

WHITE PAPER PRESENTED BY PREMIER RESEARCH

# Fit-for-Purpose: A Strategic Approach to Biomarker Method Validation for Rare Diseases



## ABSTRACT

Despite major advances in drug discovery, the number of new drug approvals has not kept pace with the increased cost of their development.<sup>1</sup> Biomarkers have the potential to reduce development time and speed new drugs to market, but widespread implementation of biomarkers in drug development may be impeded by uncertainty around how to validate biomarker measurements intended for use as surrogate endpoints in clinical trials.

# RARE DISEASE



Adequate biomarker measurement and interpretation presents its own set of difficulties, including establishing normal ranges and measuring these biomarkers in a manner that is robust enough for regulatory approval.

## Introduction

Rare diseases present a number of unique obstacles to drug developers, such as small heterogeneous patient populations, long timeframes for disease progression, a poor understanding of disease natural history, and a lack of prior clinical studies. Due to these challenges, it is often complicated or even impossible to identify suitable clinical endpoints for clinical trials. As such, biomarkers are frequently used in lieu of clinical endpoints to assess treatment effectiveness. However, adequate biomarker measurement and interpretation presents its own set of difficulties, including establishing normal ranges and measuring these biomarkers in a manner that is robust enough for regulatory approval.

As a result of these challenges, the concept of a fit-for-purpose approach to validating bioanalytical methods has become essential to successful drug development for rare diseases. This approach requires careful planning, design, and vetting with regulatory agencies to ensure smooth passage and continuity through the stages of development. In this white paper, we present a framework for validating bioanalytical methods for biomarkers intended to be used as primary endpoints in rare disease clinical trials.



### Challenges of biomarker method validation

A biomarker is a defined characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic response to an exposure or intervention. Traditionally, biomarkers have been used to monitor disease progression or the efficacy of therapeutic interventions. More recently, biomarkers have been used to provide valuable information at various stages of drug development and they have the potential to:

- + Reduce the time required to complete clinical trials
- + Reduce the sample sizes required to achieve statistical significance, which is particularly relevant for rare diseases where the eligible patient population is limited and dispersed
- + Identify patients who are most likely to respond to the investigative drug
- + Reduce uncertainty in regulatory decisions

Adequately accurate, precise, selective, and robust measurement of biomarkers is a prerequisite for the biomarker qualification as a surrogate clinical endpoint. Unfortunately, due to the intrinsic characteristics of biomarkers and the diverse nature of biomarker analysis, there is no established standard approach for the validation of biomarker assays for drug development.

### Establishing normal ranges

Many biomarkers are endogenous compounds that are present in the general population. Therefore, normal ranges need to be established for biomarkers, but these ranges often vary with gender, age, and a host of other factors, making it difficult to correlate the appropriate ranges with specific groups of patients. In addition, there are inherent bioanalytical challenges in measuring endogenous compounds, which can also be structurally different from the calibrator.

### Quantifying and interpreting biomarkers

Biomarkers can be diverse and non-homogenous. For example, lysosomal storage diseases (LSDs), which encompass dozens of individual rare and ultra-rare disorders, are characterized by the accumulation of a variety of lipids, polysaccharides, glycoproteins, and other classes of compounds, with significant chemical diversity within each class. This diversity poses major challenges in not only validating and interpreting these biomarkers, but also adequately presenting the findings in compliance with accepted regulatory requirements.

As many biomarkers have been known for a long time, the analytical methodology for measuring them often will have evolved significantly. This presents challenges in comparing and interpreting historical data and comparing between old and new clinical trial data. As such, cross-validating between different analytical methods may be essential for data interpretation.

When liquid chromatography-tandem mass spectrometry (LC/MS/MS) is used for biomarker analysis, matrix effects often represent a significant problem for accurate analysis, as stable isotope-labeled internal standards typically used to alleviate matrix effects are rarely available for biomarkers.

### An approach to biomarker validation

To provide appropriate context, there is an important distinction between biomarker analytical method validation and clinical qualification!<sup>1</sup>

- + **Validation** is the process of assessing the performance characteristics of a given assay, i.e., validating the soundness of the analytical method used to measure the biomarker and confirming that the measurement is accurate
- + **Qualification** is the evidentiary and statistical process linking biologic, pathologic, and clinical endpoints to the drug effect, or linking the biomarker to a biological or clinical endpoint

Understanding exactly what is being measured and its biological relevance is critical to the utility of biomarker data. The FDA has issued guidance for industry for bioanalytical method validation addressing validation of assays to support pharmacokinetic (PK) assessments of conventional small molecule drugs, and the Clinical and Laboratory Standards Institute (CLSI) has established standard practices for the performance of laboratory testing on humans for diagnosis, prevention, or treatment of any disease or impairment.<sup>2</sup> However, neither the FDA guidance nor the CLSI guidelines fully address the needs of drug development applications of biomarker assays. Instead, it has become clear that assay validation for drug development should be tailored to meet the intended purpose of the biomarker study, with the necessary level of regulatory rigor. This approach is known as fit-for-purpose method validation.

### Fit-for-purpose method validation

Since the objective of validation is to demonstrate that a method is reliable for the intended application, it follows that the rigor of the biomarker analytical method is highest for biomarker data intended to serve as a substitute for a clinical endpoint in a clinical trial. Increasingly, sponsors are adopting a continuous and evolving fit-for-purpose strategy to method validation which conserves resources in the exploratory stages of biomarker characterization until assay refinement is required for later stages of drug development.

### Establishing assay acceptance criteria<sup>1</sup>

Definition of the criteria for assay acceptance is a prerequisite to biomarker method development and validation. Three major factors influence the establishment of these criteria:<sup>1</sup>

1. Acceptance criteria should meet the predefined needs of the study, rather than simply reflecting the performance capabilities of the assay itself

2. The nature of the assay methodology and the data generated using that assay
3. The biological variability of the biomarker within and between study populations

### Developing a biomarker work plan<sup>1</sup>

Prior to the commencement of assay development, study objectives, the intended use of assay data, and the level of documentation of data should be defined in a biomarker work plan to aid in timely identification of reagents, controls, and experimental samples. For early-phase clinical trials, preclinical studies and literature reviews may help in the establishment of appropriate precision requirements for the assay for a given population.

### Creating standardized procedures for sample collection and handling<sup>1</sup>

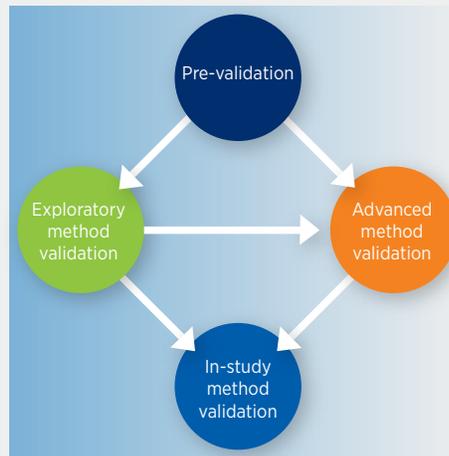
Sample integrity from collection through analysis is crucial for confirming the validity of results from biomarker assays. Defining protocols for sample collection, handling, and storage – whether it is biological fluids or tissues – is important, particularly in cases where manipulations may affect sample and/or biomarker integrity. Since the life cycle of a study sample typically includes freeze/thaw cycles, it is usually necessary to assess short-term, freeze/thaw, bench-top, and long-term stability.

### Performing method validation<sup>1</sup>

Method validation is an iterative, fit-for-purpose process that requires continuous reassessment of data and optimization of the assay method. Following are the key concepts and recommended practices for method validation.

The rigor of the biomarker analytical method is highest for biomarker data intended to serve as a substitute for a clinical endpoint in a clinical trial.

### Normal flow of biomarker development.<sup>1</sup>



#### Pre-validation

In the pre-validation phase, method feasibility studies are performed to address the likelihood that an assay will be able to achieve its intended purpose. An early objective assessment of the assay working range is essential for initial assay development. The most commonly used assessment method for macromolecule biomarkers is the “precision profile,” a tool that provides preliminary evidence whether or not an assay is capable of measuring the analyte of interest at a predetermined concentration range. Method feasibility studies should also address:

1. **Assay specificity** – the ability of the assay to unequivocally distinguish the analyte of interest from structurally similar substances
2. **Assay selectivity** – the degree to which unrelated matrix components cause analytical interference
3. **Assay accuracy** – in the presence of the drug of interest, particularly when the drug targets directly interact with the biomarker

#### Exploratory method validation

During the exploratory validation phase, the assay method is quantitatively characterized with respect to its basic analytical elements. The biomarker work plan is used to guide the investigation of method performance. It is recommended that at least three evaluation runs are carried out to provide basic assay performance including:

1. **Accuracy**, which is critical for defining the exposure-response relationship in the clinical diagnostic environment
2. **Precision** data provides information on the statistical significance of biomarker results in a study
3. **Sensitivity** is defined as the lowest analyte concentration that can be measured with acceptable accuracy and precision and must be feasible in the clinical laboratory with regulatory compliance

4. **Relative selectivity** with respect to the binding to the substrate through investigation of likely sources of interference
5. **Initial biomarker concentration ranges** in normal individuals and in the target population
6. **Assay dynamic range** reflects the lower and upper limits within which the analyte is measurable with acceptable levels of accuracy, precision, and total error
7. **Short- and long-term biomarker stability** in the expected biological matrix and under conditions mimicking those expected during the conduct of the study, including conditions at a typical clinical site, during shipment, and at all other secondary sites. A case-by-case evaluation must be made to determine whether chromatographic, immunoreactive, or biological activity assays are most appropriate for monitoring stability of a given biomarker. Keep in mind that stability measures of endogenous macromolecules may be method-dependent.

This data should be statistically evaluated to estimate whether or not the method would meet study requirements.

#### Advanced method validation

Since method validation is an iterative, evolving process, all of the performance characteristics listed in the exploratory validation should also be included in the advanced validation with additional characterization. The increased rigor of advanced validation should be undertaken in a scaled, fit-for-purpose approach as the impact of the biomarker data on decisions around critical safety, efficacy, pharmacodynamics, differentiation, or surrogate information increases. If the methods undergoing advanced validation have been validated and used for sample testing, in-study validation is an ideal source of data as it reflects accuracy and precision performance from actual assay use. Alternatively, advanced validation may be undertaken as the initial phase of formal performance characterization for the method.<sup>1</sup>

For biomarkers that are also endogenous compounds, method validation must account for the assay's ability to distinguish between the therapeutic and endogenous counterparts. More rigorous testing of potential interfering endogenous components or metabolites should be implemented during advanced validation since later-stage clinical trials typically include more diverse populations and more concomitant medications. One approach for evaluating the selectivity of the assay in the presence of endogenous substances is using blank matrices from multiple individuals of both genders.<sup>1</sup> In their draft guidance for Bioanalytical Method Validation, the FDA recommends that the calibration curve in biological fluids be compared with calibrators in buffer to detect matrix effects using at least 10 sources of blank matrix. When high-quality analytical standard of the biomarker is available, standard addition approach may also be used to establish selectivity and help alleviate matrix effects.

Advanced validation should also include assessments of freeze/thaw and long-term stability sufficient to cover the range of conditions to which samples are likely to be exposed.<sup>1</sup>

#### In-study validation

The in-study validation phase aims to ensure that the assay continues to perform according to predefined expectations. Accuracy validation entails the use of samples containing known amounts of the analytes – QC samples – typically at low, mid, and high concentrations of the analyte with at least two replicates at each level.<sup>1</sup> Precision validation should be measured using a minimum of five determinations per concentration and a minimum of three concentrations in the range of expected study sample concentrations.<sup>2</sup>

Sponsors should keep in mind that the acceptance criteria for biomarker assays will depend heavily on the intended use of the assay and should be based on physiological variability in the

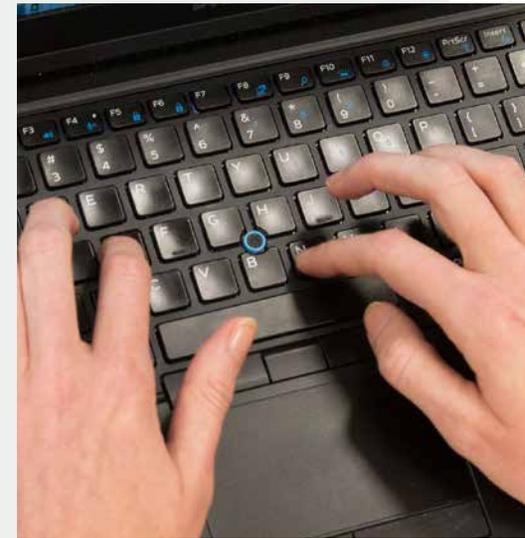
study population of interest. In other words, to determine whether a biomarker method is fit-for-purpose, sponsors should determine whether it is capable of distinguishing changes that are statistically significant based on intra- and inter-subject variation. If relevant physiological data is not available during assay validation, then healthy donor samples should be used to estimate intra- and inter-subject variation. If healthy donor samples are not available, other biological rationale should be considered.

#### The value of partnerships

Given the challenges associated with biomarker validation, sponsors would benefit from establishing strong partnerships to facilitate the validation process. Key partners might include:

- + **Subject matter experts** – Identifying clinicians and researchers with expertise in the rare disease under study can help sponsors gain a deeper understanding of the biological and clinical relevance of the selected biomarker(s)
- + **Laboratories** – Working in tandem with at least one laboratory can help to make the validation process more efficient
- + **Contract research organization** – Partnering with a CRO that has experience in analytical methodologies, validation requirements, biomarker and PK/PD interpretation, and the regulatory approval landscape can help to accelerate the validation process

Support is also available from the FDA's Biomarker Qualification Program, which was established to support the Center for Drug Evaluation and Research's (CDER's) work with external stakeholders to develop biomarkers that facilitate the drug development process.<sup>3</sup> Through this program, sponsors may request regulatory qualification of a biomarker for a particular



context of use in drug development. Context of use (COU) is a clear statement describing the manner of use, interpretation, and purpose of a biomarker in drug development.<sup>4</sup> A COU has two components:

- + **Use statement** – A concise document that includes the name and identity of the biomarker and purpose for use. Note that the term biomarker may refer to a single biomarker with a single, specific context of use, or to a composite biomarker made up of several individual biomarkers combined in a single algorithm to render a single interpretive readout.
- + **Conditions for qualified use** – A comprehensive description of conditions in which the biomarker will be used in the qualified setting

Understanding the required elements of the COU may help sponsor develop a method validation strategy that supports eventual clinical qualification of the biomarker.

Once a biomarker is qualified, it can be used in any drug development program under the context for which it obtained qualification without a need for CDER to reconfirm its suitability.

## Conclusion

Biomarkers have the potential to change the paradigm of drug development across all therapeutic areas, but especially for rare diseases. Biomarker data can yield important efficacy and safety information regarding the dose-response relationships, serving as early predictors of drug effects. However, the diverse array of intended biomarker applications presents an analytical challenge for assay or diagnostic development. In this paper, we have outlined a conceptual strategy for a fit-for-purpose approach to biomarker method development and validation which helps to avoid major pitfalls without impeding research efficiency.



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Andrew Volosov is Director of Clinical Pharmacokinetics at Premier Research providing pharmacokinetic data analysis, modelling, and interpretation for sponsors, integrating pharmacokinetic data into regulatory documents and submissions and consulting with sponsors on pharmacokinetic trial design, regulatory requirements, and study feasibility. He has over 20 years of experience in drug development within the pharmaceutical and biotechnology industries. Prior to joining Premier Research in 2014, Dr. Volosov led a group of scientists at Shire tasked with development of clinical biomarkers as endpoints for clinical trials for a variety of rare diseases.

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