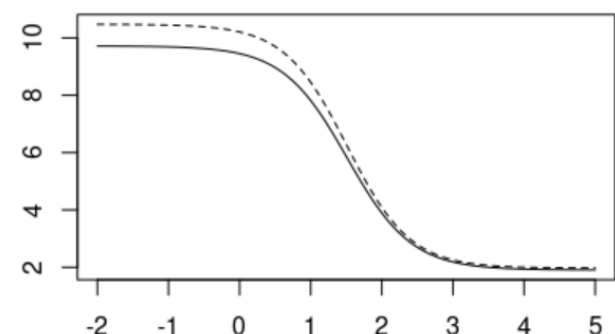
CSI_auc= 95.79



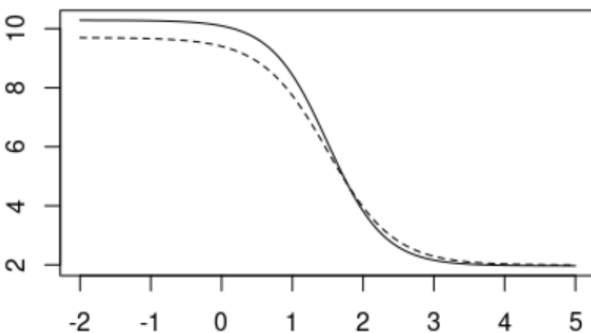
CSI_auc= 94.96



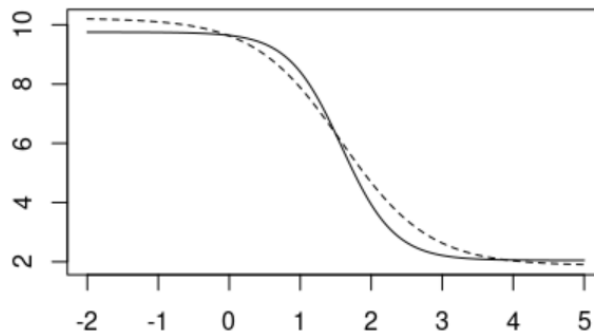
CSI_auc= 95.17



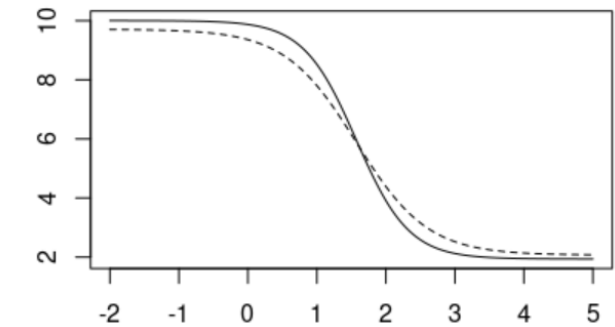
CSI_auc= 95.5



CSI_auc= 95.9

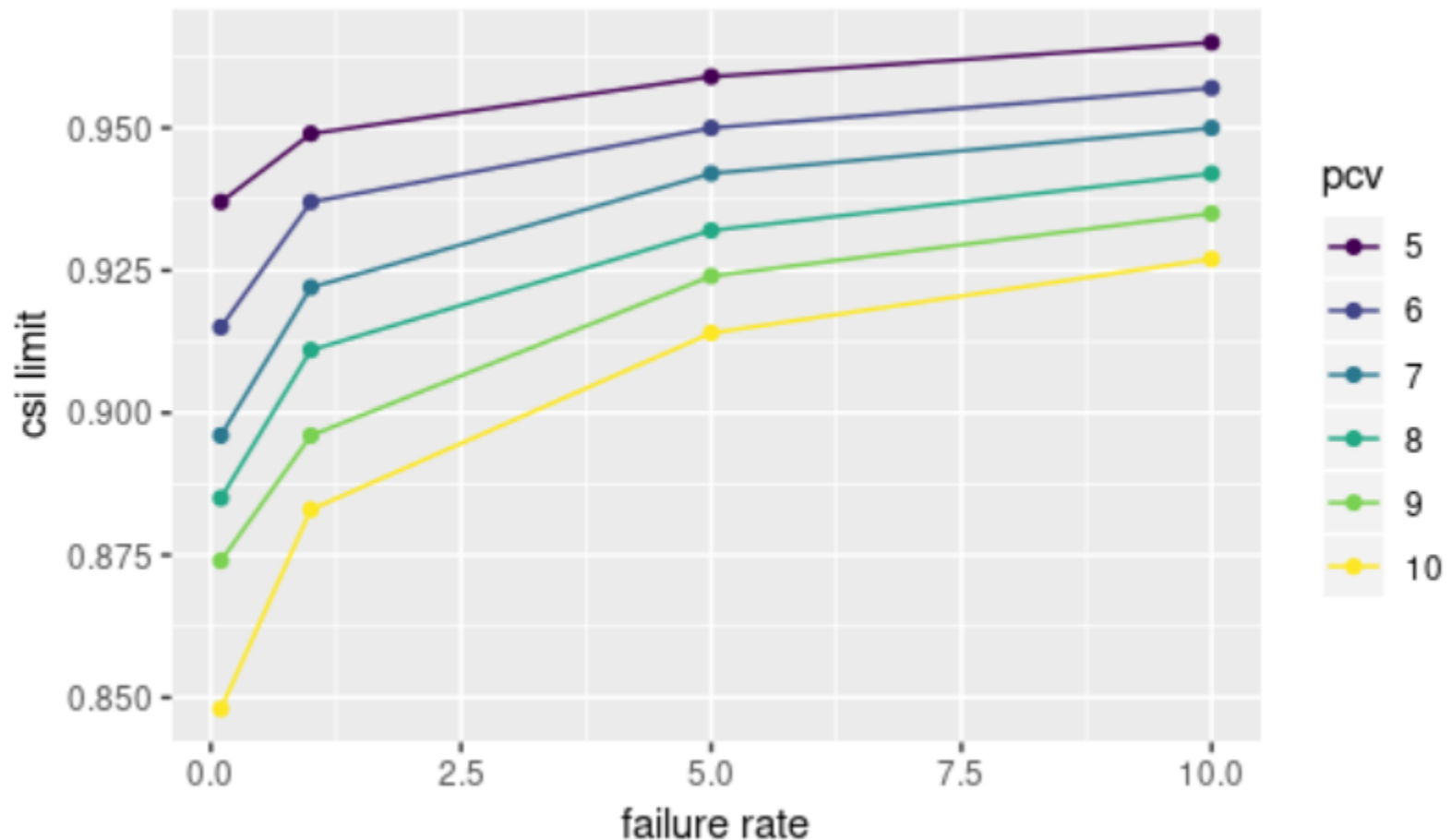


CSI_auc= 94.95



CSI Acceptance Limit

- But a method's ability to meet a $CSI \geq 0.95$ limit depends on its precision (%CV)



System vs. Sample Suitability

1. System Suitability

- Validity of the assay:
 - Standard and control parameters in their usual range
 - Standard and control data are in their usual range (residual variation)
 - Adequacy of model fit
 - Precision

2. Sample Suitability

- Validity of the potency estimate for a TS:
 - Adequacy of model fit for TS
 - Similarity to the Standard
 - Potency within range of the assay system

Sample Suitability

1. Adequacy of model fit for TS

- Could consider:
 - Lack-of-fit sum of squares (USP<1032>)
 - Relative Lack-of-Fit (Li et al., 2017)
 - %CV at each concentration

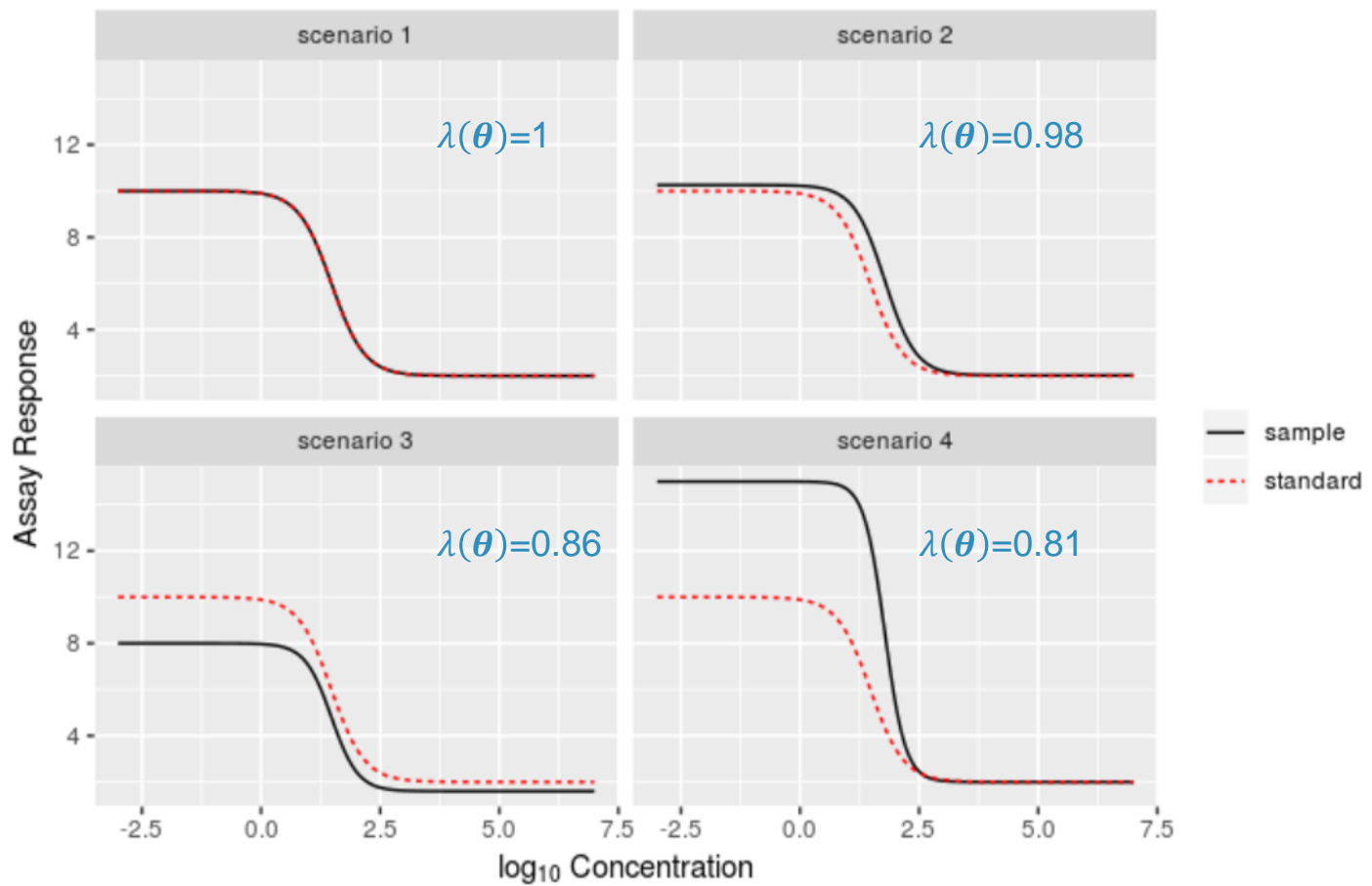
If I am satisfied with the TS model fit and the dose-response curve, then...

2. Similarity to the Standard

- $CSI \geq \delta$
- Comparing the point estimate to the limit (e.g.: 0.95)
- Measure of similarity not dependent on goodness-of-fit

3. Potency within range of the assay system

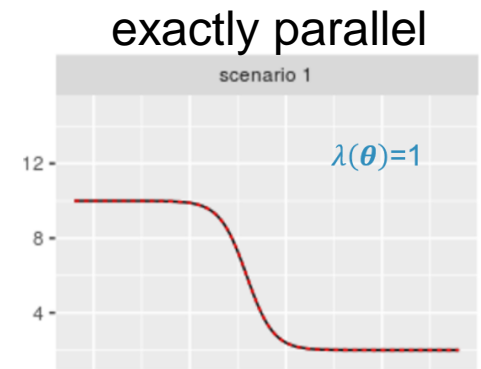
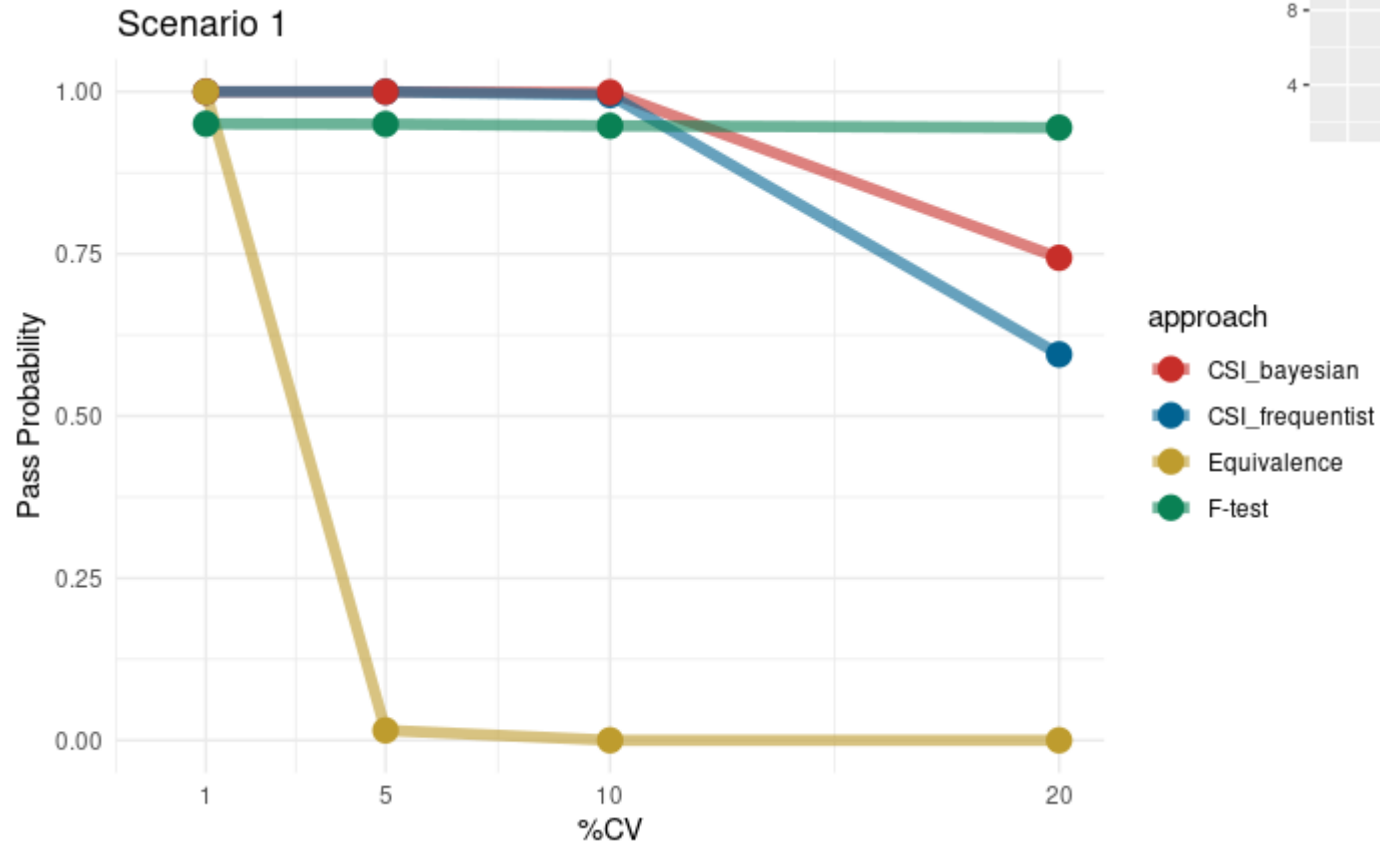
Simulation Study



Simulation Study

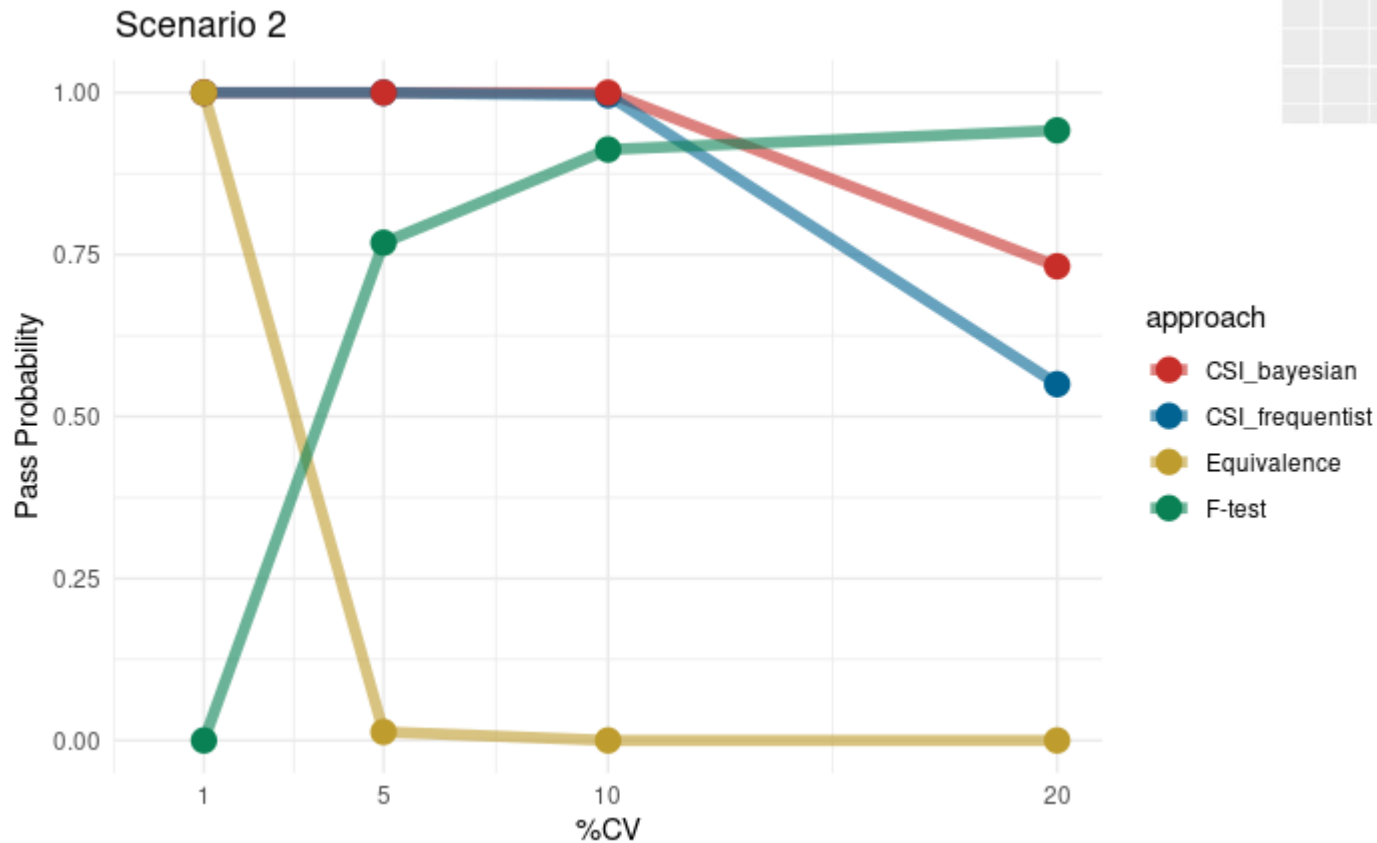
- Simulation conditions based on **hypothesis testing** approach
 - $\delta = 0.84$
 - $\alpha = 0.05$
 - $D_L = 0.8, D_U = 1.25$
 - $\%CV = (1, 5, 10, 20)$

Simulation Results

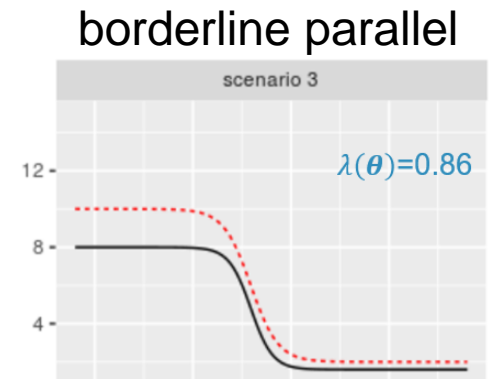
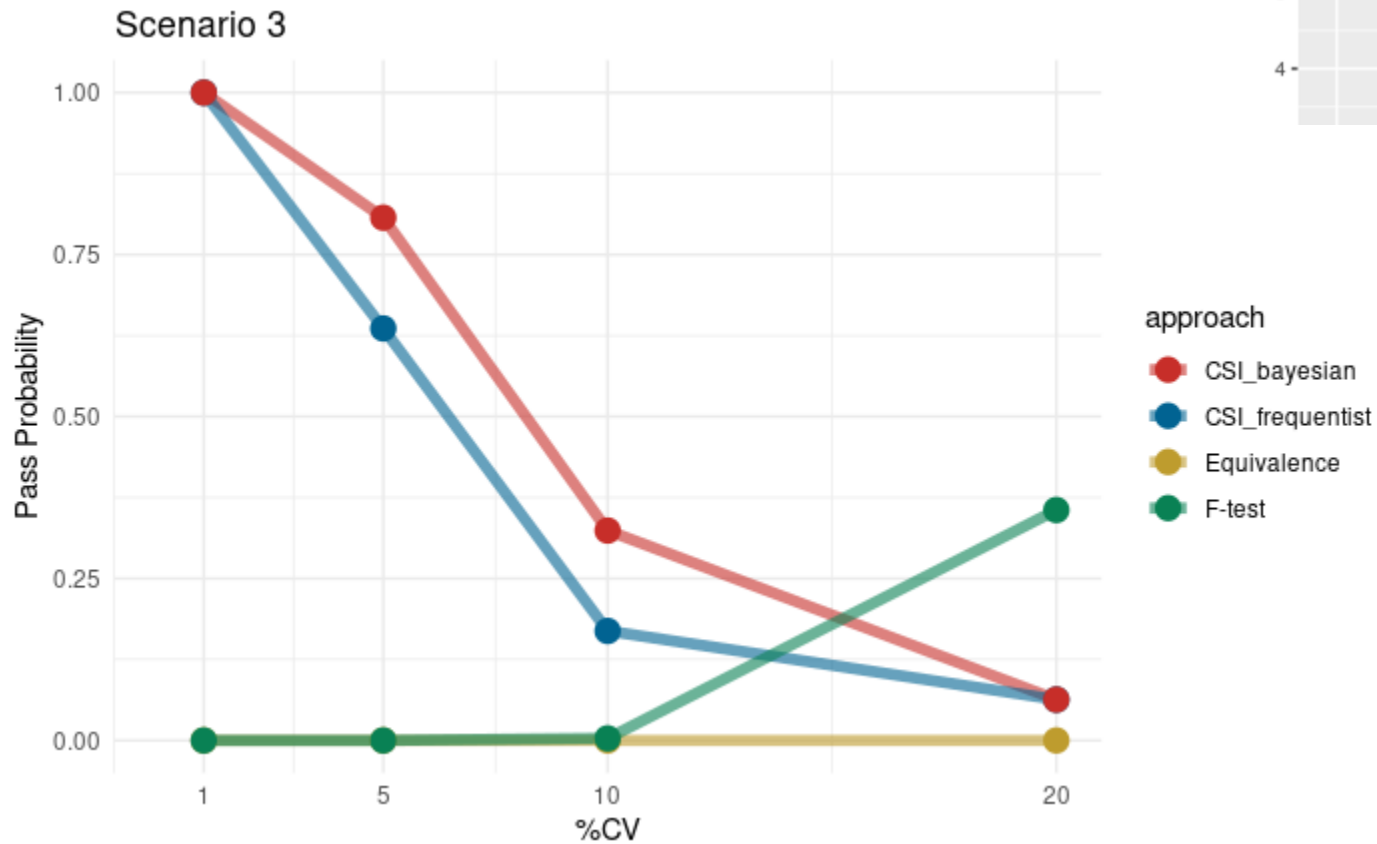


Simulation Results

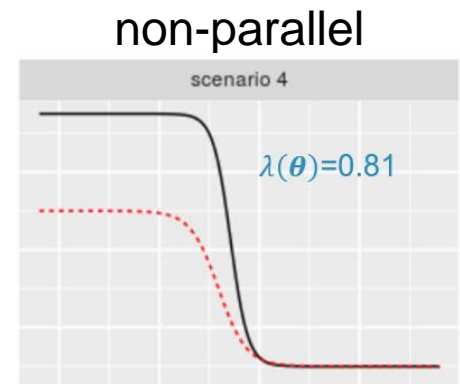
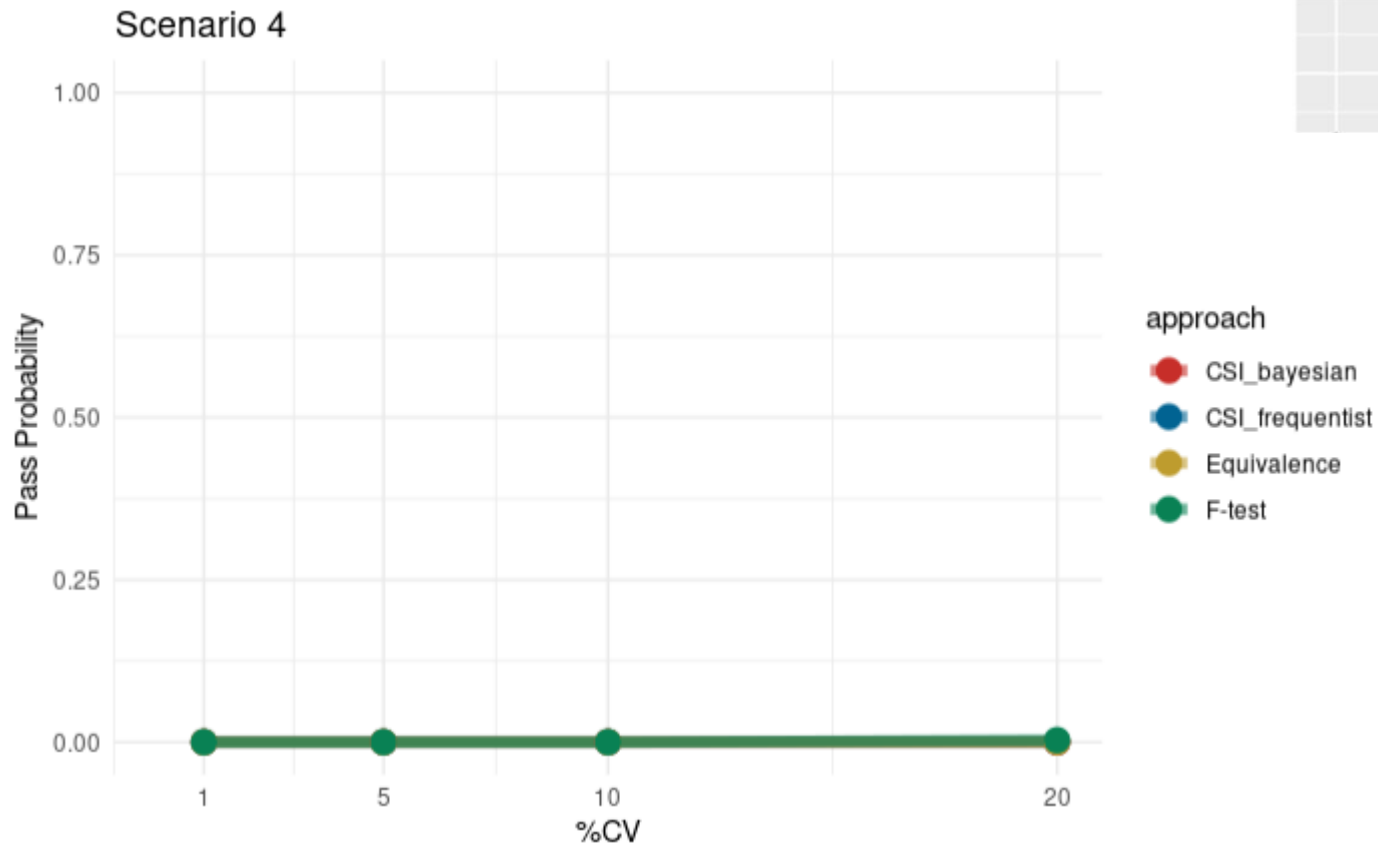
approximately parallel



Simulation Results



Simulation Results



Acknowledgements

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References

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Q&A

Questions?

Additional questions / comments can be sent to:
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