European Bioanalytical Forum
Autumn Biomarker Focus Workshop

15-17 September 2020

Day 2 Session 3

The Building Blocks of a Good Biomarker Assay

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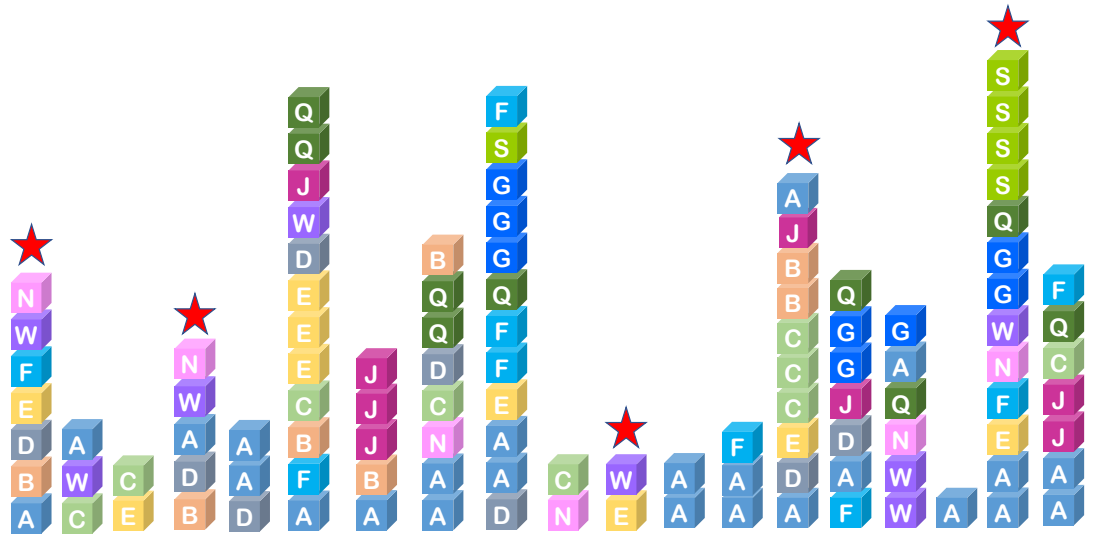
Introduction to Session 3 : The Building Blocks of a Good Biomarker Assay

- Considerations on **Matrix** Sources in Biomarker Assays
- EBF Feedback on **Critical Reagents** in LBA Biomarker Assays
- **Parallelism** – driven by COU
- Source of Biomarker **Assay Variability**

Identifying the building blocks….



Introduction to Session 3 : **The Building Blocks of a Good Biomarker Assay**

Recommendations*

- Be a Scientist
- Embrace and own Fit-for-Purpose
- Demand Context of Use (or cannot “Validate”)

*Applicable guidance in the opinion of the presenter

Information required – what and from where?

- Let's address the "**where**" first....
- Important that all stakeholders have an input
- The Biomarker/Bioanalytical/Clinical/Translational scientists at the CRO typically develops the question list
- "Clinical" Team in sponsor organization (overseeing study and those who developed study protocol)
    - Clinicians
    - Pharmacologists
    - Statisticians
    - Management / Outsourcers / QA
    - Bioanalytical/Biomarker team
- Regulators or therapeutic area KOL's for advice/consultation

Information required – what and from where?

The “**what**”…

Getting to COU:

Information required – what and from where?

- Questions for the client's stakeholders
- My checklist from 1990's onwards…
  - First Presented at International Central Lab conference 2000 and
  - International Bioanalytical Methods conference 2002

Summary - issues to consider

- Clinical and study need
  - *Safety / efficacy / PD/ exploratory?*
  - *Study specific targets / go or no-go decision criteria*
  - *Expected changes – appropriate analytical range?*
- Previous data produced or methods used?
- Stress, posture, gender, ethnicity, circadian rhythm, fasting?,seasonal variation, any additional specific factors for this Biomarker?
- Stability …>>> construct collection/preparation procedure - ? biobank matrix required if BM not detectable in normal subjects, or use incurred samples
- Commercial kit or develop in-house?
  - *Reagent source, QC?, third party reference material?*
  - *Alternatives – best fit for study requirements?*
- Endogenous BM present in matrix?? Calibrators
- Choice of Analytical Platform
- Matrix of choice, Sample volume, Turn-around time, Throughput
- Biological biomarker variations in different diseases - underlying disease status - endogenous interference?
- Concommitent drug interference / T.A. interference
- Choosing the right Biomarker for the right Species
- Population and Intra-individual variation
- Method correlations /transfer. From lab-lab or method-method
- Microplate coating & edge effects?
- Multi-analyte validation requirements
- Parallelism / matrix interference / stability -?Consent for incurred samples
- Cost

Information required – what and from where?

Questions to ask the key stakeholders

CLINICAL USE AND STUDY NEED	
• safety / Efficacy / PD / Exploratory / Stratifying / POM / POP / POC / 1° or 2° Endpoint / Other?	
• Study specific targets / go or no-go decision criteria?	★
• Expected changes - appropriate analytical range?	★
• Previous data produced or methods used?	★

PHYSIOLOGY	
• Stress, posture, gender, ethnicity, circadian rhythm, fasting, seasonal variation? (PROTOCOL!)	
• Any additional specific factors for this biomarker?	★
• what does the client know/understand about the biomarker wrt to their project?	★
• Population and intra-individual physiological variability	★

SAMPLES	
• Matrix of choice	
• Sample volume available	
• Turn-around time, Batched timings, interim reporting	
• Number of samples	
• Parallelism / matrix interference / stability - **? Consent to use incurred samples**	★

→ **These are NOT *definitions* of CoU.**

★ **Collectively answers to these questions help define the method performance criteria required to meet CoU**

Information required – what and from where?

Questions to ask the client?...*continued*

STABILITY
• ?biobank matrix required if BM not detectable in normal subjects
• Use of incurred samples or residuals -**Consent?**
• Construct collection/preparation process - cost materials

METHOD
• Commercial kit available?
• Reference Material
• Alternatives
• Has a method already been used - if so ? Correlation/transfer required?
• Multiplex possibilities?
• Choice of analytical platform

THERAPEUTIC AREA	
• Biological biomarker variations in different diseases - what does the client know about theirs?	★
• Underlying disease status (?endogenous molecule interference)	★
• Concomitant drug(s)? - Significant Test Article metabolites? *(interference check at validation)*	

COST
• Realistic timelines for PBU depending upon complexity

PLUS…

Important to FULLY understand potential impact of pre-analytical variables

Information required – key considerations

Potential Pre-Analytical Variables in the Measurement of Biomarkers in Biological Fluids

Controllable

Collection Technique:
- *Hypodermic needle gauge & speed of draw*
- *In-dwelling catheter – washouts are important!*
- *Order of draw with multiple tubes – anticoagulant contamination*
- *Stasis (tourniquet)*
- *Sampling during infusion - time and site*
- *Blood volume per tube - variation of additive concentration*
- *Positional effect - supine vs erect*
- *Type of mixing at collection*

Timing & Physiological:
- *Time of day of collection (circadian rhythm)*
- *Hydration Status*
- *Fasting or non-fasting status - Lipemia*
- *menstruation*
- *Stress – (needle phobia etc. – limited control possible)*
- *Timepoint relevant to expected change – e.g. lag times for pharmacodynamic effects*

Subject lifestyle:
- *Body Mass Index*
- *Diet*
- *Exercise*
- *Smoking*
- *Alcohol*
- *Caffeine*

Drugs:
- *Over the counter*
- *Recreational*
- *Drugs of abuse*

Sample processing:
- *Sample identification*
- *Mode of transport/storage - time and temperature*
- *Time between collection, processing and storage*
- *Storage temperature*
- *Centrifugation - temperature, speed and time*
- *Evaporation, Oxidation, Desiccation*
- *Sunlight, Artificial light, Humidity*
- *Hemolysis*

Some of these considerations may impact upon decisions to meeting COU

Uncontrollable

Personal:
- *Age*
- *Gender*
- *Race*

Physiological:
- *Pregnancy*
- *Lactation*
- *High concentrations of various circulating proteins (Oncology)*
- *Circulating antibodies (Rheumatoid factor, Human anti-mouse antibodies, etc)*
- *Stress – clinical hypertension*

Environmental:
- *Altitude*
- *Temperature*
- *Geographical location*
- *Seasonal influences*

Drugs:
- *Existing therapies*
- *Co-medications*
- *Trial drug*

Clinical Study Protocol:
- *Pre-set timings of collection*

Information required – what and from where?

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Clinical Use / Study Need	Therapeutic Area / Physiology	Samples	Analytical Method	Regulatory, Ethical & Business Matters
BM information & data gathering	**Disease state – known physiology**	**Matrix of choice – why?**	Already data from previous method?	GCP / GLP
Efficacy*	**Specific factors disease? e.g. AD vs ALS vs other Neuro**	Turn around time – Batched? Interim reporting?	**ALL** data and info is important	CDA/NDA to enable sharing of relevant details
Pharmacodynamic*	**Biomarker data/research knowledge - Client**	Whole profiles within single batch?	?Transfer ?Re-optimization	
Stratification*	**Project specific data & knowledge**	# Samples?	?New method ?Correlation required	
POM / POP / POC?	**BM variability data available?**	Volume available & back-ups collected	Commercial methods available – research	
1° or 2° Endpoint or other go/no-go criteria?		Biobanked samples available - ? Client or other	Parallelism?	
Other?		**Pre-analytical controllable variables?**	**Stability?**	**Informed consent protocol statement**

Clinical Use / Study Need	
BM information & data gathering	This is probably what is most commonly meant when assays are described as "exploratory." However, that needs to be questioned and confirmed
Efficacy Pharmacodynamic Stratification	It is important to confirm EXACT use of the data in "real-time" to ensure that CLIA or equivalent is not required. Some examples where CLIA *could* be required :- Dose escalation decisions based on efficacy / PD effect Inclusion / Exclusion based on screening/Dx data Similar to other areas in biomarker science, decision criteria may be specific to biomarker, drug, disease or study design (any or all) Ultimately, consultation & discussion with regulators in advance may be a wise course of action to ensure that there is alignment on suitable solutions to specific studies
POM / POP / POC?	
1° or 2° Endpoint or other go/no go criteria	Decision criteria help to define the precision of assay required at specific concentrations or the ability to determine specific magnitude of change (e.g. % change from baseline or X-fold change)
Other?	

Acknowledgments

- Critical thought partners: Lauren Stevenson (ILX) & Devangi Mehta (ILX)

If there's no scientific rationale, it's not science

Demand Context of Use