Comparison of Methods Used to Measure Parallelism

- Weighting and Regression Fitting
- Extra Sum of Squares Comparison (Residuals Test)
  - Full Curve and Parallel Line
  - $\chi^2$ Test and F Test
- Confidence Interval Comparison (Equivalence Test)
- Setting the Thresholds (Goalposts)

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Why Weight?

- Weighting the responses with the inverse of their variance is required for all regression fits to produce the Maximum Likelihood Estimate of the underlying curve.
- Allows each point to contribute equally to the fitted curve. Prevents the curve being fitted predominantly to the high response data points.
- Bioassay and immunoassay data are very heteroscedastic, usually 2-4 orders of magnitude, and the variance profiles vary substantially between test methods.

DEF assays

Variance = 0.0849 \times (RLU)^{1.399} \\
R^2 = 0.921

Log(Mean RLU) vs Log(Variance)

ABC assays

Variance = 0.00426 \times (FL)^{2.153} \\
R^2 = 0.934

Log(Mean FL) vs Log(Variance)

CDE assays

Variance = 10^{[-69.6 + 45.2 \times \text{Log}(FL) - 6.85 \times (\text{Log}(FL)^2) + 243.6]} \\
R^2 = 0.972

Log(Mean FL) vs Log(Variance)
Determining Variance Model

- The underlying variance profile of the responses for a test method can be determined with pooled assays. The pooled assays will also incorporate the variation in precision observed between assays. The number of assays needed for a reliable estimate of the variance profile is dependent upon the precision of the responses.

  - **Dilution Replicates.** The replicate variance of each dilution is obtained from pooled assays using the within-assay mean sum of squares from ANOVA, and these variances are fit to the variance model.

  - **Curve Residuals.** The variance model is fit from the residuals of the individual dilution curves simultaneously from the pooled assays.

- The pooled response variances are then fit using an appropriate model equation.
DEF Standard Curve

DEF-73 (Current Assay) - 5 Parameter Logistic Curve

Concentration (ng/mL)
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DEF Variance Model

Variance = 0.0849 * (RLU)^1.399

R^2 = 0.921
Curve Fitting

Least Squares Regression:
- Weight individual points
- Compute the curve fit regression parameters to minimize the residual sum of squared errors (RSSE)

Linear Regression:
- One-step least squares algorithm
- Gives exact solution for the regression parameters

Nonlinear Curve Fitting:
- Numeric iterative process to find the global minimum – a unique set of regression parameters that results in the lowest RSSE
- Different minimization algorithms may give different solutions (sets of regression parameters), so that the obtained solution estimate often depends on the algorithms used
Curve Fit Metrics

Expected Variance :
\[ \text{expected variance} = A \ast \text{resp}^B \]

Weighting :
\[ w_i = 1/(\text{expected variance})_i \]

Weighted Residual Sum of Squared Errors :
\[ \text{RSSE} = \sum_{i=1}^{N} w_i \left( y_i - \hat{y}_i \right)^2 \]

Fit Probability :
\[ \chi^2\text{Prob} = \chi^2\text{Dist} \left( \text{RSSE}, N - P \right) \]

- \(N\) : number of data points
- \(P\) : number of parameters in the curve fit equation
The logistic model is a mathematical shape function only, its parameters do not correlate with any physical properties of the bioassay or immunoassay reaction.
Asymmetric Curves: 5PL vs 4PL

The 5PL can accurately fit curves with different shaped ends, but the 4PL forces both ends to have the same shape.
Measuring Parallelism

• Measuring parallelism means comparing the shapes of two dose response curves for mathematical similarity of shape.

• To measure parallelism requires a metric that can be used to determine the distance of two sets of data from perfect parallelism.

• If one pair of data sets is considered to be more parallel (“closer” to perfect parallelism) than other pair, the parallelism metric should be smaller for the first pair than for the second pair. When two data sets are perfectly parallel, the metric should be zero.

• A parallelism metric that fits this criterion is the $\text{RSSE}_{\text{nonpar}}$ obtained from the extra-sum-of-squares ANOVA.

• This method is firmly established in the statistical and bioassay literature, and is used in many disciplines and industries to test for similarity of regressions.
Unconstrained and Constrained Regressions

**Unconstrained**

Fit the two curves to separate sets of parameters, and add their residual sum of squared errors (RSSEs).

\[
\text{SSE}_{\text{std}} (\mathbf{p}_{\text{std}}) = \sum_{i=1}^{N_{\text{std}}} w_i^{\text{std}} (y_i^{\text{std}} - f(x_i^{\text{std}}; \mathbf{p}_{\text{std}}))^2
\]

\[
\text{SSE}_{\text{uk}} (\mathbf{p}_{\text{uk}}) = \sum_{i=1}^{N_{\text{uk}}} w_i^{\text{uk}} (y_i^{\text{uk}} - f(x_i^{\text{uk}}; \mathbf{p}_{\text{uk}}))^2
\]

**RSSE_{unconst} = \min_{\mathbf{p}_{\text{std}}, \mathbf{p}_{\text{uk}}} \text{SSE}_{\text{unconst}} (\mathbf{p}_{\text{std}}, \mathbf{p}_{\text{uk}})**

**Constrained**

Fit the two curves to the same set of parameters (with an additional parameter \(r\) for the unknown relative potency) and add the RSSEs.

\[
\text{SSE}_{\text{std}} (\mathbf{p}) = \sum_{i=1}^{N_{\text{std}}} w_i^{\text{std}} (y_i^{\text{std}} - f(x_i^{\text{std}}; \mathbf{p}))^2
\]

\[
\text{SSE}_{\text{uk}} (r, \mathbf{p}) = \sum_{i=1}^{N_{\text{uk}}} w_i^{\text{uk}} (y_i^{\text{uk}} - f(rx_i^{\text{uk}}; \mathbf{p}))^2
\]

**RSSE_{const} = \min_{r, \mathbf{p}} \text{SSE}_{\text{const}} (r, \mathbf{p})**
A Direct Measure of Parallelism

\[ \text{RSSE}_{\text{nonpar}} = \text{RSSE}_{\text{const}} - \text{RSSE}_{\text{unconst}} \]

- The \( \text{RSSE}_{\text{nonpar}} \) is an actual measure of parallelism. It is not just a simple “Yes” / “No” result.
- The \( \text{RSSE}_{\text{nonpar}} \) is zero whenever the two curves are perfectly parallel.
- The \( \text{RSSE}_{\text{nonpar}} \) increases as the two curves become less parallel.
- When the data is accurately weighted, the \( \text{RSSE}_{\text{nonpar}} \) is distributed as a chi-square variable with \( P-1 \) degrees of freedom.
- Because the \( \text{RSSE}_{\text{nonpar}} \) (\( \chi^2 \) statistic) and \( \chi^2 \) probability measures nonparallelism directly, historical data can be used to determine empirically a threshold of acceptable nonparallelism.
Another extra-sum-of-squares method used to determine parallelism for linear and nonlinear curves is the F Test.

\[
\text{F Statistic} = \frac{\text{RSSE}_{\text{nonpar}}}{\text{df}_{\text{nonpar}}} = \frac{\text{RSSE}_{\text{const}} - \text{RSSE}_{\text{unconst}}}{\text{df}_{\text{const}} - \text{df}_{\text{unconst}}}
\]

- The F Statistic and F Probability are distorted by the fit of the unconstrained model, and do not measure the amount of nonparallelism directly.

- This method is often used when correct weighting is not available, because it does not absolutely require correct weighting, since the errors in the $\text{RSSE}_{\text{nonpar}}$ due to incorrect weighting are compensated by the errors in the $\text{RSSE}_{\text{unconst}}$. 

Equivalence Test

- Two curves were fit using the formula:

\[
Ln(\text{Response}_i) = (D - S_i \times dD) + \frac{(A - S_i \times dA) - (D - S_i \times dD)}{1 + \text{Exp}((B - S_i \times dB) \times (\text{LC} - S_i \times dLC - \text{Ln}((\text{Concentration}_i)))}.
\]

- \{S_i, \text{Concentration}_i, \text{Response}_i\} are combined data sets (Std and Uk)
- \(S_i = 1\) for Std data points and 0 for Uk data points
- A, D, B and LC are the 4PL curve parameters for the Uk curve fit
- \(dA, dD, dB\) and \(dLC\) are the respective parameter differences between the Uk and Std curve fits
Equivalence Confidence Intervals

- The 90% confidence intervals (CI) were computed for the dA, dD and dB parameters using the profile method.

- A pair of dilution curves are considered non-parallel if the 90% CI for any of the dA, dD or dB parameters extends outside their respective CI thresholds (goalposts).

- The confidence interval goalposts for parameters:
  - 1.5-fold difference for the upper response asymptote: Cutoff = \( \ln(1.5) = 0.405 \)
  - 2-fold difference for the lower response asymptote: Cutoff = \( \ln(2) = 0.693 \)
  - The confidence interval goalpost for parameter dB: Cutoff = 0.3

<table>
<thead>
<tr>
<th>Assays</th>
<th>Curve Slope</th>
<th>dA Goalposts</th>
<th>dD Goalposts</th>
<th>dB Goalposts</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABB-#, ABD-#</td>
<td>Descending (A &lt; D)</td>
<td>-0.693</td>
<td>0.693</td>
<td>-0.405</td>
</tr>
<tr>
<td>RN3-#</td>
<td>Ascending (A &gt; D)</td>
<td>-0.405</td>
<td>0.405</td>
<td>-0.693</td>
</tr>
</tbody>
</table>
ABD-12: Uk-3 (150% Potency Control)

5PL, $\chi^2$ Test

Relative Potency $= 1.599$

Fit Probs:
- Std: 0.941
- Uk-3: 0.978

Parallelism:
- $X^2$ Stat $= 5.297$
- $X^2$ Prob $= 0.258$

RSSE$_{\text{unconst}}$

RSSE$_{\text{nonpar}}$

Unconstrained

Constrained
ABD-12: Uk-3 (Lin Regr, F Test)

Relative Potency = 1.675

<table>
<thead>
<tr>
<th>Test of Regression (F-Test)</th>
<th>( F_{\text{Regression}} )</th>
<th>276.705</th>
<th>( F_{\text{Fictile}} (98.0%) )</th>
<th>7.188</th>
</tr>
</thead>
<tbody>
<tr>
<td>This test passes if ( F_{\text{Regression}} &gt; F_{\text{Fictile}} )</td>
<td>Test Passed!</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test of Linearity (F-Test)</th>
<th>( F_{\text{Non-linearity}} )</th>
<th>3.341</th>
<th>( F_{\text{Fictile}} (98.0%) )</th>
<th>5.516</th>
</tr>
</thead>
<tbody>
<tr>
<td>This test passes if ( F_{\text{Non-linearity}} &lt; F_{\text{Fictile}} )</td>
<td>Test Passed!</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test of Parallelism (F-Test)</th>
<th>( F_{\text{Non-parallelism}} )</th>
<th>0.388</th>
<th>( F_{\text{Fictile}} (99.0%) )</th>
<th>9.330</th>
</tr>
</thead>
<tbody>
<tr>
<td>This test passes if ( F_{\text{Non-parallelism}} &lt; F_{\text{Fictile}} )</td>
<td>Test Passed!</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## ABD-12: Uk-3 (4PL, Equivalence)

### Parameter Estimates and Confidence Intervals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>ApproxStdErr</th>
<th>Lower CL</th>
<th>Upper CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>6.4903631792</td>
<td>0.01692638</td>
<td>6.45827398</td>
<td>6.52082463</td>
</tr>
<tr>
<td>d</td>
<td>9.1824336538</td>
<td>0.01123147</td>
<td>9.16196489</td>
<td>9.20360486</td>
</tr>
<tr>
<td>b</td>
<td>1.9816224621</td>
<td>0.03971287</td>
<td>1.90934852</td>
<td>2.05557032</td>
</tr>
<tr>
<td>lc</td>
<td>2.3459474498</td>
<td>0.01005577</td>
<td>2.32740285</td>
<td>2.36477893</td>
</tr>
<tr>
<td>da</td>
<td>0.0632295981</td>
<td>0.03247974</td>
<td>0.00415074</td>
<td>0.1249726</td>
</tr>
<tr>
<td>dd</td>
<td>-0.057832479</td>
<td>0.01450578</td>
<td>-0.0847167</td>
<td>-0.0307303</td>
</tr>
<tr>
<td>db</td>
<td>0.001877459</td>
<td>0.05851344</td>
<td>-0.1069601</td>
<td>0.11046596</td>
</tr>
<tr>
<td>dlc</td>
<td>-0.448336224</td>
<td>0.01664522</td>
<td>-0.4796278</td>
<td>-0.4178319</td>
</tr>
</tbody>
</table>

**Notes:**
- da passed
- dd passed
- db passed
- dlc passed
ABD-21: Uk-1 (100% Potency Control)

**5PL, $\chi^2$ Test**

- **RSSE**\textsubscript{unconst}:
  - Std: 0.981
  - Uk-1: 0.904
  - Relative Potency = 1.040
- **RSSE**\textsubscript{nonpar}:
  - Parallelism: $X^2$ Stat = 5.207
  - $X^2$ Prob = 0.267

Fit Probs:
- Std: 0.981
- Uk-1: 0.904
ABD-21: Uk-1 (Lin Regr, F Test)

Relative Potency = 1.058

<table>
<thead>
<tr>
<th>Test of Regression (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{regression}}$</td>
<td>573.807</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{critical}}$ (98.0%)</td>
<td>7.188</td>
<td>Test Passed!</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test of Linearity (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{non-linearity}}$</td>
<td>1.074</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{critical}}$ (98.0%)</td>
<td>5.516</td>
<td>Test Passed!</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test of Parallelism (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{non-parallelism}}$</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>$F_{\text{critical}}$ (99.0%)</td>
<td>9.330</td>
<td>Test Passed!</td>
</tr>
</tbody>
</table>
### ABD-21: Uk-1 (4PL, Equivalence)

![Graph showing confidence intervals for parameters db, da, and dd with passed and failed results.]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>ApproxStdErr</th>
<th>Lower CL</th>
<th>Upper CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>6.3616209242</td>
<td>0.07789988</td>
<td>6.20206382</td>
<td>6.49175876</td>
</tr>
<tr>
<td>d</td>
<td>9.431374732</td>
<td>0.02008377</td>
<td>9.39548995</td>
<td>9.46949016</td>
</tr>
<tr>
<td>b</td>
<td>1.9093056416</td>
<td>0.09148277</td>
<td>1.74609833</td>
<td>2.07947877</td>
</tr>
<tr>
<td>lc</td>
<td>2.9263585835</td>
<td>0.0334876</td>
<td>2.86869134</td>
<td>2.99393084</td>
</tr>
<tr>
<td>da (passed)</td>
<td>-0.20376594</td>
<td>0.09440572</td>
<td>-0.3874071</td>
<td>-0.0353282</td>
</tr>
<tr>
<td>dd (passed)</td>
<td>0.0602486677</td>
<td>0.02612591</td>
<td>0.01195331</td>
<td>0.10932652</td>
</tr>
<tr>
<td>db (failed)</td>
<td>-0.453136519</td>
<td>0.13966805</td>
<td>-0.7162445</td>
<td>-0.1948245</td>
</tr>
<tr>
<td>dlc</td>
<td>0.0386188721</td>
<td>0.04086976</td>
<td>-0.0346532</td>
<td>0.11722683</td>
</tr>
</tbody>
</table>

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ABD-21: Uk-2 (Isoform Impurity)

5PL, $\chi^2$ Test

RSSE$_{\text{unconst}}$

Unconstrained

Constrained

Fit Probs:
Std: 0.981
Uk-2: 0.768

Relative Potency = 1.356

Parallelism:
$X^2$ Stat = 46.898
$X^2$ Prob = <0.0001
**ABD-21: Uk-2 (Lin Regr, F Test)**

Relative Potency = 1.545

---

### Test of Regression (F-Test)

<table>
<thead>
<tr>
<th></th>
<th>F_{Regression}</th>
<th>F_{Critical (98.0%)}</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>348.951</td>
<td>7.188</td>
<td>This test passes if $F_{Regression} &gt; F_{Critical}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Test Passed!</strong></td>
</tr>
</tbody>
</table>

### Test of Linearity (F-Test)

<table>
<thead>
<tr>
<th></th>
<th>F_{Non-linearity}</th>
<th>F_{Critical (98.0%)}</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.485</td>
<td>5.516</td>
<td>This test passes if $F_{Non-linearity} &lt; F_{Critical}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Test Passed!</strong></td>
</tr>
</tbody>
</table>

### Test of Parallelism (F-Test)

<table>
<thead>
<tr>
<th></th>
<th>F_{Non-parallelism}</th>
<th>F_{Critical (99.0%)}</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.542</td>
<td>9.330</td>
<td>This test passes if $F_{Non-parallelism} &lt; F_{Critical}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Test Passed!</strong></td>
</tr>
</tbody>
</table>
ABD-21: Uk-2 (4PL, Equivalence)

### Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>ApproxStdErr</th>
<th>Lower CL</th>
<th>Upper CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>6.3829542743</td>
<td>0.07983509</td>
<td>6.21733766</td>
<td>6.51427418</td>
</tr>
<tr>
<td>d</td>
<td>9.2674048106</td>
<td>0.02619901</td>
<td>9.2203663</td>
<td>9.31886673</td>
</tr>
<tr>
<td>b</td>
<td>1.8610164827</td>
<td>0.1091648</td>
<td>1.66400789</td>
<td>2.06818246</td>
</tr>
<tr>
<td>lc</td>
<td>2.7833465469</td>
<td>0.038094</td>
<td>2.71858317</td>
<td>2.85959562</td>
</tr>
<tr>
<td>da</td>
<td>-0.182434303</td>
<td>0.10239092</td>
<td>-0.3799487</td>
<td>0.002909</td>
</tr>
<tr>
<td>dd</td>
<td>-0.103720706</td>
<td>0.03301365</td>
<td>-0.1648954</td>
<td>-0.0404282</td>
</tr>
<tr>
<td>db</td>
<td>-0.501430687</td>
<td>0.16737389</td>
<td>-0.8194687</td>
<td>-0.1901856</td>
</tr>
<tr>
<td>dlc</td>
<td>-0.104392766</td>
<td>0.0473757</td>
<td>-0.1892034</td>
<td>-0.0143556</td>
</tr>
</tbody>
</table>
ABB-181: Uk-1 (100% Potency Control)

5PL, $\chi^2$ Test

Parallelism: $X^2$ Stat = 2.831
$X^2$ Prob = 0.587

Relative Potency = 1.065
ABB-181: Uk-1 (Lin Regr, F Test)

Relative Potency = 1.076

<table>
<thead>
<tr>
<th>Test of Regression (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| $F_{\text{Regression}}$     | 1681.933 | This test passes if $F_{\text{Regression}} > F_{\text{critical}}$
| $F_{\text{critical}}$ (98.0%) | 6.674 |

<table>
<thead>
<tr>
<th>Test of Linearity (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| $F_{\text{Non-linearity}}$ | 2.016 | This test passes if $F_{\text{Non-linearity}} < F_{\text{critical}}$
| $F_{\text{critical}}$ (98.0%) | 3.974 |

<table>
<thead>
<tr>
<th>Test of Parallelism (F-Test)</th>
<th></th>
<th></th>
</tr>
</thead>
</table>
| $F_{\text{Non-parallelism}}$ | 0.434 | This test passes if $F_{\text{Non-parallelism}} < F_{\text{critical}}$
| $F_{\text{critical}}$ (99.0%) | 8.531 |
### ABB-181: Uk-1 (4PL, Equivalence)

#### Parameter Estimates and Confidence Intervals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>ApproxStdErr</th>
<th>Lower CL</th>
<th>Upper CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>10.187898132</td>
<td>0.06616825</td>
<td>10.0641854</td>
<td>10.2987088</td>
</tr>
<tr>
<td>d</td>
<td>14.242125652</td>
<td>0.03081444</td>
<td>14.1883799</td>
<td>14.2978492</td>
</tr>
<tr>
<td>b</td>
<td>1.512838565</td>
<td>0.07370185</td>
<td>1.38826975</td>
<td>1.64685506</td>
</tr>
<tr>
<td>lc</td>
<td>2.8766543623</td>
<td>0.03624092</td>
<td>2.81370132</td>
<td>2.94292246</td>
</tr>
<tr>
<td>da</td>
<td>0.2868585482</td>
<td>0.1038622</td>
<td><strong>0.10709987</strong></td>
<td><strong>0.47146349</strong></td>
</tr>
<tr>
<td>dd</td>
<td>-0.014942036</td>
<td>0.04365775</td>
<td><strong>-0.092181</strong></td>
<td><strong>0.06230548</strong></td>
</tr>
<tr>
<td>db</td>
<td>0.0787050764</td>
<td>0.10094856</td>
<td><strong>-0.095309</strong></td>
<td><strong>0.25450923</strong></td>
</tr>
<tr>
<td>dlc</td>
<td>-0.171603728</td>
<td>0.05341827</td>
<td>-0.2667786</td>
<td>-0.0774443</td>
</tr>
</tbody>
</table>

* da and dd passed.*
ABB-181: Uk-5 (UV Degraded)

5PL, $\chi^2$ Test

RSSE$_{unconst}$

RSSE$_{nonpar}$

Parallelism: $X^2$ Stat = 77.782
$X^2$ Prob = <0.0001

Relative Potency = 1.612

Fit Probs: Std: 0.675
Uk-5: 0.999

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ABB-181: Uk-5 (Lin Regr, F Test)

**Test of Regression (F-Test)**

<table>
<thead>
<tr>
<th>Regression</th>
<th>F (98.0%)</th>
<th>1290.988</th>
<th>This test passes if F_{Regression} &gt; F_{critical} Test Passed!</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{critical}</td>
<td>6.974</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test of Linearity (F-Test)**

<table>
<thead>
<tr>
<th>F_{non-linearity}</th>
<th>F (98.0%)</th>
<th>2.225</th>
<th>This test passes if F_{non-linearity} &lt; F_{critical} Test Passed!</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{critical}</td>
<td>3.974</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test of Parallelism (F-Test)**

<table>
<thead>
<tr>
<th>F_{non-parallelism}</th>
<th>F (99.0%)</th>
<th>55.098</th>
<th>This test passes if F_{non-parallelism} &lt; F_{critical} Test Failed!</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_{critical}</td>
<td>8.531</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Relative Potency = 2.134
ABB-181: Uk-5 (4PL, Equivalence)

Parameter | Estimate | ApproxStdErr | Lower CL | Upper CL
--- | --- | --- | --- | ---
a | 9.6335499197 | 0.08370461 | 9.47557182 | 9.77084277
b | 14.01878692 | 0.03646215 | 13.9553129 | 14.0858123
lc | 1.3275330705 | 0.06791094 | 1.21169067 | 1.45103465
lca | 2.8763464654 | 0.0432597 | 2.80268029 | 2.95554505
\textbf{da passed} | -0.267495092 | 0.11931236 | -0.4770097 | -0.0584199
\textbf{dd passed} | -0.238279408 | 0.04907462 | -0.325001 | -0.1505584
\textbf{db passed} | -0.106606034 | 0.09989685 | -0.2800848 | 0.06616517
dl | -0.171909706 | 0.060071 | -0.2778009 | -0.0657556
RN3-18: Uk-1 (50% Potency Control)

5PL, $\chi^2$ Test

Unconstrained

RSSE_{unconst}

Constrained

RSSE_{nonpar}

Relative Potency = 0.555

Fit Probs:
- Std: 0.874
- Uk-1: 0.131

Parallelism:
- $X^2$ Stat = 0.976
- $X^2$ Prob = 0.913
Relative Potency = 0.565
### RN3-18: Uk-1 (4PL, Equivalence)

#### Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>ApproxStdErr</th>
<th>Lower CL</th>
<th>Upper CL</th>
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<tbody>
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#### Confidence Interval

- **db**: Failed
- **dd**: Failed
- **da**: Failed
- **dlc**: Passed
RN3-18: Uk-3 (Vaccine B)

5PL, $\chi^2$ Test

RSSE_{unconst}

RSSE_{nonpar}

Parallelism: $X^2$ Stat = 0.905

$X^2$ Prob = 0.923

Relative Potency = 60.8
## Relative Potency = 61.9

### Test of Regression (F-Test)

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This test passes if $F_{\text{Regression}} > F_{\text{critical}}$

**Test Passed!**

### Test of Linearity (F-Test)

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This test passes if $F_{\text{Linearity}} < F_{\text{critical}}$

**Test Failed!**

### Test of Parallelism (F-Test)

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This test passes if $F_{\text{Parallelism}} < F_{\text{critical}}$

**Test Passed!**
## RN3-18: Uk-3 (4PL, Equivalence)

### Confidence Interval

![Confidence Interval Graph](image)

<table>
<thead>
<tr>
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<th>Estimate</th>
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<th>Lower CL</th>
<th>Upper CL</th>
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## Parallelism Analysis: Lin Regr, F Test

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## Parallelism Analysis: 4PL, Equivalence

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<td>-∞</td>
<td>+∞</td>
<td>0.405</td>
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</tr>
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<td>RN3-10</td>
<td>Uk-3</td>
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<td>+∞</td>
<td>0.405</td>
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</tr>
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## Parallelism Analysis Results Comparison

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample</th>
<th>Material Tested</th>
<th>Parallelism Test Outcome</th>
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<tr>
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<td>Expected Result</td>
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<tr>
<td>ABD-12 Receptor-ligand binding inhibition assay</td>
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<td>Reference standard</td>
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<td>ABD-21 Receptor-ligand binding inhibition assay</td>
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<td>ABD-75 Receptor-ligand binding inhibition assay</td>
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</tr>
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<td>Uk-3</td>
<td>Structural isoform impurity</td>
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<td>ABB-150 Cell proliferation assay</td>
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<td>ABB-181 Cell proliferation assay</td>
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<td>RN3-18 Vaccine antigen assay</td>
<td>Uk-1</td>
<td>Reference standard</td>
<td>Pass</td>
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</tr>
<tr>
<td></td>
<td>Uk-3</td>
<td>Accepted vaccine batch</td>
<td>Pass</td>
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</tbody>
</table>
Parallel Line and F Test

- **Linear Regression for Nonlinear Curves**
  - Most bioassays do not generate a linear dose response curve.
  - Fails to detect dissimilar behavior appearing only at high or low concentrations.
  - Linear regions of both curves must bridge same response range.
  - Many curves do not have a "linear" region.

- **F Test**
  - Very good regression fits cause parallel curves to fail parallelism.
  - Poor regression fits cause dissimilar samples to pass parallelism.
  - Increasing the degrees of freedom (data points) brings the F probability closer to the $\chi^2$ probability.
Computing Nonlinear Confidence Regions

• Nonlinear curve parameters are coupled, and require computing the full joint multidimensional non-linear confidence regions...a difficult problem!

“In the linear model case [confidence contours]...consist of concentric ellipses. When the model is nonlinear, the contours are sometimes banana-shaped, often elongated. Sometimes the contours stretch to infinity and do not even close, or they may have multiple loops surrounding a number of stationary values.”


“This discussion has focused on 2 parameter models, but nonlinear models with many more parameters occur and, unfortunately, standard contouring methods are not easily extended beyond $P = 2$.”


• Methods which depend on computation of confidence regions of nonlinear curves are unworkable.

“The usual tests appropriate in the linear model case are, in general, *not* appropriate when the model is nonlinear.”


“So far we have assumed that linear approximations provide adequate summaries of the inferential results of nonlinear analysis. Unfortunately, in many nonlinear analyses they will be woefully inadequate.”


Example of 2 parameter non-linear confidence region for Biochemical Oxygen Demand (BOD) equation.

Inaccurate Confidence Region Bounds

Projections (in 2D) of the true 95% confidence regions of 2 parameter pairs of a (4D) 4PL curve. 95% of possible curve fits (black dots) of a symmetrical dose-response curve are enclosed.

Separating parameters from nonlinear curves and then examining individual parameter confidence intervals independently result in large errors in parallelism determinations. (Note difference between the true 95% region and the rectangular approximation obtained by separating the parameters.)
The RSSE\textsubscript{nonpar} ($\chi^2$ statistic/probability) is a direct measure of the amount of nonparallelism between the two curves.

- Parallelism thresholds can be established empirically.
- Parallelism thresholds can be made lenient or strict, depending upon the needs and cost/benefit determinations.
- Parallelism thresholds are universal for all bioassays.
Different Bioassay Behavior...

\[ \text{DEF} \]
\[ \text{Variance} = 0.0849 \times (\text{RLU})^{1.399} \]
\[ R^2 = 0.921 \]

\[ \text{ABC} \]
\[ \text{Variance} = 0.00426 \times (\text{FL})^{2.153} \]
\[ R^2 = 0.934 \]

\[ \text{EEG} \]
\[ \text{Variance} = 6.272 \times (\text{FL})^{1.511} \]
\[ R^2 = 0.889 \]

\[ \text{CDE} \]
\[ \text{Variance} = 10^{(69.6 + 45.2 \times \log(\text{FL}) - 6.85 \times (\log(\text{FL}))^2)} + 243.6 \]
\[ R^2 = 0.972 \]

\[ \text{VUT} \]
\[ \text{Variance} = 10^{(27.1 + 18.0 \times \log(\text{FL}) - 2.63 \times (\log(\text{FL}))^2)} + 22.7 \]
\[ R^2 = 0.998 \]
...Same RSSE_{nonpar} ($\chi^2$ Statistic) Behavior

DEF (203 curve pairs)

ABC (100 curve pairs)

EEG (131 curve pairs)

CDE (115 curve pairs)

VUT (211 curve pairs)

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F Test Parallelism Thresholds

- The F probability (F statistic) does not correlate with the amount of nonparallelism between the samples.
  - The F Statistic and F Probability are distorted by the fit of the unconstrained model.
  - Thresholds cannot be established empirically.
Equivalence Goalposts

- Curve coefficient confidence intervals do not directly correlate with the amount of nonparallelism between the samples.

  - Establishing goalposts is always subjective, even when derived from historical data. There are no scientifically established methods to compute the goalposts.

  - Goalposts must be established separately for each parameter of each bioassay from a historical pool of assays comparing similar samples.

  - There are multiple options available for choosing each parameter’s goalposts, and each goalpost can be chosen independently from the other goalposts.
Summary

• The extra sum of squares (residuals) method for determining the similarity of regressions is firmly established in the mathematical and bioassay literature, and is widely used in many disciplines and industries.

• This classical statistical approach should be included in the USP <111> chapter as it is in the European Pharmacopeia 5.3.

• Comparing “linear regions” of nonlinear curves can fail to detect substantial dissimilarity between samples. Many bioassays do not have linear regions.

• The lack of computational methods to determine the confidence regions for complex nonlinear curves explains why the equivalence method failed to give the correct answer for 10 of the 20 samples examined.

• When correctly weighted with an adequate regression model, such as the 5PL for asymmetric dose-response curves, the extra sum of squares method yields reliable parallelism metrics, $\text{RSSE}_{\text{nonpar}} (\chi^2 \text{ statistic})$ and $\chi^2 \text{ probability}$, that are accurate measures of the amount of parallelism between two curves, and solves the problems inherent in other approaches.

• The data from this study are available on www.brendan.com.