Management of Analytical Methods
Life Cycle for Biotechnology Products:
A Regulatory Perspective

Rashmi Rawat, Ph.D.
Product Quality Reviewer
Office of Biotechnology Products
Division of Monoclonal Antibodies
OPS/CDER

WCBP 2012: 16th Symposium on the Interface of Regulatory and Analytical Sciences
for Biotechnology Health Products, Jan. 23-25, 2012, San Francisco, CA
Presentation Outline

- Life cycle of analytical methods
- Analytical development through product life cycle
- Regulatory considerations for change in analytical method
- Case Studies: Change, replacement and transfer of analytical method

Disclaimer

Some of the views expressed during this presentation are my own and may not necessarily reflect the official opinion of FDA
Major Stages in the Method’s Life Cycle

Selection/Development

Qualification

Validation
Selection and Design of Analytical Methods

The selection and design of analytical method should be based on a systematic approach:

- **Intended use of the assay**
  - Release test, stability test, product characterization, in process testing etc.

- **Identify sources of analytical variation**
  - Method variability, risk from analyst, reagents, and instruments

- **Define reportable results**
  - Independent tests, mean of replicates etc.
Rational Assay Development/Qualification

- Investigate and identify the assay’s critical characteristics and parameters.
- Method robustness studies are performed to evaluate its reliability during conditions of use.
- Detailed assay SOP, with system suitability requirements, that incorporates assay’s characteristics and parameters into a clear, concise procedural plan.
- Use this information as a basis from which to develop a scientifically sound validation plan that will demonstrate that the assay does what it is intended to do on a routine basis.
Analytical Method Qualification

• Qualification studies will identify/refine method performance capabilities such as specificity, linearity, accuracy, precision, robustness, stability etc. where applicable.

• Provides a sufficient foundation for the development of a scientifically sound validation protocol.

• Limited pre-determined method performance specifications.

• A method cannot fail qualification; it gets is re-optimized until it achieves acceptable performance or it is rejected for the intended application.
Analytical Method Validation

- Validation trials are run according to an established validation protocol.

- Method performance specifications are pre-established, documented and confirmed during validation trial.

- These specifications must be met by every validation trial.

- A method can fail validation; if it does, assignable cause for the failure must be investigated, resolved and the assay re-validated.
Qualification Vs. Validation

- Assay Qualification:
  **Determining** whether an assay is suitable for its intended purpose
    - Limited pre-determined performance criteria

- Assay Validation:
  **Assuring** the assay is suitable for its intended purpose on a routine basis.
    - Pre-defined assay performance criteria
Why Validate Assay?

21 CFR 211.165

“The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented in accordance with 21 CFR 211.194(a)(2).”

21 CFR 211.194(a)(2)

“[The firm] shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested ….. The suitability of all testing methods used shall be verified under actual conditions of use.”
What Assays Need to be Validated

- **Regulatory (Compendial)**: Procedures used to evaluate a defined characteristic of the drug substance or drug product that are legally recognized under 21 USC 501(b) (USP/NF). Generally, will need no or only partial validation (e.g., need to be verified for use).

- **Alternative (Non-Compendial)**: Procedures proposed by the applicant for use instead of or in addition to the regulatory analytical procedure. Generally, will need full validation.

- **Stability**: Procedures that can detect changes with time in the pertinent properties of the drug substance and drug product will need full validation unless they fall under regulatory.
Analytical Method Validation ICH Q2 (R1)

• ICH Q2 (R1) describes the validation parameters that should be validated for different analytical procedure:
  • Identification Tests
  • Quantitative test for Impurity Content
  • Limit test for the Control of Impurities
  • Quantitative Test for Active Moiety in samples

• Revalidation of an analytical method may be required if:
  • Change in the synthesis of drug substance
  • Change in the composition of the finished product
  • Changes in the analytical procedure
Where is Assay Validation Required?

• Lot release assays
• Stability methods for defining expiration dates/holding times
• Assays for significant process related impurities (e.g., host cell proteins, residual DNA, protein A, etc.)
• Analytical in-process tests
• Excipient and raw material testing (generally compendial)
Expectations For Methods During Product Development

• Ensure safety of the product

• Assay is providing meaningful results

• Assurance that analytical information gained in development can be reliably related to commercial manufacturing

• Determine method performance capabilities including specificity, linearity, accuracy, precision, robustness, and stability
Assay Validation During Product Development

- FDA Process Validation Guidance (2011)
  - “Validated analytical methods are not necessarily required during product- and process-development activities or when used in characterization studies.”
  - “…analytical methods should be scientifically sound (e.g., specific, sensitive, and accurate) and provide results that are reliable.”
  - Clinical supply production should follow the CGMPs appropriate for the particular phase of clinical studies.

- Interpretation:
  Review staff focus on the adequacy of non-compendial safety tests for early phase clinical supply material
Life Cycle of Analytical Methods

**Pre-Clinical**
- Selection
- Development/optimized

**Phase I**
- Safety tests validated, qualified methods, set tentative release and stability acceptance criteria

**Phase 2**
- Assay optimization/qualification-refine lot release criteria
- Set tentative validation acceptance criteria
- Delineate/initiate assay validation parameters

**Phase 3 and BLA**
- Validation strongly recommended for phase 3
- Full assay validation for BLA

**Post-Licensure**
- Trend analysis, Performance review
- Method Replacement (supplement)
Regulatory Considerations for Changes in Analytical Methods
Regulatory Considerations for Changes in Analytical Methods

21 CFR 601.12, 314.7 and GFI: Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products [1997])

Changes in analytical methods that have high risk of impacting product quality need to be approved by the FDA prior to the methods implementation.

For Example:
When a new or revised analytical procedure is used for release or stability do not eliminate a test or relax an acceptance criterion, that was approved by FDA in your application, unless FDA informs you that the proposed change is acceptable.
Regulatory Considerations for Changes in Analytical Methods

Any modification or replacement of an existing method requires a comparability or equivalency study (21CFR 610.9):

• To demonstrate that changes in the analytical procedures improve or do not significantly change analytical procedure characteristics that are relevant to the type of analytical procedure, its validation, and its intended use.
• Modified or new methods should be validated.
• Homogenous samples from the same batches should be included in the studies.
• Statistical analyses should be performed to demonstrate the comparability or equivalency of the modified or the new method with the existing method.
Case Studies

Changing or Replacing Analytical Methods
Changes in Analytical Methods For Purity and Charge Assays

• New methods to detect purity and charge (cSDS, cIEF, CEX, etc.) are used to replace old methods such as SDS-PAGE or IEF method.

• The new assays have higher sensitivity detecting product-related impurities, at release or during stability studies, that were not detected by the original method.

• Use of these new methods raises the question whether the impurities are new or the new assay is better than the old assay detecting product degradants.
Case Study #1: Change from IEF to cIEF

- Sponsor seeks to replace a qualitative (IEF) assay with a quantitative (cIEF) assay.

- The IEF assay had acceptance criteria of “compares to reference” while the cIEF assay had quantitative acceptance criteria for the major peaks.

- Using these acceptance criteria, stability samples were failing by the IEF assay at earlier time points than by the cIEF assay.

- It appeared that the new assay was not as stability indicating as the old assay.

- The stability failures by IEF appear to be due to the appearance of a particular isoform compared to the reference standard. In case of cIEF, same isoform is consistently present at very low levels and is detected by the cIEF, even in the reference standard.
Case Study #1: Change from IEF to cIEF

**Recommendation**
The cIEF was allowed for use in stability testing based on the data and risk assessment provided by the sponsor that showed:

- Detection by the IEF was the result of a less than 0.5% change in its level. Because this was considered a ‘change’ from reference, it was considered a stability failure by IEF, but did not actually represent a significant change in product quality.

- Impurity was identified and justified not to impact product efficacy or safety.
Case Study #2: Change from IEF to cIEF

At the time of licensure, sponsors seeks to replace an IEF assay with a cIEF assay.

• New method was shown to have comparable or better performance compared to the old method.

• There was however, limited data from drug product lots using the new method upon which to base quantitative release and/or stability acceptance criteria.

Recommendation
New method run concurrently with old method until sufficient data accumulated with which to establish relevant acceptance criteria.
Case Study#3 : Host cell protein (HCP) assays

HCP assay updated during development from a third-party anti-HCP assay to a product specific assay.

- Third party assay: The anti-HCP antiserum is raised against a ‘generic’ cell line (e.g., CHO, NS0, E. coli).

- Specific assay: The anti-HCP antiserum is raised against a cell line that is the same as that used for transfection. Generally a vector transfected cell line.
Commercial versus Specific HCP Assays

Third party HCP Assay Antibody

Specific HCP Assay Antibody

Silver stain of HCP assay standards

Western blots
## Non-Specific Vs. Specific HCP Assays

<table>
<thead>
<tr>
<th>Host Cell Protein</th>
<th>Historical process Results</th>
<th>Current process Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. ± Std. Dev.</td>
<td>933 ± 271</td>
<td>452 ± 112</td>
</tr>
<tr>
<td>Range</td>
<td>4 – 106</td>
<td>20 – 74</td>
</tr>
<tr>
<td>Avg. ± Std. Dev.</td>
<td>29 ± 25</td>
<td>49 ± 19</td>
</tr>
</tbody>
</table>

Recommendation: Implement specific assay early in development
Considerations for Replacing an Existing Analytical Methods

- Performance parameters of the new assay should be the same or better than the existing assay.

- The stability indicating properties of the new assay should be the same or better than the existing assay.

- If new product related variants or process related impurities are seen with the new assay, information from retained samples should be provided demonstrating that the variants/impurities are not new.

- If making a major change in how a quality attribute is measured:
  - Assess consistency and comparability across multiple lots.
  - Release and stability data from multiple lots will be required for establishment of a commercial specification.
Case Studies: Analytical Method Transfer
Case Study#4: Analytical Method Transfer

Following change in manufacturing process and site change, the SDS-PAGE method for purity of the drug substance (DS) was transferred to new site.

• At new site a different densitometer was used to quantify protein gels.

• Result on DS lots from the new site with new densitometer showed a decrease in product purity for non-reduced SDS-PAGE gels.
Case Study#4: Analytical Method Transfer

• To demonstrate that the decrease in product purity was not due to the process change the sponsor performed side-by-side testing as follow:
  - DS batches produced by new manufacturing process were tested at the previous analytical laboratory site and at the new analytical site.
  - DS lots from original and new manufacturing process were tested at the new analytical site.

• Results from these side-by-side comparisons showed that the apparent decrease in purity was due to the use of the new densitometer and not due to change in product quality.
Case Study #5: Analytical Method Transfer

Sponsor submitted a PAS for the transfer of multiple analytical methods for drug product (DP) testing. The following deficiencies were noted in the method transfer study:

- It did not include comparison on percent monomer - a lot release and stability specification.
- The method transfer included only one analyst at the recipient site.
- The results of the DP lots used to support the transfer of the method used to detect impurities were predominantly reported as < LOQ. No data was provided to demonstrate that LOQ of the transferred method at the recipient site is comparable to the original site.
Case Study #5: Analytical Method Transfer

Recommendations:
• To compare monomer results from both facilities

• Method transfer should include two operators at both sites or provide data from additional testing sites to demonstrate that assay is not subject to operator bias

• Include analysis of additional lots with detectable impurities. The analysis of spiked samples with known amount of impurities could also be used
Considerations for Analytical Methods Transfer

• A statistically relevant number of DS or DP lots should be analyzed at both sites.

• Forced degradation samples should be analyzed at both sites, if applicable.

• Recipient lab should meet the assay performance parameter as set in the method transfer protocol:
  - Number of replicates within each run
  - Intermediate precision of the method at the new site with respect to analysts, equipment, and days if applicable, or justify if the criterion is not met
References on Analytical Methods Validation

1. ICH Guidelines Q2(R1) [2005]
2. FDA draft Guidance on Analytical Procedures and Methods Validation [2000]
4. FDA-GFI: Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products [1997]
5. FDA guidance on Content and format of IND for Phase 1 studies of Drug, including Well-Characterized, Therapeutic, Biotechnology products [2000]
6. FDA-GFI: INDs for phase 2 and phase 3 studies, CMC Information [2003]
Acknowledgements

• Dr. Barbara Rellahan, PhD
• Colleagues at DMA/FDA