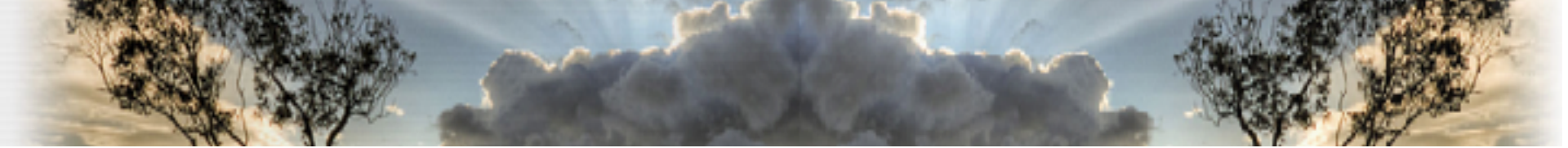

Guideline paragraphs anticipated not needing a discussion

Robert Nelson, on behalf of the EBF

Paragraph from ICH M10 covered in plenary

- The following topics were discussed in plenary on Day 1:
  - **Scope**
  - **Method development**
  - **Full & partial validation**
  - **Cross-validation**
  - **Stability testing**
    - Benchtop & freeze/thaw
    - Blood stability
    - Long term

Paragraph from ICH M10 covered in plenary

- The following topics will discussed in plenary on Day 3:
  - **ISR**
  - **Documentation**
  - **Glossary**
  - **Repeat analysis**

Paragraph from ICH M10 for LBA

- The following topics were discussed in the Morning Parallel Sessions:
  - **Commercial & diagnostic kits**
  - **New & alternative technologies**

- The following topics will be discussed in this LBA Break-Out Session:
  - **Reference standards** & **critical reagents**
  - **Use of surrogate matrix** in calibration, dilution and QCs
  - **Analytes that are also endogenous compounds**

Paragraph from ICH M10 for LBA

- The following topics will be discussed in the Afternoon LBA Break-Out Session:
  - **Stability** assessments
  - **Calibration curve & range**
  - **Specificity** & **selectivity**
  - **Dilutional linearity**, **hook effect** & **parallelism**
  - **Documentation** & **glossary**

Paragraph from ICH M10 for LBA

- The following topics will **not** be covered specifically during the LBA breakout sessions:
  - **Minimum required dilution (MRD)**
  - **Accuracy** & **precision**
  - **Carry over**

- In this presentation, we will present a brief overview on these topics

Have we missed something…?

- If you feel we have not covered sometime important in the Focus Workshop, or we did not cover LBA aspects in enough detail for one of the common topics, please speak up during the **panel discussions** at the end of each break-out session…

Minimum Required Dilution (MRD)

MRD - Paragraphs from ICH M10

7.4 Minimum Required Dilution

- MRD is a dilution factor employed in samples that are **diluted with buffer** solution **to reduce the background signal or matrix interference** on the analysis using LBA.
- The MRD should be identical for all samples including calibration standards and the QCs and it should be determined during method development.
- If **MRD is changed** after establishment of the method, **partial validation is necessary**.
- MRD should be **defined in the Validation Report** of the analytical method.

MRD - Feedback from EBF Strategy Meeting

- More flexibility would be desirable. If absence of any matrix effect is shown during validation e.g. overlapping calibration curves independent of the MRD, varying sample dilutions during sample analysis would be made possible.
  - Example: if a sample is BLQ in an assay running in 10% matrix (1/10 dilution), reanalysis at a lower dilution (higher % of matrix) such as 50% matrix (1/2 dilution) or 100% matrix (no dilution) would be possible to increase the sensitivity.
  - This would be highly valuable for dose escalation studies, particularly when MABEL approach is applied.

MRD - Feedback from Delegates

MRD should be identical for all samples including calibration standards and the QCs

- Agree that QCs should be treated in the same way as samples, but may be occasions when calibration standards are prepared differently

- e.g. For an assay with 1/10 MRD, may prepare calibrators in 10% matrix and not apply a dilution to respect 3R principles

MRD should be ~~defined~~ ***documented*** in the Validation Report of the analytical method

- Defined in Method Development, confirmed in validation

MRD – EBF Comments to EMA/EWG

- EBF proposes no changes to this section

  - As written, it provides appropriate framework for performing assessment
    - Strongly support wording that MRD is assessed in method development

Accuracy & Precision (A&P)

A&P - Paragraphs from ICH M10

4.2.4 Accuracy and Precision

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 dilution series for the preparation of the QCs should be completely independent from the dilution series for the preparation of calibration standard samples. They may be prepared from a single stock provided that its accuracy has been verified or is known. The QCs should be prepared at a minimum of 5 concentration levels within the calibration curve range: 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***.

A&P - Paragraphs from ICH M10

4.2.4.2 Evaluation of Accuracy and Precision

- Accuracy and precision should be determined by analysing the QCs within each run (**within-run**) and in different runs (**between-run**). Accuracy and precision should be evaluated using the same runs and data.
- Accuracy and precision should be determined by analysing **at least 3 replicates per run** at each QC concentration level (LLOQ, low, medium, high, ULOQ) in **at least 6 runs over 2 or more days**.
- Reported method validation data and the determination of accuracy and precision should **include all results obtained**, except those cases where ***errors are obvious and documented***.

A&P - Paragraphs from ICH M10

4.2.4.2 Evaluation of Accuracy and Precision

- Within-run accuracy and precision data should be reported for each run. 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. Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.
- The **overall within-run** and **between-run accuracy** at each concentration level should be **within ±20% of the nominal values**, except for the LLOQ and ULOQ, which should be within ±25% of the nominal value.
- **Within-run** and **between-run precision** of the QC concentrations determined at each level **should not exceed 20%**, except at the LLOQ and ULOQ, where it should not exceed 25%.
- Furthermore, the total error (i.e., sum of absolute value of the errors in accuracy (%) and precision (%)) should be evaluated. The **total error should not exceed 30%** (40% at LLOQ and ULOQ).

A&P - EBF position on the subject

- Toward decision-based acceptance criteria for Bioanalytical Method Validation: a proposal for discussion from the European Bioanalysis Forum
  - Timmerman et al. Bioanalysis (2018) 10(16): 1255–1259
    - https://www.future-science.com/doi/10.4155/bio-2018-0131

- EBF Focus Workshop, Lisbon 2017:
  - Steve White, Run Acceptance Criteria
    - http://www.e-b-f.eu/wp-content/uploads/2018/06/fw201709-11.-Steve-White-Run-acceptance-criteria.pdf

A&P- Feedback from EBF Strategy Meeting

- It is not mentioned whether fresh QC are needed for Accuracy and Precision runs
- It is not clear what is meant in the sentence “accuracy and precision should be evaluated using the same runs and data”.
  - Does it mean that the same data set should be used to assess inter- and intra-assay variation?

A&P- Feedback from EBF Strategy Meeting

- Several comments on "***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***."
  - Please define "overall within-run"
  - Intention of sentence not clear.
  - We are not sure what this sentence means. What should an overall estimate be? How would the reporting look?
  - Should an ANOVA be used to calculate the overall within-run, between run and total precision and accuracy?

A&P- Feedback from Delegates

It is stated that QCs should be prepared within the calibration curve range. How does this comply with 4.3.3 Calibration Range: At least two QCs sample levels should fall within the range of concentrations measured in study samples

A&P might fail in a target capture assay/free drug assay if the ligand containing matrix is used for QC preparation!

A&P- Feedback from Delegates

- Several comments on "***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***."

Not clear what is meant by "overall estimate of …" - Same as between-run?

Please consider to include a full example table as Appendix

What does an overall estimate mean? Add examples

Please specify what is meant by "overall estimate of …"

Carry-Over

Carry-Over - Paragraphs from ICH M10

4.2.5 Carry-over

- Carry-over is generally not an issue for LBA analyses. However, if the assay platform is prone to carry-over, the potential of carry-over should be investigated by placing blank samples after the calibration standard at the ULOQ.
- The response of blank samples should be below the LLOQ.

Carry-Over - Feedback from EBF Strategy Meeting

- Carry-over is generally not an issue for LBA analyses. However, if the assay platform is prone to carry-over, the potential of carry-over should be investigated by placing blank samples after the ***highest calibration standard***.
  - Highest standard suggested instead of standard at ULOQ, just in case of anchor point sample(s) above the ULOQ.

Carry-Over - Feedback from delegates

Lack of description for LBA; adapt to LBA

Suggested comment to EMA/EWG

Final recommendation from this presentation, which combines the original recommendation enhanced with the discussions from the panel discussions during the meeting, are captured in the summary slide deck: Recommendations from the EBF Spring FW 2019

Acknowledgements and questions

- The EBF community for survey data and feedback
- Workshop delegates for feedback ahead of the meeting
- Further questions to info@e-b-f.eu before 31 May