Use of surrogate matrices in LBA PK assays

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Focus Workshop

(*In collaboration with the AAPS and JBF*)

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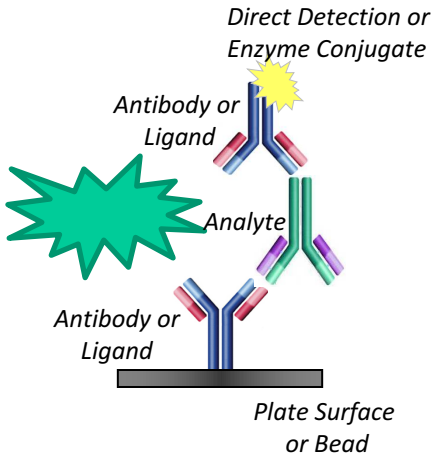
Use of surrogate matrices in LBA PK assays

Surrogate Matrix -definition

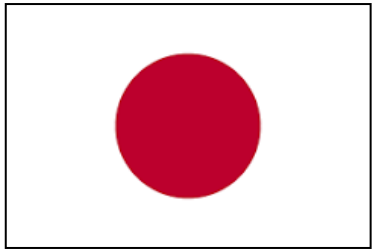
- Substitute matrix used to prepare calibrators that is free of the target analyte and mimics (or is representative for) the behavior of the biological sample matrix

Facts about LBA



- Ligand binding assays rely on high-affinity binding between the analyte and the capture & detection reagents
- LBA do not use a sample extraction step
- Other components in the matrix can interact with the assay reagents => affect the accurate quantification of the analyte by either decreasing or enhancing the signal

Bioanalytical Guidance/Guidelines



FDA, 2013 draft	EMA, 2011	MHLW, 2014
Calibrators should be prepared in the same matrix as the study samples. If an alternate matrix is used, proper justification should be provided.	The calibration standards should be prepared in the same matrix as the matrix of the intended study samples by spiking the blank matrix with known concentrations of the analyte	A surrogate matrix may be used to prepare calibration standards and QC samples. However, the use of a surrogate matrix should be justified as much as possible in the course of establishing the analytical method.

Use of surrogate matrices

➢ FDA draft guidance

A Calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking matrix with known concentrations of the analyte. In rare cases, matrices may be difficult to obtain (e.g. cerebrospinal fluid). In such cases, calibration curves constructed in surrogate matrices should be justified.

Use of surrogate matrices

➢ EMA guideline

7.1.1.5. Matrix selection

The measurement of some macromolecules may not be possible in complex matrices **without extraction due to high interferences with high levels of structurally related endogenous compounds**. Although the use of extracted matrix (e.g. **charcoal, immuno-affinity**) or alternative matrix (e.g. **protein buffers, dialysed serum**) is not recommended, the use of such matrices may be necessary when there is no other strategy to quantify the analyte of interest. The calibration standard curve may be prepared in these surrogate matrices. QC samples should be prepared in the actual sample matrix and the accuracy should be calculated to demonstrate the absence of matrix effect.

Calibrator curve

Sample Matrix

Surrogate Matrix

Always

Except when

Rare matrices
High level endogenous protein

QCs ?

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Surrogate Matrix

- Rare matrices
  - Cerebrospinal fluid
  - Tissue
  - Synovial fluid

- High levels of structurally related endogenous compounds
  - Replacement therapy (Hormone, enzyme, protein)

Challenges with surrogate matrix

- Calibrator matrix
  - Buffer, Ab-stripped matrix, charcoal matrix, alternative species matrix?
- QC matrix
  - Sample matrix?
- Assignment of nominal value for QC samples
  - How to assign nominal values → Accuracy?
- LLOQ determination
  - How to determine LLOQ when endogenous counterpart is present >LLOQ?

EBF Topic Team TT-51

- High levels of structurally related endogenous compounds

- Burning questions for how to do
  - Matrix selection for preparation of calibration standards
  - Preparation of QC samples
  - LLOQ

… more burning questions

- Generate specific reagents to the analyte
  - But what if this is not possible?
- Screen multiple individual lots of matrix to generate a pool with low endogenous level of analyte
  - But how do you ensure sufficient rare matrix for long-term studies?
- A solution is to raise the assay LLOQ
  - But what if a sensitive assay is needed?
- High level endogenous analogue?
  - A challenge to subtract pre-dose from post-dose

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Recommendations PK-assay

Based upon data from EBF survey and discussion within EBF

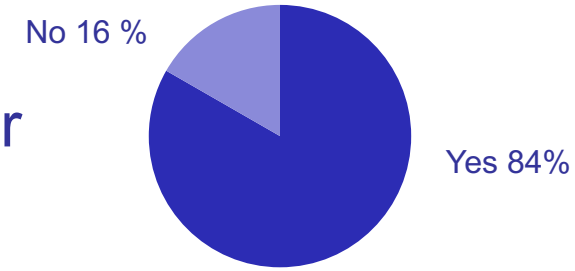
- Calibrator matrix
- QC matrix
- Assignment of a nominal QC value
- LLOQ determination

Calibrator matrix - challenges

- Extracted matrix
  (e.g. charcoal, immuno-affinity)
- Protein-based matrix

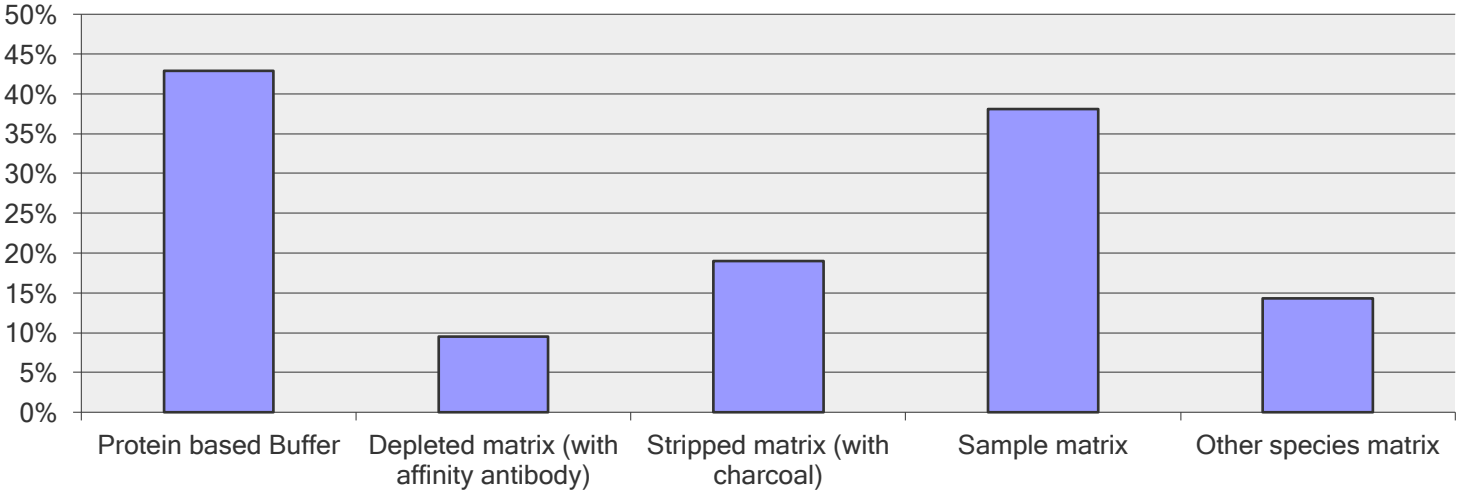
Calibrator matrix

In case you use a surrogate matrix for either calibrator or QC do you describe the scientific rationale for this in e.g. Validation plan/Report?



Which Matrix do you most often use for preparation of Calibrator?

For high endogenous proteins



Have you received feedback from regulatory authorities regarding use of surrogate matrix?

- No: 83.3%
- Yes: 16.7 %

but always positive (well accepted)

TT-51 – Endogenous protein

Antibody depleted serum to be used to prepare calibrator & QC matrix

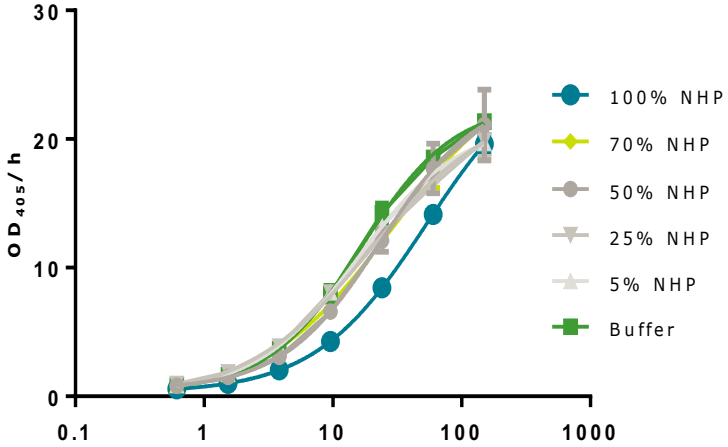
- Procedure
  - Sepharose-beads with non-competing antibody were used
  - Depleted matrix was compared to buffer curve and was parallel to this
- Issues
  - Poor recovery
  - Matrix depleted again but now with less output
  - Can be difficult to obtain non-competing Ab

TT-51 – Endogenous protein

CASE STUDY

BSA-Buffer for preparation of Calibrators

- Procedure
  - Calibrators prepared in BSA-buffer
  - QCs prepared in matrix



- Issues
  - None !
  - Recovery of QCs was acceptable
  - Samples successfully analysed

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Recommendations for Calibrator matrix

- Protein based buffer* (PBB) first choice if neat sample matrix is not an option
  - Sample matrix mostly used because this is described in guidelines/white papers, but there is no scientific rationale for this.
  - It can be difficult to get consistency when using depleted matrix and it is not equal to sample matrix anyway
  - Based on EBF members experience with authorities it seems to be accepted to use PBB also for PK assays

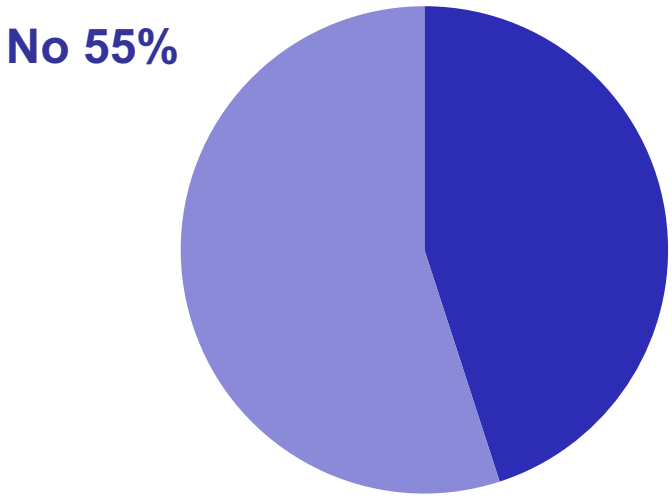
* PBB = Protein based buffer (e.g. PBS + BSA)

QC matrix - challenges

- Extracted matrix (e.g. charcoal, immuno-affinity)
- Individual matrix with low endogenous levels

QC matrix

- Have you ever used other matrix than sample matrix for QC samples?



No regulatory feedback on using a surrogate matrix for QC preparation

TT-51 – Endogenous protein

Charcoal depleted serum from a commercial supplier

- Procedure
  - Determine endogenous level in matrix pool in order to make QC pool
  - LLOQ prepared by dilution of QC pool with depleted serum
- Issues
  - when ordering of new depleted pool it was not the same as the first pool although CoA say so….

TT-51 – Endogenous protein

Screening of individual matrix

- Procedure
  - Screen matrix from several individuals against buffer curve
  - Buy enough of low level matrices to prepare a large pool for future studies
  - Spike QCs into pools
- Issues
  - Cumbersome

Recommendations for QC matrix

- Sample matrix should always be used

- Problems arise when endogenous levels are higher than the LLOQ of the assay – especially for Low QC
- The low QCs should be prepared in:
  (in order of preference)
  1. Individual matrix with low endogenous concentration
  2. Analyte depleted matrix
  3. Alternative species matrix

- Alternative matrix for rare matrices may be considered, e.g. protein based buffer

The QCs should resemble your sample

Recommendations: Assignment of nominal value for QCs

- Endogenous < LLOQ:
  - Use the spike level plus the endogenous level
  - Assign the “nominal values” in min 3 runs with 6 replicates
- Endogenous > > LLOQ
  - Use the endogenous levels in matrix for preparation of the 3 levels of QCs
  - Screen individual samples (n=10-100) to be used as QC samples
  - Assign the “nominal values” in 3 runs with 6 replicates
- Endogenous > LLOQ
  - Use a combination: low QC = endogenous, Mid and High = spike + endogenous level
- Endogenous > ULOQ
  - Consider new assay range!

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Recommendations for LLOQ determination

- Endogenous > LLOQ (or close to LLOQ)
  (in order of preference)
  1. Use lowest calibrator point for precision and assay range determination (in buffer)
  2. Use analyte depleted matrix
     - Spike LLOQ at lowest calibrator level
  3. Dilute sample (with buffer) down to LOD and spike at LLOQ
     (not commonly used in EBF community)
- Endogenous < LLOQ:
  - Use the spike level plus the endogenous level
  - Assign the “nominal” value in min 3 runs with 6 replicates

- Endogenous << LLOQ
  - Spike and use the spike level as nominal value

Conclusion & recommendations Surrogate Matrix

For rare matrices and high endogenous counterpart

- Calibrator matrix
  - Protein based buffer (e.g. PBS+ BSA)
- QC matrix
  - Sample matrix as starting point
  - Alternative matrix for rare matrix may be considered
- Assignment of nominal value for QC samples
  - Assign nominal value on total content (spiked plus endogenous) in QC matrix
- LLOQ determination
  - Use lowest calibrator point

Acknowledgement

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