Biologics Development

The Power of Proper System Suitability Tests - A Case Study of cGMP Method Improvement

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Outline

• Problem statement
• Method assessment
• Method improvement: system suitability updates
• Summary
Problem Statement

• A legacy GMP bioassay method suffers from higher than desirable assay and sample failure rate
  – High assay invalid rate, i.e., failed system suitability tests (SST) that applied to reference and/or control sample
  – Additional sample repeats due to similar sample acceptance failures

The method must be assessed and improved
Potential Root Causes for High Assay Failure Rate

- Undesirable assay data quality
  - E.g., due to non-optimal assay design, assay conditions, etc.
- Inappropriate statistical model and/or data analysis
- Operational errors
  - E.g., due to dilution, instrument, analyst training
- Improper system suitability criteria
  - System suitability parameter
    - Not reflective of assay data quality
    - Can not effectively differentiate good vs. bad assays
    - Not robust. E.g., only applicable to a subset of labs / instruments
  - Acceptance range
    - Not based on representative data set and appropriate evaluation
Method Assessment

- Reviewed relevant documents and data to identify potential root cause for high assay failure rate

<table>
<thead>
<tr>
<th>Data / documents reviewed</th>
<th>Observation</th>
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<tbody>
<tr>
<td>Method validation report and long-term method performance trending data</td>
<td>Great accuracy and precision</td>
</tr>
<tr>
<td>Method procedure, assay development / optimization DOE data and analysis</td>
<td>No major concern with assay design and conditions</td>
</tr>
<tr>
<td>Large amount of existing assay outputs, including:</td>
<td>- Reasonable statistical model</td>
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<tr>
<td>- Numerical results (curve fit parameters, SST, potency)</td>
<td>- Acceptable data quality in general (goodness of fit, variability)</td>
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<tr>
<td>- Graphs (dose-response data and fitted curves)</td>
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<tr>
<td>Preliminary review of SST results</td>
<td>- Some SST parameters do not effectively control assay quality</td>
</tr>
<tr>
<td>- Existing system suitability parameters and ranges</td>
<td>- Some critical SST parameters are missing</td>
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<tr>
<td>- Data used to set / justify the SST criteria</td>
<td></td>
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<tr>
<td>- Outputs of failed and passed assays</td>
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- Potential primary root cause: improper SST criteria

**Method improvement plan: thoroughly re-evaluate and re-establish system suitability criteria**
Re-Evaluation of System Suitability Criteria

• Review each existing system suitability parameter and the acceptance range
  – Parameter
    – What is the intended purpose of the parameter?
    – Is the intended purpose directly related to the quality of assay results?
    – Does the parameter provide meaningful assessment for its intended purpose, i.e., effectively differentiate desirable vs. unacceptable assay data?
    – Do all the parameters together provide adequate system suitability assessment?
    – Are there any redundant parameters?
  – Acceptance range
    – How was the range determined?
    – What data set and analysis were used to set / justify the range?
    – Was the data set representative? Was the analysis appropriate?
Re-Establishment of System Suitability Criteria

- Based on the re-evaluation, existing system suitability criteria were added, replaced, removed or retained as appropriate

<table>
<thead>
<tr>
<th>Re-Evaluation Observation</th>
<th>Decision / Action</th>
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<tbody>
<tr>
<td>Critical SST assessments missing</td>
<td>Add new criteria to fill the gaps</td>
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<tr>
<td>Ineffective SST criteria</td>
<td>Replace existing criteria with properly defined new criteria *</td>
</tr>
<tr>
<td>Redundant / non value added criteria</td>
<td>Remove with appropriate justification</td>
</tr>
<tr>
<td>Properly defined SST parameters and ranges</td>
<td>Retain existing criteria</td>
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* An example of SST criteria replacement will be presented in the following slides
Example: Replacement of Lack-of-Fit SST Criterion

- Lack-of-fit (LOF) P-value based on ANOVA F test was used in the legacy method to assess goodness of fit
- LOF P-value was the most contributing criterion to assay failures
- The legacy P-value approach has known limitations
  - Tends to over-sensitively reject precise data with adequate fit and retain noisy data with poor fit
- A new LOF criterion (relative LOF error) was established to replace the legacy P-value criterion to provide more meaningful assessment
Original Parameter: LOF P-Value

- LOF P-value based on F test

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

- Assay fails if the LOF term is statistically significant (small P-value)

Notations
- \( y_{i,j} \): Individual response value
- \( \bar{y}_i \): Local mean of individual response values at given concentration
- \( \hat{y}_i \): Fitted value at given concentration
- \( SS_{LOF} \): Sum of squares of LOF error \( (\bar{y}_i - \hat{y}_i) \)
- \( SS_{PE} \): Sum of squares of pure error \( (y_{i,j} - \bar{y}_i) \)
- \( DF \): Degrees of freedom
Original Parameter: LOF P-Value (cont.)

- **Intended purpose**
  - Assess the adequacy of the dose-response model

- **How does LOF P-value work?**
  - Compare LOF error to pure error (PE)
  - Assay fails if LOF error is too large compared to pure error

- **Limitation of LOF P-value**
  - Tends to penalize precise data (with small PE) and propensity to retain undesirable noisy data (with large PE)

**LOF error**: difference between local mean and fitted value (measures the closeness of the fitted curve to the observed data)

**Pure error**: difference between individual value and local mean (measured the precision of observed data)
New Parameter: Relative LOF Error

- Relative LOF error

\[ \frac{\sqrt{SS_{LOF}/N}}{A_{ref} - D_{ref}} \times 100\% \]

- LOF error normalized against reference curve window (upper asymptote A - lower asymptote D)
- Assay fails if relative LOF error is too large

- A more robust measurement of lack-of-fit
- Independent of pure error and thus overcomes the shortcomings of LOF P-value
- Independent of the magnitude of response readings

Example: Comparison of Original and New LOF Criteria - Representative Assay Plots

LOF P-value: Failed
Relative LOF error: Passed

Acceptable fit, precise data

LOF P-value: Passed
Relative LOF error: Passed

Acceptable fit, less precise data

LOF P-value tends to over-sensitively reject precise assays with acceptable fit
Relative LOF error retains assays with acceptable fit regardless of noise level
Example: Comparison of Original and New LOF Criteria - Representative Assay Plots

LOF P-value could retain noisy data with undesirable fit
Relative LOF error rejects assays with unacceptable fit regardless of noise level
Example: Comparison of Original and New LOF Criteria - Passed vs. Failed Results

**Blue**: Distribution of QC potency recovery results that passed LOF test

**Red**: Individual QC potency recovery results that failed LOF test

(Data source: Method validation)
Outcomes of Method Improvement

The method was significantly improved with updated SST criteria (added, replaced, removed or retained)

- Adequate and more meaningful SST assessment
- Overall assay invalid rate reduced by more than 60%
- Same great accuracy and precision
  – Based on retrospective analysis of historical data
Implementation of the New SST Criteria

- Documentation of SST updates and justification
- Data analysis software updates and re-validation
- Validation amendment
  - Re-assess existing validation data (with updated SST applied) against validation criteria
- Method change control and filing
Summary

• A legacy cGMP bioassay suffered from high assay failure rate
• Improper system suitability tests was identified as primary root cause
• Without any wet lab work, the quality and success rate of the legacy method were significantly improved by implementing state-of-the-art updated system suitability criteria
• The case study clearly illustrated the power of proper system suitability tests
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