Adaptation of fit-for-purpose biomarker assay validation using commercial kits: A CRO perspective

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A The biomarkers have been the focus of increased attention and interest throughout the pharmaceutical and biotech industry. There has been an increase in the number of scientific meetings and conferences devoted to this subject. More recently at 2010 BIO International Convention, in Chicago there were ten breakout sessions addressing a variety of pertinent issues including their utility in making personalized medicine a reality and accelerating the drug development process.

Over the last decade, the rate of approval of new drugs by regulatory agencies has lagged far behind the rate of increase in investment of time and money by both private and public sectors. As a result, there has been an increased emphasis to accelerate the drug development process at all levels. Interest in identifying and utilizing biomarkers to enhance the efficiency of drug development process was remarkably increased after the publication of 'Critical Path Imitative' white paper (1) from US Food and Drug administration (FDA) in 2004 that was followed by the launch of 'Biomarker Consortium (BC)' in 2006 as a part of FDA's Critical Path Initiative. As defined by BC," Biomarkers are characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention." In this article our focus is mainly on protein based biomarkers.

Today, **biomarkers** have become an integral part of drug development strategy primarily aimed to identify and eliminate the poor drug candidates at the early stage and redirect the investment of resources to more promising drug candidates making the process more efficient and cost effective. Consequently many pharmaceutical companies and Contract Research Organizations (CRO) invested heavily in bionalytical support services for quantification of biomarkers especially protein biomarker. Although there are a number of FDA guidance documents that address the regulatory expectations for validation and implementation of a variety of bionalytical methods for quantification of drug entities there is not much available for the validation of bionalytical methods for biomarkers.
To fill this void members of the Ligand Binding Assay Bionalytical Focus Group Biomarker subcommittee at the American Association of Pharmaceutical Scientists (AAPS) developed a 'Fit-for-Purpose' approach for the validation of biomarker assays for use in drug development projects (2). This approach is based on the notion that the intended use of the data should dictate the level and rigor of the method validation that ranges from exploratory to advanced method validation. Since the publication of the white paper in 2006, there have been numerous scientific conferences and publications on adaptation and further refinement of this approach.

It is interesting to note, that there are a large number of commercially available kits for a wide variety of biomarkers. These kits are available in mainly two categories i) diagnostic kits with FDA 510(k) approval for use in clinical settings, and ii) Research

Use Only (RUO) kits that are available for a wider variety of biomarkers in a variety of human and non-human biological fluids for use in research laboratories. It should be noted that none of these kits are designed and developed to support drug development projects.

In our experience the RUO kits are more readily adapted for quantification of biomarkers in drug development projects, mainly because of the availability of a wide variety of esoteric assays, perceived ease of use and cost effectiveness. However, selection of a trust-worthy kit vendor, a reliable kit, acquisition of material for quality control preparation in biological matrix, and adaptation of kit to meet the intended needs of sensitivity and reproducibility of biomarker measurement poses a unique set of challenges that are beyond the scope of this article. Here we are limited to give an overview of the adaptation of 'Fit-for-Purpose' method validation strategy for commercially purchased kit(s) based assay validation.

At KCAS we have simplified this approach and are able to offer customized "Fit-for-Purpose' services based on client needs. In view of varying needs of clients, intended use of data, cost and time constrains and number of samples available for analysis, we recommend two additional levels i.e., Kit performance verification, and kit performance qualification, besides the exploratory and advanced method validation originally proposed in the white paper. A summary is outlined in Table I.

Table I – Fit-for purpose validation of kit based biomarker assays

	Kit verification	Kit qualification	Exploratory method validation	Advanced method validation
INTENDED USE	Exploratory information gathering	Exploratory information gathering	Internal decision making	Critical decision making
	Small number of samples	Large number of samples		Supporting Regulatory filing
CALIBRATORS				
Matrix	Kit calibrators / Buffer-Protein solution	Buffer-Protein solution / Biological matrix	Kit calibrators / Buffer-Protein solution	Buffer-Protein solution / Biological matrix
Number	Kit provided	6 or more	6 or more	6 or more
Range of quantification	Manufacturer provided	Evaluate with spiked LLOQ and ULOQ calibrators	Evaluate with spiked LLOQ and ULOQ calibrators	Evaluate with spiked LLOQ and ULOQ calibrators
CONTROLS				
Matrix	Kit QC in proprietary matrix	Buffer-Protein / Biological matrix	Buffer-Protein	Biological matrix
Number	As supplied in kit	3	3	5
Sample control	No	Evaluate	2 level	2 level
REFERENCE MATERIAL				
Non-kit source	No	Yes	Yes	Yes
ACCURACY AND PRECISION RUNS	1 to 3	3	3	6
Inter-day	1 or more	2 or more	2 or more	2 or more
LOD	Compute	Compute	Compute	Compute
LLOQ	Lowest acceptable non-zero calibrator	Verify by spiked sample	Verify by spiked sample	Verify by spiked sample
ULOQ	Highest acceptable non-zero calibrator	Verify by spiked sample	Verify by spiked sample	Verify by spiked sample
Dilutional linearity	Optional	Optional	Determine	Determine
Parallelism	Optional	Optional	Determine	Determine
Normal range	No	No	Determine	Determine
Disease sate range	No	No	Determine	Determine
Selectivity	No	Determine	Determine	Determine
ANALYTE STABILITY				
Room temperature	Optional	4-6 hrs	4-6 hrs	4-6 hrs
Freeze-Thaw	Optional	Optional	3 cycles or more	3 cycles or more
Storage	No	No	Yes	Yes
DOCUMENTATION				
Protocol	Yes	Yes	Yes	Yes
Test procedure (TP)	Kit Insert / Abbreviated TP	Yes	Standard TP	Standard TP
Report	Data summary	Data summary	Full report	Full report

KCAS offers a variety of bionalytical services for both large and small molecule drug development projects. This includes but not limited to: assay development for novel biomarkers, fit-for-purpose biomarker assay validation, and custom assay development for immunogenicity evaluation, sample analysis using RIA, ELISA and ECL-based assays in singleplex and multiplex format.

REFERENCES

1) ***Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products***; U.S. Department of Health and Human Services, Food and Drug Administration, March 2004

2) Lee J.W. *et al.* "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" ***Pharm. Research*** **2006**, *23* (2), 312-28; DOI: 10.1007/s11095-005-9045-3