Autumn Focus Workshop
Biomarkers/CoU - Sharing Experience through Examples

The BM toolbox…did you know there is more in life than LBA & LC/MS?

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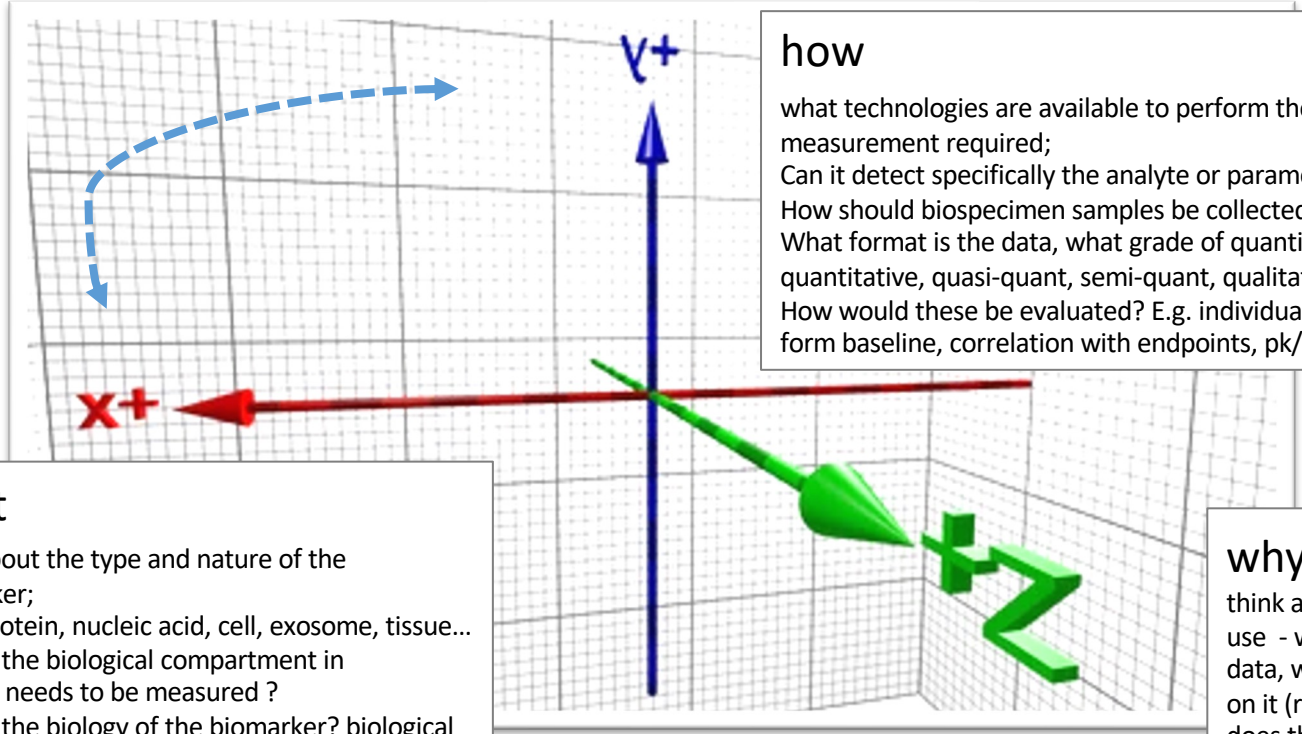
The concepts associated with applying CoU to your BM validation is three dimensional



how

what technologies are available to perform the type of measurement required;
Can it detect specifically the analyte or parameter of interest?
How should biospecimen samples be collected and prepared?
What format is the data, what grade of quantification? Relative quantitative, quasi-quant, semi-quant, qualitative…
How would these be evaluated? E.g. individual/average change form baseline, correlation with endpoints, pk/pd modelling….

what

think about the type and nature of the biomarker;
Is it a protein, nucleic acid, cell, exosome, tissue…
What is the biological compartment in which it needs to be measured ?
What is the biology of the biomarker? biological variability, interactions, transformations….

why?

think about the details of the context of use - what is the intended use of the data, which kind of decisions will base on it (risk assessment)?
does the chosen technology fit this?

The complexities of biomarker assays is compounded by the different types of endogenous molecules measured on a variety of different analytical technology platforms

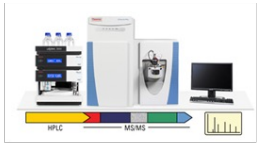
Whole blood
Serum & Plasma
Urine
Saliva
CSF
Bone Marrow
Tissue homogenates
Cell lysates

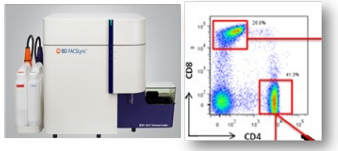
Protein
Small molecule
Cell exosome
Tissue
RNA & DNA

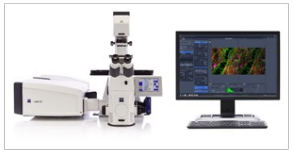
Enzyme /activity
LBA
LC-MS
Cytometry
- Flow and FACS
- Image / Chip
- Mass

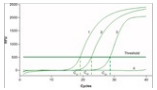
ELISpot
DLS/ NTA
IHC
FISH
MRI/PET /CT
RT-qPCR
Transcriptomics & Genomics
NGS

Platforms





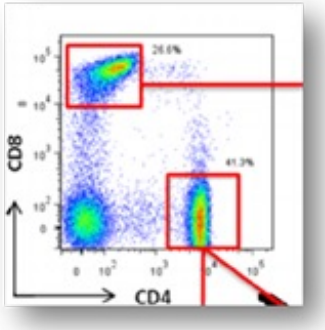


Different validation parameters apply for different analytical platforms

Platform	Read-out	Performance characteristics							
		Accuracy	Trueness (bias)	Precision	Assay range	Sensitivity	Reproducibility	Specificity	Parallelism
Mass spectrometry	Definitive / relative quant	✓ / ✗	✓	✓	LLOQ - ULOQ	✓	✓	✓	✓
LBA	Relative quant / quasi-quant	✗ / ✗	✓ / ✗	✓ / ✓	LLOQ – ULOQ / LOD	✓ / ✓	✓ / ✓	✓ / ✓	✓
Flow	quasi-quant	✗	✗	✓	✓	✓	✓	✓	✗
IHC	Semi-quant / qualitative	✗ / ✗	✗ / ✗	✓ / ✗	✓ / ✗	✓ / ✓	✓ / ✓	✓ / ✓	✗
qPCR	Relative quant	✗	✓	✓	✓	✓	✓	✓	✗

Different technologies can present unique challenges when it comes to specific validation parameters

Flow Cytometry



- There are several white papers
  - O'Hara 2011
  - CSLI H62 document
- Challenges to validation
  - Logistics
    - Sample processing
    - Stability
    - Activation/fixation
  - Clear data analysis/gating strategy
    - Compensation
    - Instrument settings
  - Inter-instrument and inter-lab precision is valuable
  - Analyte availability
    - Panel evaluation
    - Performance/instrument/gating controls
    - Precision of rare events

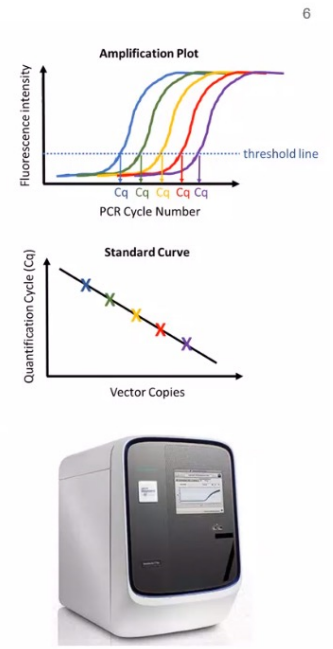
Different technologies can present unique challenges when it comes to specific validation parameters

IHC

- Guidance from pathology sector
- Challenges to validation
  - Identification and/or availability of suitable cell and tissues
  - Choose an appropriate IHC method
    - Detection system (fluor vs chromogenic)
    - Sample type (frozen vs FFPE)
    - Antibody selectivity / specificity for target epitope
  - Appropriate level of analytical validation
    - Specificity using appropriate controls and based on expected staining patterns
    - Precision – how many sections, how many individuals
      The intrinsic variability of serial tissue sections needs to be considered when defining the acceptance criteria
  - Appropriate level of validation for data analysis
    - IHC image analysis: either manual or digital
    - Train multiple analysts or algorithm, with pathologist oversight, to ensure reproducibility in clinical use

Different technologies can present unique challenges when it comes to specific validation parameters

qPCR



- There are several white papers
  - Gorovits et al. 2019 Bioanalysis
  - Ma et al. 2020 Molecular Therapy
- Several consortia recommendations, including 2 EBF papers
- Challenges to validation
  - Extensive pre-analytical workflow
    - Homogenization (tissues only)
    - DNA extraction efficiency & quantitation
  - Primer design and optimization
    - Careful target sequence selection
    - Optimized concentration for lowest Ct value

Additional challenges to focus on in the non-PK BMV world


Annoyance


Up-hill battle


Massive headache


No problem!

	Endogenous QC	Pre-analytical variability?	Sample preparation requirement	Specialist Data analytics	Critical Reagent monitoring	CRO availability	Throughput/ Automatability
Flow							
IHC							
RT-qPCR							
LBA							
LC-MS							

Conclusions

- One biomarker assay may look very different to that of another because of the nature of the analyte, the matrix, the indication…hence assays may have different applications and the challenges will be unique in each case.
- The range of technologies being employed to measure biomarkers is considerably broader than just LBA and LC/MS – but each comes with their own specific advantages, disadvantages or limitations.
- You must undertake careful consideration of the strengths/limitations of the chosen technology, and how these impact its suitability. The selected technology platform must be appropriate to support the CoU.
- For many technologies, falling back into a validation plan based on PK-level criteria is not practical, and in most instances, would not even be possible.
- Start with a blank page and the CoU instead of a PK method validation template!

Acknowledgements

Lien Dejager
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Contact Information

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