Workshop

Towards harmonised implementation of the ICH M10 Guideline

Chapter 4

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Themes/questions discussed today

- Singlicate sample analysis in LBA assays
- Reference standard
- Critical reagents
- QC sample preparation and selection of concentrations
- Dilutional linearity
- Stability
- Selectivity
- Surrogate matrix

Singlicate sample analysis in LBA assays

4.2 Validation

Most often, microtiter plates are used for LBAs and study samples can be analyzed using an assay format **of one or more well(s) per sample**. The assay format should be specified in the protocol, study plan or SOP. **If method development and method validation are performed using one or more well(s) per sample, then study sample analysis should also be performed using one or more well(s) per sample, respectively.** ...

EBF position:

European Bioanalysis Forum recommendation on singlicate analysis for ligand binding assays: time for a new mindset

Matthew Barfield, Joanne Goodman, John Hood & Philip Timmerman, Bioanalysis 2020

EBF proposal for implementation

- Implement singlicate well analysis as appropriate
- If assay validation was performed in duplicate yet data show that singlicate analysis is robust then singlicate sample analysis is justified

Reference standard

4.1.1. Reference standard

"It is recommended that the manufacturing batch of the reference standard **used for the preparation of calibration standards and QCs** is **derived from** the **same batch of drug substance** as that **used for dosing** in the nonclinical and clinical studies whenever possible"

EBF proposal for implementation:

If CMC assessments show comparability of drug substance batches, the reference standard is deemed appropriate for bioanalytical purposes

Critical reagents

4.1.2 Critical Reagents

"Critical reagents, including binding reagents ... have direct impact on the results of the assay and, therefore, their quality should be assured. Critical reagents bind the analyte and, upon interaction, lead to an instrument signal corresponding to the analyte concentration. **The critical reagents should be identified and defined in the assay method… ."**

"A critical reagent lifecycle management procedure is necessary to ensure consistency between the original and new batches of critical reagents. Reagent performance should be evaluated using the bioanalytical method. ..."

"It can be extended beyond the expiration date from the supplier."

EBF position and EBF proposal for implementation:

- It is positive that these considerations were included in M10
- EBF recommendation on practical management of critical reagents for PK ligand-binding assays

Susanne Pihl, Barry WA van der Strate, Michaela Golob, Laurent Vermet, Birgit Jaitner, Joanne Goodman, Marianne Scheel Fjording & Philip Timmerman, Bioanalysis 2018

QC sample preparation and selection of concentrations

4.2.4.1 Preparation of Quality Control Samples

- The QCs are intended to mimic study samples and should be prepared by spiking matrix with a known quantity of analyte, **stored under the conditions anticipated for study samples** and analysed to assess the validity of the analytical method.
- **The analyte should be spiked at the LLOQ, within three times of the LLOQ (low QC), around the geometric mean of the calibration curve range (medium QC), and at least at 75% of the ULOQ (high QC) and at the ULOQ**.

EBF proposal for implementation:

- Confirm that QCs are spiked correctly before freezing
- Use QCs that reflect samples for validation assessment
- It is positive that geometric mean is used for the mid QC

QC sample preparation and selection of concentrations

4.2.4.2 Evaluation of Accuracy and Precision

"If the within-run accuracy or precision criteria are not met in all runs, an overall estimate of within-run accuracy and precision for each QC level should be calculated."

EBF proposal for implementation:

- Follow DeSilva et al (2003)

Pharmaceutical Research, Vol. 20, No. 11, November 2003 (© 2003)

Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules

Binodh DeSilva,[1] Wendell Smith,[2] Russell Weiner,[3] Marian Kelley,[4,11] JoMarie Smolec,[5] Ben Lee,[6] Masood Khan,[7] Richard Tacey,[8] Howard Hill,[9] and Abbie Celniker[10]

Table VIIA. Precision and Accuracy Numerical Example. Replicate Results are Analytical Data from an Immunoassay for a Therapeutic Protein. Statistics were Calculated in a Excel Spreadsheet by an Analysis of Variance (ANOVA). Symbolic Notation for all Data Values are Listed in Table VIIB with Formulae Defined in Appendix A.

		Replicate results			Intrabatch (within-run) statistics					
Sample	Batch run	1	2	3	n	Mean	SD	%CV	%RE	Ancillary statistics
QC 4 50 (ng/mL)	1	47.6	48.1	52.2	3	49.3	2.52	5.0	−1.4	MS_w = 9.320
	2	42.0	41.4	43.7	3	42.4	1.19	2.4	−15.3	MS_b = 59.444
	3	72.4X	53.1	45.8	2	49.5	5.16	10.3	−1.1	MS_t = 24.984
	4	53.4	55.3	54.5	3	54.4	0.95	1.9	8.8	s_t = 4.998
	5	45.6	42.6	51.5	3	46.6	4.53	9.1	−6.9	s_b = 4.213
	6	46.5	42.3	40.8	3	43.2	2.95	5.9	−13.6	p = 6
	Intrabatch (within-run) statistics (Pooled):				2.88	47.4	3.05	6.1	−5.1	
	Interbatch (between-run) statistics (ANOVA):				17	47.5	5.20	10.4	−5.0	

X—Analytical error, value omitted from computations.

QC sample preparation and selection of concentrations

4.3.1 Analytical Run

*"The QCs should be **placed in the run** in such a way that the accuracy and precision of the whole run is ensured taking into account that study samples should always be bracketed by QCs."*

EBF proposal for implementation:

- In this instance bracketing means physical placement and not concentration

Dilutional linearity

4.2.6 Dilution Linearity and Hook Effect

"The same matrix as that of the study sample should be used for preparation of the QCs for dilution."

"For each dilution factor tested, **at least 3 independently prepared dilution series** should be performed using the number of replicates that will be used in sample analysis."

"The **dilution factor(s) applied during study sample analysis should be within the range of dilution factors evaluated** during validation."

EBF proposal for implementation:

- The use of surrogate matrix may be justified (e.g., free drug) with supporting data
- It is positive that it is accepted to use 3 independently prepared dilution series
- Dilution QCs do not need to be included in sample analysis if within range of dilution factors tested in validation

Stability

4.2.7 Stability

“Stability evaluations should be carried out to ensure that **every step taken during sample preparation**, processing and analysis as well as the storage conditions used do not affect the concentration of the analyte.”

EBF proposal for implementation:

- Whole blood stability is generally not necessary for large molecules if stability in plasma/serum has been demonstrated under the same conditions unless the analyte is known to behave differently in the presence of blood cells
- Stability does not have to be repeated in a second laboratory if conditions and matrix have not changed. The stability report needs to be available and reviewed.

Stability

4.2.7 Stability

“Since sample dilution may be required for many LBA methods due to a narrow calibration range, the concentrations of the study samples may be consistently higher than the ULOQ of the calibration curve. If this is the case, the **concentration of the QCs** should be adjusted, considering the applied sample dilution, to **represent the actual sample concentration range**.”

“For biological drugs, a bracketing approach can be applied, e.g., in the case that the stability has been demonstrated at -70/-80C and at -20C, then it is not necessary to investigate the stability at temperatures in between those two points at **which study samples will be stored**.”

EBF proposal for implementation:

- A single high level stability QC should represent the expected sample concentration (eg close to Cmax)
- The length of the stability assessment must cover the storage time and temperature.
- A bracketing approach can be used between -20C and -80C

Selectivity

4.2.2 Selectivity

"For lipaemic and haemolysed samples, tests can be evaluated once using a single source of matrix. Selectivity should be assessed in s**amples from relevant patient populations** (e.g., renally or hepatically impaired patients, inflammatory or immuno-oncology patients if applicable). **In the case of relevant patient populations, there should be at least five individual patients."**

EBF proposal for implementation:

- In cases where insufficient samples are available for validation consider in study selectivity assessment
- 1 lipaemic and 1 haemolysed sample can be included in the 10 individuals
  European Bioanalysis Forum: recommendation on dealing with hemolyzed and hyperlipidemic matrices. Benno Ingelse, Begona Barroso, Nicholas Gray, Verena Jakob-Rodamer, Clare Kingsley, Corinna Sykora, Petra Vinck, Martina Wein, & Stephen White. Bioanalysis 2014.

Surrogate matrix

2.2.1. Full validation

"In some cases, it may be difficult to obtain an identical matrix to that of the study samples (e.g., **rare matrices** such as tissue, cerebrospinal fluid, bile **or** in cases where **free drug** is measured). In such cases, **surrogate matrices may be acceptable** for analytical method validation."

EBF proposal for implementation:

- The use of surrogate matrix for calibrators, QCs and sample dilution may be justified with supporting data.

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Contact Information

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