

Europ. Pharm., 5th Ed. (2005), Ch. 5.3, 5.4.1.a - Four-parameter logistic curve analysis, A serological assay of tetanus sera

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Calculation

Calculation performed: 02.06.2014 12:13:11, Matthias Schmitt (PLA 3.0.0 Build 623, nbmsc03, 10014)
Report generated: 02.06.2014 12:13:19, Matthias Schmitt (PLA 3.0.0 Build 623, nbmsc03, 10014)

Signatures

.....
Responsibility

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Approval

.....
Review



DOCUMENT-52



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Overview

General Properties

| Analysis | General |
|---|-------------------------------|
| Response Adjustment | None |
| Response Transformation | None |
| Model | 4 Parameter Logistic Fit |
| Multiplex Assay | Yes |
| Potency Estimation Confidence Interval | 95.0% |
| Calculate mean potency estimate of test samples | No |
| ANOVA Model | ANOVA (Pure Error Separation) |
| ANOVA with consideration of additional factors | Yes |
| Logarithm Base | Natural logarithm (base e) |
| Invert Potency | No |

Documentation

| General | |
|---------|---------------------|
| Date | 25.02.2014 14:58:22 |

Assay

Setup

Sample Setup

| Setup | Standard Sample: Standard S | Test Sample: Preparation T |
|--------------------------|-----------------------------|----------------------------|
| Preparation Scheme | PreparationScheme-1 | PreparationScheme-1 |
| Step Count | 10 | 10 |
| Replicate Count | 2 | 2 |
| Potency Definition | By Stock Solution | By Stock Solution |
| Assigned Assumed Potency | 0.4 IU/ml | 0.4 IU/ml |
| Dilution Scale | n-Fold Sequence | n-Fold Sequence |
| Factor | 0.1 | 0.1 |
| Base | 2.0 | 2.0 |
| Analysis | Standard Sample: Standard S | Test Sample: Preparation T |
| Data Selection Scheme | DataSelectionScheme-1 | DataSelectionScheme-1 |
| Outlier Detection | None | None |
| Range | Full | Full |
| Optimization | No | No |

Observations

Response values in bold are used for analysis as a result of your configuration.

Standard Sample: Standard S

Selected Steps: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

| Dose Value | 0.10000 | 0.05000 | 0.02500 | 0.01250 | 0.00625 | 0.00313 | 0.00156 | 0.00078 |
|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Dose Step | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Response 1 | 2.912 | 2.579 | 2.13 | 1.651 | 1.073 | 0.585 | 0.463 | 0.266 |
| Response 2 | 2.917 | 2.654 | 2.212 | 1.638 | 0.973 | 0.666 | 0.356 | 0.234 |
| Mean | 2.91450 | 2.61650 | 2.17100 | 1.64450 | 1.02300 | 0.62550 | 0.40950 | 0.25000 |
| SD | 0.00354 | 0.05303 | 0.05798 | 0.00919 | 0.07071 | 0.05728 | 0.07566 | 0.02263 |
| CV[%] | 0.12131 | 2.02687 | 2.67079 | 0.55898 | 6.91209 | 9.15678 | 18.47629 | 9.05097 |

| Dose Value | 0.00039 | 0.00020 |
|------------|--------------|--------------|
| Dose Step | 9 | 10 |
| Response 1 | 0.228 | 0.176 |
| Response 2 | 0.197 | 0.215 |
| Mean | 0.21250 | 0.19550 |
| SD | 0.02192 | 0.02758 |
| CV[%] | 10.31544 | 14.10597 |

Test Sample: Preparation T

Selected Steps: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

| Dose Value | 0.10000 | 0.05000 | 0.02500 | 0.01250 | 0.00625 | 0.00313 | 0.00156 | 0.00078 |
|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Dose Step | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Response 1 | 3.017 | 2.801 | 2.401 | 1.918 | 1.364 | 0.861 | 0.497 | 0.34 |
| 2 | 2.987 | 2.808 | 2.45 | 1.963 | 1.299 | 0.854 | 0.496 | 0.344 |
| Mean | 3.00200 | 2.80450 | 2.42550 | 1.94050 | 1.33150 | 0.85750 | 0.49650 | 0.34200 |
| SD | 0.02121 | 0.00495 | 0.03465 | 0.03182 | 0.04596 | 0.00495 | 0.00071 | 0.00283 |
| CV[%] | 0.70664 | 0.17649 | 1.42850 | 1.63977 | 3.45189 | 0.57723 | 0.14242 | 0.82703 |

| Dose Value | 0.00039 | 0.00020 |
|------------|--------------|--------------|
| Dose Step | 9 | 10 |
| Response 1 | 0.242 | 0.178 |
| 2 | 0.217 | 0.125 |
| Mean | 0.22950 | 0.15150 |
| SD | 0.01768 | 0.03748 |
| CV[%] | 7.70269 | 24.73707 |

Result

Analysis of Variance (ANOVA)

Total number of observations: 40

| Source of Variation | d.f. | Sum of Squares | Mean Squares | F-Ratio | Probability |
|-----------------------|------|----------------|--------------|------------|-------------|
| Treatments | 19 | 42.20601 | 2.22137 | | |
| Preparation | 1 | 0.23058 | 0.23058 | | |
| Regression | 3 | 41.94963 | 13.98321 | 9785.48210 | 7.91391E-32 |
| Non-Parallelism | 3 | 0.00210 | 0.00070 | 0.48912 | 0.69374 |
| Non-Linearity (LoF) | 12 | 0.01068 | 0.00089 | 0.62266 | 0.79912 |
| Residual (Pure) Error | 20 | 0.02858 | 0.00143 | | |
| Total | 39 | 42.23459 | 1.08294 | | |

Regression

| Restricted Model (Common Slope and Asymptotes) | | | |
|--|----------|---------|-----------------------|
| Parameter | Estimate | Error | Quality of Regression |
| Upper Asymptote (A) | 3.19599 | 0.03596 | Iterations 32 |
| Lower Asymptote (D) | 0.14546 | 0.01567 | FTol 1.00000E-10 |
| Slope (B) | 1.12452 | 0.03234 | PTol 1.00000E-10 |
| Standard S log Inflection Point Parameter (C) | -4.30679 | 0.03019 | |
| Preparation T log Inflection Point Parameter (C) | -4.68444 | | |
| Preparation T log Relative Potency Parameter (R) | 0.37765 | 0.02395 | |
| Unrestricted Regression | | | |
| Parameter | Estimate | Error | Quality of Regression |
| Standard S Upper Asymptote (A) | 3.16754 | 0.05629 | Iterations 33 |
| Standard S Slope (B) | 1.15812 | 0.04896 | FTol 1.00000E-10 |
| Standard S Inflection Point (C) | -4.31549 | 0.04261 | PTol 1.00000E-10 |
| Standard S Lower Asymptote (D) | 0.16194 | 0.02050 | |
| Preparation T Upper Asymptote (A) | 3.22068 | 0.04812 | |
| Preparation T Slope (B) | 1.09051 | 0.04420 | |
| Preparation T Inflection Point (C) | -4.68236 | 0.03815 | |
| Preparation T Lower Asymptote (D) | 0.12426 | 0.02454 | |

Validity Tests

Overview:

| | Passed | Failed (Rejected) | Failed (Warning) | Passed (Info) | Not Calculated |
|---------------------|--------|-------------------|------------------|---------------|----------------|
| Assay Suitability | 0 | 0 | 0 | 0 | 0 |
| Sample Suitability | 2 | 0 | 0 | 0 | 0 |
| Overall Test Result | Passed | | | | |

Sample Suitability Tests

| | | |
|--|--------------------|--------|
| F-Test (Hypothesis Test): Significance of Non-Linearity Standard S, Preparation T | | Passed |
| Test Severity | Warning | |
| F _{critical} (95.0%) F | 2.27758 0.62266 | |
| F-Test (Hypothesis Test): Significance of Non-Parallelism Standard S, Preparation T | | Passed |
| Test Severity | Warning | |
| F _{critical} (95.0%) F | 3.09839 0.48912 | |

Potency Estimation

| Relative Potency | Preparation T | Standard S |
|---|--------------------------|---------------|
| Potency Ratio | 1.45886 | |
| 95% Confidence Interval | 1.38777 - 1.53359 | |
| Relative Confidence Interval | 95.13% - 105.12% (9.99%) | |
| Stock Solution | | |
| Assumed/Assigned Potency | 0.40000 IU/ml | 0.40000 IU/ml |
| Factor rel. Estimated Sample Potency | 1.45886 | 1.45886 |
| Estimated Sample Potency (Stock Solution) | 0.58354 IU/ml | |
| 95% Confidence Interval | 0.55511 - 0.61343 IU/ml | |
| Relative Confidence Interval | 95.13% - 105.12% (9.99%) | |

Graphics

