Fit–for–Purpose Biomarker Assay Validation: From Concept to Practices

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Outline of discussions

- Introduction - Concept of Fit-for-Purpose method validation of biomarker ligand binding assay

- Practices
  - Pre-analytical considerations
  - Major challenges of biomarker method validation that is different from those of protein biotherapeutics.
  - Method feasibility and optimization
  - Exploratory vs. Advanced method validation
  - Illustrations of critical analytical issues
Acknowledgement

- American Association of Pharmaceutical Scientists (AAPS) Ligand Binding Assay Bioanalytical Focus Group.
- Colleagues at Amgen PKDM
- Ron Bowsher
Motivation & Purposes of Biomarker Assay Validation

- Biomarkers can play an important role in evaluating the safety and/or effectiveness of a new medical product
- It is critical to ensure the integrity of data from these assays
- A fit-for-purpose approach should be used for the wide varieties of purposes during drug development
- Full Validation required to support a regulatory action (e.g., pivotal safety or effectiveness, labeled dosing instructions)
- Validation of appropriate extent for early drug development (e.g., candidate selection, go-no-go decisions, proof-of-concept)
- The majority of biomarkers are endogenous proteins, which are generally analyzed by ligand binding assays (LBA)

FDA Draft Guidance for Industry Bioanalytical Method Validation 2013
Fit-for-Purpose Concept
'Fit-for-Purpose' Biomarker Assay Validation

DOI: 10.1007/s11095-005-9045-3

Research Paper

Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement

Jean W. Lee,1,16,17 Viswanath Devanarayan,2 Yu Chen Barrett,3 Russell Weiner,3 John Allinson,4 Scott Fountain,5 Stephen Keller,6 Ira Weinryb,7 Marie Green,8 Larry Duan,9 James A. Rogers,10 Robert Millham,10 Peter J. O’Brien,11 Jeff Sailstad,12 Masood Khan,13 Chad Ray,14 and John A. Wagner15

Received July 28, 2005; accepted October 7, 2005

Abstract. Despite major advances in modern drug discovery and development, the number of new drug approvals has not kept pace with the increased cost of their development. Increasingly, innovative uses of biomarkers are employed in an attempt to speed new drugs to market. Still, widespread adoption of biomarkers is impeded by limited experience interpreting biomarker data and an unclear regulatory climate. Key differences preclude the direct application of existing validation paradigms for drug analysis to biomarker research. Following the AAPS 2003 Biomarker Workshop (J. W. Lee, R. S. Weiner, J. M. Sailstad, et al. Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development. A conference report. Pharm Res 22:499–511, 2005), these and other critical issues were addressed. A practical, iterative, “fit-for-purpose” approach to biomarker method development and validation is proposed, keeping in mind the intended use of the data and the attendant regulatory requirements associated with that use. Sample analysis within this context of fit-for-purpose method development and validation are well suited for successful biomarker implementation, allowing increased use of biomarkers in drug development.
Conceptual diagram of Fit-for-Purpose method validation

**Pre-validation:**
- Pre-Analytical and Analytical Method Feasibility
- Method Optimization

**Method Validation:**
- Continuous, evolving & iterative
- Graded process of refinement, dependent on the **intended use**

Lee et al., *Pharm Res* 2006; 23:312-328.
Biomarker Analytical Validation

- **Exploratory** – Method validation that is less rigorous, but adequate to meet study needs
  ....*not for submission to regulatory agencies.*

- **Advanced** – Method validation of more intensity and thorough investigation both in the validation tasks and documentation.
  ...*for submission to regulatory agencies for drug approval.*

Lee et al., Pharm Res 2006; 23:312-328.
Practices
Biomarkers related to disease state and drug effects

Selectivity against similar molecules

Specific target pathway

On-target biomarker

BMKa

BMKb

Ligands

Proximal biomarkers

BMKl

Drug Exposure

BMKn,m

Biomarkers from other pathways

Downstream Distal biomarkers

BMKi,j

Disease outcome

Toxicity biomarkers

BMKt

Direct effect

Indirect, downstream effect

Lag time of PD effect after dosing, single vs. multiple doses
Exploratory & Advanced method validation during drug development

Khan et al. Bioanalysis 7(2) 229-42, 2015
Example: Biomarkers during early clinical development of Denosumab

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Commercial kit or in-house</th>
<th>Intended Purpose</th>
<th>Method validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target biomarker a</td>
<td>In-house</td>
<td>PoM</td>
<td>Exploratory</td>
</tr>
<tr>
<td>Proximal biomarker b</td>
<td>Research kit</td>
<td>PoM</td>
<td>Exploratory</td>
</tr>
<tr>
<td>Distal biomarker c</td>
<td>Diagnostic kit</td>
<td>PoB, dose selection</td>
<td>Advanced</td>
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Schematic of decisions & processes

Work Plan
Define purpose of study & biomarker measurements
Which biomarker(s) and in what biologic matrix to be included in the study?
Exploratory or advanced application?

Pre-analytic sample integrity

Method development & validation
Reference standard & reagents
Calibrator matrix
Initial assay range & sensitivity
Quality Controls
Selectivity, parallelism
Precision & Accuracy
Matrix controls
Factors affecting biomarker dynamics and data interpretation

- Sample collection or storage conditions:
  - Convert upstream forms to the biomarker of interest, resulting in overestimation
  - Degradation of the biomarker, resulting in low levels.

- Binding of a biotherapeutic to the target biomarker requires the decision whether to measure “free” or “total” (free + bound) forms
  - “Free” levels are very low – challenge in method development
  - “Total” may provide information on compensatory feedback or drug protection effect of the biomarker

- *In vivo* conversion to downstream forms
  - Is any of these forms bioactive?
  - Options to develop “bioactive” (or epitope-specific specific immuno) assays vs. chemical (or species-specific immuno) assay of each bioactive species.

These should be considered and discussed with the project team
Basic Pre-Analytical Considerations

- Information from literature, vendor and discovery team
- Disparity of biomarker concentrations/activities in targeted disease population from normal; expected contraction from drug effect
- Biomarker concentration fluctuation with time (daylight and season), age, gender, body mass (or fat).
- Available methods and reagents
- How important will be the decision to the company?
Sources of biomarker LBA methods and reagents

Developed by Pharma companies
• Originated in discovery-research programs
• Typically for target and/or proximal biomarkers
• Method validated by the users for intended purpose

Apply Clinical Diagnostic Commercial Device or Kits

Clinical-grade Diagnostics
• FDA approved and CLIA regulated
• 510 (K) or equivalent
• Closed system, no modification

Research Use Only (RUO)
• Not FDA approved
• Manual assays
• Intended for research only
• Flexible for modifications
What are the main challenges of biomarker assay that are different from those of biotherapeutics?

- Subjected to biological variability (e.g. diurnal effect, diseases)
- Reference standard limitations – impure, not well characterized, not fully represent the endogenous biomarker
- Analyte-free biological matrix may not be found, a substituted matrix is used for calibrators
- Parallelism required to show equivalent performance of: recombinant reference ≈ endogenous; substitute matrix ≈ biological
- Matrix Controls (MC) should be made to reflect those of incurred samples
- Lot-to-lot variability of diagnostic kit performance
Kit reference standard may be inconsistent & lack documentation - Example

Advantage of In-house ref standard:
• Ample supply
• Lot consistency
• Documentation: Certificate of stability and purity/structural analysis
Validation samples and quality controls of biotherapeutics and biomarkers

- **Purposes**
  - Validation sample (VS) – Used during pre-study validation to characterize the method being validated, at least 4 levels including LLOQ, Low, mid and high QCs.
  - Quality control sample (QCs) – Low, mid and high QCs used during in-study validation to monitor the performance, and to accept/reject an assay run.

- **Preparation**
  - In the same biological matrix as subject samples. *This may not be possible for biomarkers with substantial endogenous amounts.*
  - Concentration range should cover the expected levels of target populations
  - Test parallelism to justify using spike samples as VS/QC

- **Matrix Controls (MC) for biomarkers** – Pooled from authentic samples to reflect study samples, at least 2 levels.
**Biological biomarker variations in different diseases**

**Example of an inflammatory biomarker IL6**

- Sepsis
- Bacterial Infection
- Moderate Pancreatitis
- Acute Appendicitis
- Trauma
- Viral Infection
- Active Crohn's Disease
- Rheumatoid Arthritis
- Healthy Subject

*Calibrator ranges and MCs can be different for different target disease*

*From Dr. Ron Bowsher*
Exploratory Validation basic elements

**What are the minimum requirements?**

**Recommendations:**

- Parallelism, or spike recovery
- Validation samples reflecting clinical sample matrix and target range
- Assay working range established with standards and validation samples
- Sample collection integrity
- Bench-top stability
- Precision & Accuracy data from 3 validation runs
Example of Parallelism Tests

Biomarker A

Results acceptable

Biomarker B

Results failed

Multiple matrix lots
Multiple dilution factors
Dilute with standard matrix
Essential considerations for advanced validation and in-study validation

- **Advanced Method Validation**
  - Reagents and reference material stability & inventory control over time span of the program
  - Target range from incurred samples, relative accuracy/recovery from multiple donors
  - Assay performance evaluation from more validation runs (≥6)
  - Long term stability
  - Extensive testing of assay interferences (matrices, concomitants)
  - Robustness (reagent & change control)
  - Learn from in-study validation data of pilot study
  - Beware of biological variability from commercial or in-house samples.

- **In-Study Method Validation**
  - Statistic tools for Acceptance criteria
  - MC from incurred samples vs. spiked samples
Matrix Controls in an Advanced Validation - Example

Each color represents a separate kit lot

Matrix Controls in an Exploratory Validation – Example of RUO kit lot differences

**Pool 1**

<table>
<thead>
<tr>
<th>Kit lot no.</th>
<th>N</th>
<th>Mean</th>
<th>%CV</th>
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Example - Pharmacodynamic profiles of CTx % Change in patients dosed with denosumab

Dose related suppression was shown by the PD biomarker
Summary highlights

- Target-oriented working plan to guide biomarker investigation
- Assays to meet the intended purpose of the study, instead of following biotherapeutic LBA guideline for PK studies.
- Exploratory Method Validation
  - Range finding to determine appropriate range and representative QC/MC levels
  - Parallelism experiments
- Advanced Method Validation
  - Proactive plans for consistent supply of critical reagents
  - Matrix Controls to monitor assay performance and stability
- Learn from initial study data to statistically assess drug effect over the basal levels.
Thank you for your attention

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Supplemental Information
Another way of Parallelism Tests
Spike recovery of multiple lots from healthy normal and patients.

Spiking 150 mU
References