EBF European Bioanalysis Forum

What Should Not be in the Guideline for LBA?

Presenter: Jo Goodman
on behalf of the EBF

Focus Workshop

(*In collaboration with the AAPS and JBF*)

Industry input into ICH M10: Experimental data as the cornerstone for a science driven bioanalytical guideline

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The problem statement today

- LBA assays have distinct differences from chromatographic methods
- Guidance for Ligand Binding Assays (LBA) grew from existing language rather than starting with a blank page
  - Trying to fit into an existing framework
  - Examples of chromatography “creep”
  - Different regions have different requirements
  - Whilst some documents cover LBA relatively well, others do not include it or have irrelevant components
- Guidance has evolved over time
  - For example trying to fit biomarkers to PK requirements
  - Will all LBA method types find their way into guidance?

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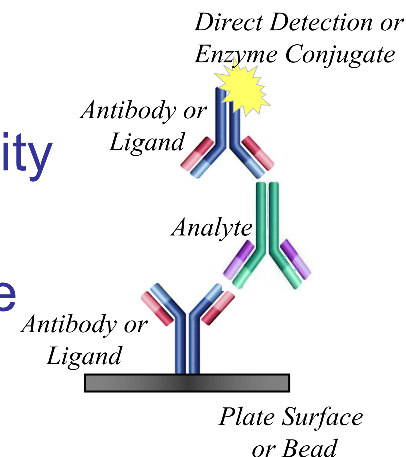
Ligand biniding assays (LBA) differ from chromatography methods



- LBA is generally the method used for large molecule quantification as well as biomarkers and immunogenicity
- Variety of platforms and reporter systems
- Require specifically developed reagents for the analyte based on binding events
- Specific assays for free, total or bound analyte
- Reduced sample run capacity (usually plate/CD/beads)
- In the majority of cases there is no prior extraction of the molecule before analysis
- Samples generally run in duplicate although there is a move to a single replicate
- Generally non-linear curves with anchor points
- Range can be narrow
- Samples undergo a minimal required dilution (MRD) and may be further diluted into assay range
- Prone to matrix/sample interferences

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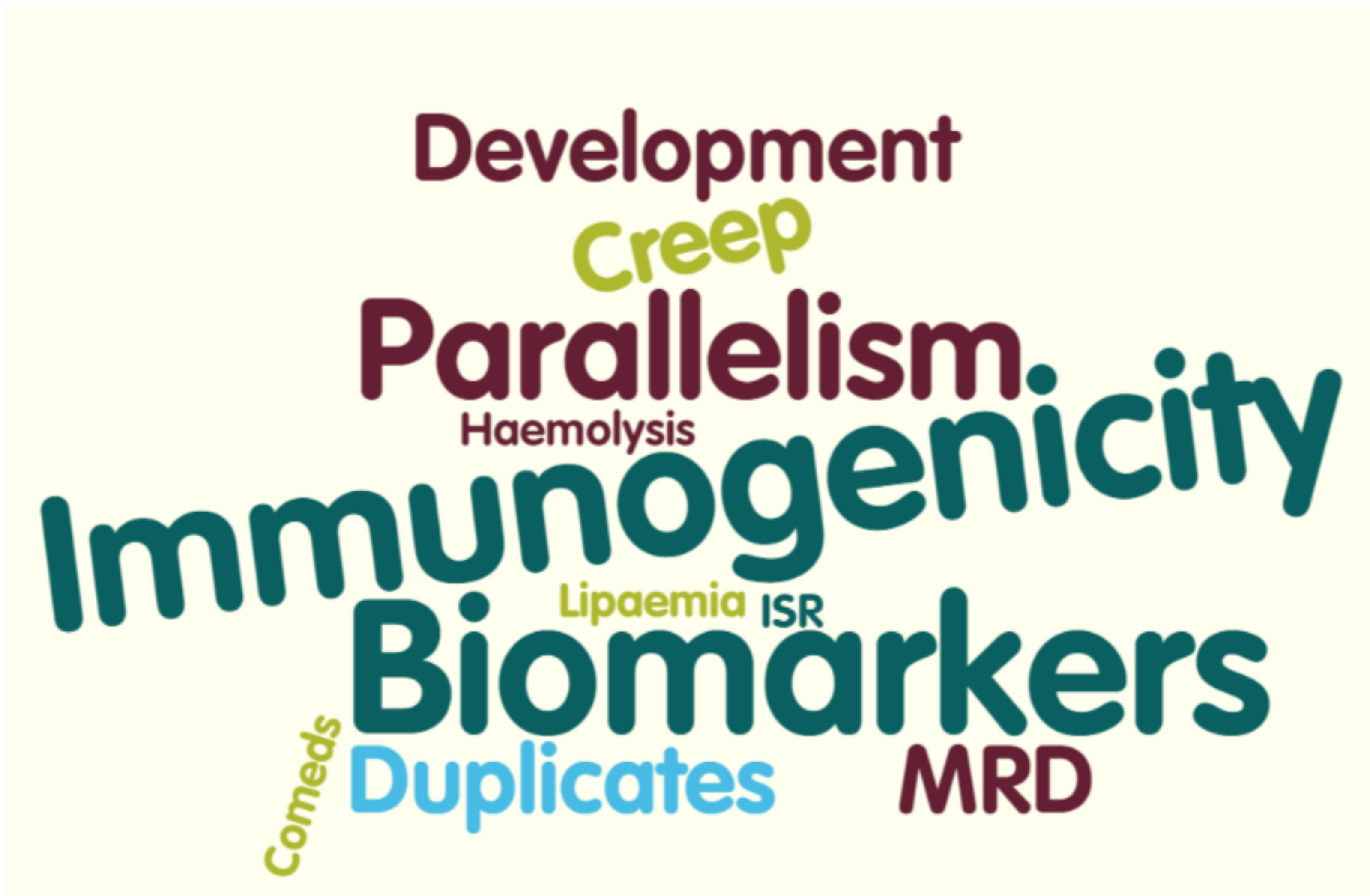
Our members said ....



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Biomarkers assays are not PK assays

Table 1. Examples of common differences for PK and PD immunoassays, where an antibody is the biotherapeutic drug molecule.

Attribute	PK assay for antibodies	PD for biomarkers
Molecule detected	▪ Drug	▪ Antigen/ligand
Quantitative	▪ Considered fully quantitative	▪ Generally considered not fully quantitative
Sensitivity commonly required	▪ µg/ml to ng/ml	▪ ng/ml to pg/ml
Capture reagent	▪ Anti-IgG antibody ▪ Anti-idiotype antibody ▪ Target antigen	▪ Anti-analyte antibody ▪ Soluble receptor
Reference calibrator	▪ Drug molecule itself ▪ Usually well characterized	▪ Often recombinant form of the antigen ▪ May change from vendor-to-vendor or lot-to-lot ▪ Occasionally available in purified endogenous form
Analyte stability	▪ Generally stable during multiple freeze–thaw cycles	▪ May be more prone to analyte instability, especially on initial freezing or repeated freeze–thaw cycles
Calibrator matrix	▪ Same as the sample type at minimal required dilution	▪ Substitute matrix may be used ▪ Matrix depleted of the analyte of interest is beneficial when significant circulating endogenous levels of antigen exist
Minimal required dilution	▪ Often higher than in PD assays in order to overcome matrix interference	▪ Dilution kept to a minimum to avoid disrupting equilibrium
Examples of interfering substances	▪ Soluble target ▪ Anti-drug antibodies ▪ Endogenous IgG	▪ Anti-drug antibodies ▪ Soluble receptor ▪ Binding proteins
Kit availability	▪ Universal IgG assays can be developed or available as kits	▪ Kits for specific analytes may be available ▪ Singleplex or multiplexed assay with analytes within a similar physiological range
Regulation	▪ Regulated	▪ Not regulated but required to be 'fit-for-purpose'

Goodman and Agoram (2013)

➢ *Recommendation: biomarkers should not be part of Guideline*

Immunogenicity assays are not PK assays

Component	Quantitative Immunoassays (PK)	Immunogenicity Assays
Reference standard	• Fully characterised • Dosed molecule	• **Surrogate positive control** (PC) • **Negative control** (NC)
Standard curve	• Constructed with reference material • Used in sample testing	• Serial dilution of surrogate PC • Not used in sample testing
Sensitivity	• Defined by accuracy and precision of a QC	• Uses a threshold measurement (**cut point**) defined on variability
Accuracy and precision	• Both determined	• Accuracy is not relevant • Only precision is determined • PCs and NCs
Interferences	• Matrix components • Other medications • ADA	• Free (dosed) drug • Endogenous counterpart to drug • Target interference • Matrix components • Pre-existing antibodies

➤ *Recommendation: Guideline is not expanded to cover immunogenicity*

LBA are not chromatographic methods

- Examples of creep (not exhaustive)
  - "tracers"
  - "cross-reactivity of metabolites, concomitant medications, and their significant metabolites …."
  - "Where possible, LBA should be compared with a validated reference method (such as LC-MS)"
  - "sample extraction"
  - ULOQ requiring 20% RE rather than 25%
  - "The calibration curve should cover the expected study sample concentration range"
  - "…. the standard curve should be extended and revalidated"
  - "QCs should be interspersed with study samples"
  - "If the study sample concentrations are clustered in a narrow range of the standard curve, additional QCs should be added"
  - "All study samples from a subject should be analyzed in a single run"

- *Recommendation: separate and entire sections for LBA and chromatography and any contamination from LC-MS is removed*

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LBA does not necessairly require duplicates

- Duplicates for LBA came about due to precision issues (or perceived issues)
- Established LBA methods are generally robust
- The exclusion of sample results due to poor precision is generally low
- ISR will flag any issues
- Consideration: what is the impact on TK/PK parameters (AUC, CL, $T_{1/2}$) for your method if a single replicate is used

- *Recommendation: flexibility should be built into the guidance to allow the use of single replicates*

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Method development defines the assay that goes into validation

- Validation is confirming what you already know!
  - Passing validation is dependent on robust method development
  - Parameters that are to be tested in validation are usually assessed in method development
- LBA PK assays utilise a minimal required dilution (MRD)
  - Matrix component interference
  - Set in method development and does not change in validation
- *Recommendations:*
  - *Method development is not required in guideline*
  - *MRD is not a validation parameter but a component of the actual assay method*
  - *Provide a one page summary of method development and assay history (should you wish to answer regulatory questions)*

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Parallelism is important for biomarker assays but necessarily PK assays

- "*A performance characteristic that is typically evaluated during in-study validation. It is **conceptually similar to dilutional linearity** except that it is assessed with **multiple dilutions of actual study samples or samples that represent the same matrix and analyte combination** as those that will be generated during a study*" DeSilva *et al.* (2003)

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Parallelism should not be a routine assessment for PK assays

- Rarely possible in pre-study validation due to unavailability of incurred samples
  - Requires appropriate informed consent language to allow “future use”
- Generally rare - examples of PK non-parallelism are generally seen with non-mAb products (selectivity experiments are performed)
- If samples are not expected to be >ULOQ then parallelism will be redundant
- EMA requests Cmax samples diluted to 3 concentrations (within 30% precision)
- Consideration: Should it be tested in the first clinical single-dose study of until the final dosing paradigm has been established? Should different populations be assessed? Samples vs. Pools and if they fail is it the sample or the assay that has failed? Stevenson and Purushothama (2014)
- Consideration: dilution of samples can be enough to dissociate complexes which makes the assay non-parallel
- Does it make sense to always include parallelism as a routine parameter?
  - 88% of EBF members are performing this regardless

GBC L2 recommends that parallelism is not a routine validation parameter

- GBC L2 recommendation: *"the need to perform a parallelism assessment for a given biotherapeutic depends upon the **characteristics of the drug, its binding partners, and assay reagents' specificity**"*
  - Aggregation, presence of anti-drug antibodies, how stable the drug is *in vivo*, presence of endogenous binding partners and which form is being detected *in vivo* (free or bound)

- *Recommendation: Perform parallelism on a case by case basis and not as a routine parameter. Utilise a priori decision trees for when to assess.*

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Lipemic and hemolysed samples may not always be needed in validation

- Depends on the biology
- Depends on the matrix of the sample being tested
- May depend on the stage of development

- *Recommendation: should be assessed when there is a scientific reason for evaluation and not as a checklist exercise*

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Summary and key messages

- Exclude contamination from Biomarker and Immunogenicity BMV
  - They are not PK assays!
- Flexibility for single replicate analysis should be built into guidance for LBA methods
- Method development activities should be outside the scope of ICH M10
- Chromatographic principles that do not apply to LBA should be removed
  - Entire and separate sections should be authored for chromatography and LBA to avoid cross referencing
- The requirement for routine assessment of parallelism for PK assays should be on a risk-based approach as per GBC L2 recommendations
- Routine assessments in haemolysed and hyperlipidaemic matrices for LBA are not required unless there is scientific basis

Acknowledgements

- EBF community
- EBF steering committee

Contact: info@europeanbioanalysisforum.eu

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