EBF

EBF Focus Workshop

(*sister meeting, in collaboration with AAPS/CBF/JBF*)

ICH M10 - Public consultation & Industry Feedback

Summary of recommendations from the EBF Spring FW 2019

Barcelona, 20-22 May 2019

Introduction – 1/2

- This slide deck summarises the consolidated recommendation referred to in the final slides of each presentation from the meeting

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

22

The meeting agenda

Plenary: Introduction, objective, background/scope of Guideline, Comparison of draft ICH M10 with existing Guidance/Guidelines

Scope of ICH M10 – learnings from EBF Strategy Meeting, ICH M3 (R2) and metabolite quantification

Plenary: Did we consider the world around us?

The Value of Decision-based acceptance criteria!
3Rs and surrogate matrix
Informed consent and GCP
Clinical vs. Preclinical

ICH M10: a global harmonised BMV Guideline - Regulatory perspective

Plenary: General principles of Method Development/Validation, Partial and Cross Validation

Method Development
Full & Partial Validation – LBA and CHROM
Cross validation
General Principles of Stability Testing
Benchtop and F/T
Blood stability testing
F/T and LT stability testing: intra- or extrapolation?
ISR
General aspects of Documentation & Glossary
Repeat Analysis

Breakout sessions: Chromatography

Guideline paragraphs anticipated of not needing a discussion
Considerations from the JBF for general requirements (with focus on Chromatographic assays)
Considerations on reference standards for chromatographic assays
Haemolysed/hyperlipidaemic – matrix effects
Stability assessment: considerations on FDC
Considerations on specificity and selectivity for MS/MS assays
Value of Dilution QC in batch analysis
QC samples – considerations on geometric vs. arithmetic placement of the midQC
Considerations on re-injection
Considerations from the JBF for requirements specific to chromatographic assays
Documentation & Glossary – Specific to Chromatographic assays

Breakout session: Ligand Binding Assays

Guideline paragraphs anticipated of not needing a discussion
Considerations for reference standards and key reagents
Scientific aspects for the use of surrogate matrix in calibration, dilution and QC
Analytes that are also Endogenous Compounds – Focus on LBA
Stability assessments
Considerations on calibration range during validation & sample analysis
Specificity and Selectivity
Partial validation and/or Dilution linearity & Parallelism
Documentation & glossary – Specific to LBA

Workshops

A. Dried Matrix Methods
B. New or Alternative Technologies
C. Commercial and Diagnostic Kits

Common theme throughout the workshop

- Geographical differences in implementation and execution
  - In some regions, discussions are (historically) encouraged vs. other regions will implement ICH M10 as part of their law
  - Will this effect open scientific interaction in less straightforward programs?
- Training
  - Common ICH training program is essential, for industry, regulators and inspectors, for successful implementation of the guideline.
- Ambiguity fueling continues risk of ISRc
  - Again….need for training and communication

Scope

From the survey:

- the scope definition is ambiguous
- many mention the scope is too wide.
  - may lead to virtually all studies, matrices and analytes coming in scope

Recommendation:

- Intensive training will be needed to further clarify and agree scope
  - Suggested rewording for “studies” → see next slide
  - Further clarification needed on “other matrices” re: ‘partial validation’ vs. ‘alternative approaches’
  - Interpret: “supporting a submission” (= all) vs. “make regulatory decisions” (= less)
- training should include all stakeholders (BA, industry and HA stakeholder)

Potential Scope Narrowing and Clarification

- This guideline describes the method validation that is expected for bioanalytical assays that are submitted to support regulatory submissions. The guideline is applicable to the validation of bioanalytical methods used to measure concentrations of chemical and biological drug(s) and their metabolite(s) in biological samples (e.g., blood, plasma, serum, other body fluids or tissues) obtained in *nonclinical TK studies falling under the scope of the GLPs that are used to make regulatory decisions, nonclinical PK studies that are conducted as surrogates for clinical studies, and all phases of clinical trials in regulatory submissions for which a primary objective of the study is to assess, compare or characterise drug exposure.* Full method validation is expected for the primary matrix(ces) intended to support regulatory submissions. Additional matrices should be partially validated as necessary. The analytes that should be measured in nonclinical and clinical studies and the types of studies necessary to support a regulatory submission are described in other ICH and regional regulatory documents.

Additional considerations as per discussions in the session: ‘**did we consider the world around us?'**

1. Acceptance criteria
2. GCP considerations
3. 3Rs

1. Acceptance criteria

EBF would like the industry and HA to consider an open and science based discussion on the added value of integrating harmonized decision-based acceptance criteria for PK bioanalytical assays

In this way, we create a transparent platform to facilitate the use of new technologies in the toolbox of the regulated bioanalytical scientist

2. GCP considerations

- Challenges within the bioanalytical lab to be resolved through continuous improvement and advancement of relevant GCP processes and trainings

Recommendation to EMA/EWG

Current text in ICH M10 draft	Suggested change
For studies that are subject to Good Laboratory Practice (GLP) or Good Clinical Practice (GCP) the bioanalysis of study samples should also conform to their requirements.	Add: For GCP, this implies that Sample analysis to be conducted in accordance with study protocol and only to include work covered by the informed consent

3. 3R's in the bioanalytical community

The EU based BA community feels that a (global,) science based guideline should consider animal welfare and not require unnecessary use of animals

- Replace
  - Surrogate matrix used when valid
    - Sample dilutions
    - Calibrators
- Reduce
  - Using smaller volumes of sample or matrix
    - Consider less replicates in preclinical assays
    - Reduce requirement for non-serial sampling or satellite groups
- (Refine
  - Microsampling to reduce stress)

Summary and recommendations from surveys and presentations this week

Recommendations from: **Method development**

M10 current text	Suggested changes
146-148 However, the applicant should record the changes to procedures as well as any issues and their resolutions to provide a rationale for any changes made to validated methods immediately prior to or in the course of analysing study samples for pivotal studies.	The applicant should record the changes to procedures as well as any issues and their resolutions to provide a rationale for any changes made to validated methods (i.e. Method Evolution) immediately prior to or in the course of analysing study samples for pivotal studies. Formal reporting is not required, however a proper use of the CTD detailing rationale for any changes is encouraged.
□ Reference standards □ Critical reagents □ Calibration curve □ Quality control samples (QCs) □ Selectivity and specificity □ Sensitivity □ Accuracy □ Precision □ Recovery □ Stability of the analyte in the matrix □ Minimum Required Dilution (MRD)	Delete this list In addition to deleting the list panel also suggested to delete/replace the word *optimised* (line 220) and *optimisation* (line 225) – there should not be an expectation that all parameters are fine tuned to an optimum

Recommendations from: Full Validation

No recommendation were made at the meeting. Comments were given as part of EBF pre-meeting surveys and these comments will be survey will be summarised and provided to EWG via EMA

Recommendations from: Partial Validation

No recommendation were made at the meeting. Comments were given as part of EBF pre-meeting surveys and these comments will be survey will be summarised and provided to EWG via EMA

Recommendations from: **Cross Validation**

Current text in draft M10	Suggested changes
186-189 Data from different fully validated methods across studies ….	Where data are obtained from different methods utilizing different detection platforms (LC/MS vs LBA) or sample preparation methods (LLE vs PPT) (e.g. ligand binding → mass spec) across clinical studies. comparison of those data is needed and a cross validation of the applied analytical methods should be carried out. Cross validation for preclinical assays should be considered on a case-by-case basis.
1016-1020 Bias can be assessed by Bland-Altman plots or Deming regression. Other methods…may be used too.	More discussion will be needed to understand the intention and the practical implementation of this new requirement
1013-1015 Cross validation should be assessed by measuring the same set of QCs (low, medium and high) in triplicate and study samples that span the study sample concentration range (if available n≥30) with both assays or in both laboratories.	Re-phrase to: Cross validation should be assessed by measuring the same set of QCs (low, medium and high) in triplicate **or** study samples that span the study sample concentration range (if available n≥30) with both assays or in both laboratories.

Suggestion made at the meeting: add statement that methods using the same platform don't need to be cross validated. If 2 datasets from different studies in the same program are different, then we need to investigate why.

Comment from the panel – there were lots of different interpretations of the cross validation section from the start – important to highlight back to EWG and to push for this to be addressed as part of the training

Recommendations from: **general principles stability testing – 1/3**

Current text in draft M10	Suggested changes
Line 392 – 395 and Line 795-798 Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint.	Stability of the analyte in the studied matrix is evaluated using low and high concentration stability QCs. Aliquots of the low and high stability QCs are analysed at time zero and after the applied storage conditions that are to be evaluated. A minimum of three replicates ~~stability QCs~~ should be ~~prepared and~~ analysed per concentration level/storage condition/timepoint.

Recommendations from: **general principles stability testing – 2/3**

Current text in draft M10	Suggested changes
9. Glossary (1272-1483) Sample, Aliquot and Replicate terms not defined	**Sample:** a small quantity (of something) from which the general quality (of the whole) may be inferred **Aliquot:** In analytical chemistry, the term aliquot is generally used to define any representative portion of the sample. **Replicate Analysis or Measurement:** The repeated analysis or measurement of the variable of interest performed as identically as possible.

Recommendations from: **general principles stability testing – 3/3**

Current text in draft M10	Suggested changes
Lines 393 3.2.8 stability Aliquots of the low and high stability QCs are analysed at time zero...	A minimum of three replicates stability QCs should be prepared and analysed per concentration level/storage condition/timepoint.
Line 399-401 3.2.8 stability If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range.....”	Take out the sentence on ultra high QC

Recommendations from: **Benchtop & FT stability**

Current text in draft M10	Suggested changes
Lines 428 - 430 'The number of freeze-thaw cycles validated should be equal or exceed that of the freeze-thaw cycles undergone by the study samples, but a minimum of three cycles should be conducted.'	'The number of freeze-thaw cycles validated should be equal or exceed that of the freeze-thaw cycles undergone by the study samples. , ~~but a minimum of three cycles should be conducted'.~~
Line 435 'Low and high stability QCs should be thawed in the same manner as the study samples and kept on the bench top at the same temperature and for at least the same duration as the study samples.'	'Low and high stability QCs should be thawed in the same manner as the study samples and stored under the same conditions and for at least the same duration as the study samples.'
Lines Multiple Use of the term freshly…..	Definition to be included in ICH M10 – freshly mean…..' Definition of fresh(ly) – prepared on the day of analysis and analysed within stability and the using intermediates which are within known stability (or to be proven stability).'

Recommendations from: **Blood Stability – LBA/CHROM**

Current text in draft M10	Suggested changes
6) Whole blood stability (line 546) […] If the matrix used is **plasma or serum**, the stability of the analyte in blood should be evaluated during method development (e.g., **using an exploratory method in blood**) or during method validation. The results should be provided in the Validation Report.	➢ 6) Whole blood stability (line 546) […] Include that whole blood stability assessment should be case-by case: **”A demonstration of this stability may be needed on a case-by-case basis, depending on the structure of the analyte”**
Line 787-789: “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.” (Whole blood stability is not specifically mentioned in the LBA chapter)	Include a negative statement that WBS is not required for LBA assays

Recommendations from: **FT and LT stability testing - intra or extrapolation**

Current text in draft M10	Suggested changes
Line 453-456 and 815-819 Paragraph. *For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.* ***What is the scientific rational behind this difference?***	Action to be taken, e.g. to be replaced by: *It is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C)* ***As stability data for biologics support this approach***

Recommendations from:
paragraphs we didn't discuss in detail CHROM – 1/2

Current text in draft M10	Suggested changes
Lines 346 – 349 ***3.2.5.2 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.	***Clarification needed on red*** ***3.2.5.2 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.

Recommendations from:
paragraphs we didn't discuss in detail CHROM – 2/2

Current text in draft M10	Suggested changes
<Line 1146> 7.3 Recovery For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated.	For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated **during method development.**
Integration and Reintegration throughout the document.	Propose to define in glossary: suggestion could be: The process of chromatographic integration can be further subdivided into the "initial integration" and "reintegration," which are quite distinct processes and need clear definition and control. It is our recommendation that initial integration is defined as the process by which the area (or height) of a chromatographic peak is adequately defined by trained personnel using the most appropriate parameters prior to regression. With regard to "reintegration," it is proposed that this is defined as the process that occurs post regression, i.e., after the back-calculated concentration, acceptance of calibration and QC samples, and acceptance or rejection of the whole run has been made.

Recommendations from: **FDC**

Current text in draft M10	Suggested changes
Line 403 – 405: If multiple analytes are present in the study samples (e.g., studies with a fixed combination, or due to a specific drug regimen) the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes.	If multiple analytes are present in the samples from studies with a fixed combination the stability test of an analyte in matrix should be conducted with the matrix containing all of the analytes. This is only a requirement for BE studies. Alternative approaches to demonstrate stability in a fixed dose combination regimen for BE studies can be utilised.

Clarification made at the meeting: alternative approaches include CMC, ISS, paper assessment on likelihood of chemical interaction

Recommendations from: **value of dilution QC – 1/2**

Current text in draft M10	Suggested changes
Line 370-382 Dilution integrity ……The same matrix from the same species used for preparation of ……..should not exceed 15%. In the cases of rare matrices use of a surrogate matrix for dilution may be acceptable, as long as it has been demonstrated that this does not affect precision and accuracy	 Dilution integrity …… The same matrix ~~from the same species~~ used for preparation of ……..should not exceed 15%. ~~In~~ The ~~cases of rare matrices~~ use of a surrogate matrix for dilution may be acceptable, as long as it has been demonstrated that this does not affect precision and accuracy

Recommendations from: **value of dilution QC – 2/2**

Current text in draft M10	Suggested changes
Line 535-540 Analytical runs containing samples that are diluted and reanalysed should include dilution QCs to verify the accuracy and precision of the dilution method during study sample analysis. The concentration of the dilution QCs should exceed that of the study samples being diluted (or of the ULOQ) and they should be diluted using the same dilution factor. The within-run acceptance criteria of the dilution QC(s) will only affect the acceptance of the diluted study samples and not the outcome of the analytical run.	Analytical runs containing samples that are diluted ~~and reanalysed~~ within the limits of a validated dilution factor do not require in-study dilution QC samples. However, in those cases where a dilution factor has not been validated, in-study dilution QCs to verify the accuracy and precision of a dilution may be used. The concentration of the dilution QCs should exceed that of the study samples being diluted ~~(or of the ULOQ)~~ and they should be diluted using the same dilution factor. The acceptance criteria of those dilution QC(s) will only affect the acceptance of the diluted study samples and not the outcome of the analytical run.

Recommendations from: **placement of midQC**

Current text in draft M10	Suggested changes
Line 331- 333, Paragraph 3.2.5.1 During method validation the QCs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around 30 - 50% of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).	Action to be taken: Correct text During method validation the QCs should be prepared at a minimum of 4 concentration levels within the calibration curve range: the LLOQ, within three times of the LLOQ (low QC), around geometric mean of the calibration curve range (medium QC) and at least 75% of the ULOQ (high QC).
Line 735- 739, Paragraph 4.2.4.1 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.	Action to be taken: NONE

Recommendations from:
Haemolysed & hyperlipidaemic matrix effects

Current text in draft M10	Suggested changes
Lines 273-277 The matrix effect should be evaluated by analysing at least 3 replicates of low and high QCs, each prepared using matrix from at least 6 different sources/lots. The accuracy should be within ±15% of the nominal concentration and the precision (per cent coefficient of variation (%CV)) should not be greater than 15% in all individual matrix sources/lots. Use of fewer sources/lots may be acceptable in the case of rare matrices.	2 levels x 3 replicates x 6 sources (+ haemolysed + lipaemic) = excessive for ALL validations at all stages of development… reflect on 3Rs (purpose bred – 1 per species will do) Copy in lines 240_242: The evaluation of lipeamic matrices is not necessary for preclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidaemic'
Lines #278-282 The matrix effect should also be evaluated in relevant patient populations or special populations (e.g., hepatically impaired or renally impaired) when available. An additional evaluation of the matrix effect is recommended using haemolysed or lipaemic matrix samples during method validation on a case by case basis, especially when these conditions are expected to occur within the study.	Application for BA/BE studies only… For all other studies, a within study assessment of IS response is a scientifically sound approach

Recommendations from: **Selectivity & specificity CHROM – 1/3**

Current text in draft M10	Suggested changes
Line 233 – 234 For the investigation of selectivity in lipaemic matrices at least one source of matrix should be used.	If required the investigation of selectivity in lipaemic matrices at least one source of matrix should be used.
Line 243 – 244 For the investigation of selectivity in haemolysed matrices at least one source of matrix should be used.	If required the investigation of selectivity in haemolysed matrices at least one source of matrix should be used.
Line 252 – 257 If the presence of related substances is anticipated in the biological matrix of interest, the impact of such substances should be evaluated during method validation, or alternatively, in the pre dose study samples. In the case of LC-MS based methods, to assess the impact of such substances, the evaluation may include comparing the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte.	If the presence of related substances is anticipated in the biological matrix of interest, the impact of such substances should be evaluated during method validation, or alternatively, in the pre dose study samples. In the case of MS based methods, to assess the impact of such substances, the evaluation may be done by comparing the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte.

Recommendations from: **Selectivity & specificity CHROM – 2/3**

Current text in draft M10	Suggested changes
Line – 226 Use of fewer sources may be acceptable in the case of rare matrices.	Use of fewer sources may be acceptable in the case of rare matrices *and when scientifically justified for non-clinical matrices.*
Line 243 - 245 For the investigation of selectivity in haemolysed matrices at least one source of matrix should be used. Haemolysed matrices are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.	For the investigation of selectivity in haemolysed matrices at least one source of matrix should be used. Haemolysed matrices are obtained by spiking matrix with haemolysed whole blood (at least 2% V/V).
Line 235 – 236 A naturally lipaemic matrix with abnormally high levels of triglycerides should be obtained from donors.	A naturally lipaemic matrix with abnormally high levels of triglycerides (> 300 mg/dL (3.4 mM/L)) should be obtained from donors.

Recommendations from: **Selectivity & specificity CHROM – 3/3**

Current text in draft M10	Suggested changes
Line 224 - 232 Selectivity is evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least 6 individual sources/lots (non-haemolysed and non lipaemic). Use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated. The evaluation of selectivity should demonstrate that no significant response attributable to interfering components is observed at the retention time(s) of the analyte or the IS in the blank samples. Responses detected and attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample for each matrix.	Suggested acceptance criteria: 5 out of 6 should pass

Recommendations from: **reference standard CHROM – 1/2**

Current text in draft M10	Suggested changes
General comment	Acknowledge the molecular diversity (which includes peptide and proteins) in chromatography and the impact on reference standards. Current section 3.1 is written around NCE.
Section 3.1 It is important that the reference standard is well characterised and the quality (purity, strength, identity) of the reference standard and the suitability of the IS is ensured	It is important that the reference standard is well characterised and the quality of the reference standard and the suitability of the IS is ensured
Section 3.1 A certificate of analysis (CoA) or an equivalent alternative is required to ensure quality and to provide information on the purity, storage conditions, retest/expiration date and batch number of the reference standard	A certificate of analysis (CoA) or an equivalent alternative is required to ensure quality and to provide information on the purity, storage conditions, retest or expiration date, batch or lot number and manufacturer or source of the reference standard
Documentation – at analytical site CoA or equivalent alternative to ensure quality (including purity), stability/expiration/retest date(s), batch number, and manufacturer or source	CoA or equivalent alternative to ensure quality and to provide information on the purity, storage conditions, retest or expiration date, batch or lot number and manufacture or source of the reference standard

Recommendations from: **reference standard CHROM – 1/2**

Current text in draft M10	Suggested changes
Section 3.1 Calibration standards and QCs ……. the stock solution have been verified. A suitable internal standard (IS) should be added to all calibration standards, QCs and study samples during sample processing. The absence of an IS should be technically justified.	Calibration standards and QCs ……. the stock solution provided the accuracy and stability of the stock solution have been verified. *It is recommended to add a* suitable internal standard (IS) to all calibration standards, QCs and study samples during sample processing.
Section 3.1 Stock and working solutions can only be prepared from reference standards that are within the stability period as documented in the CoA (either expiration date or the retest date in early development phase).	Stock and working solutions can only be prepared from reference standards that are within the stability period as documented in the CoA (either expiration date or the retest date).
Section 3.2.8 Stability The routine practice of making stock and working solutions from reference standards solely for extending the expiry date for the use of the reference standard is not acceptable	Remove this statement

Recommendations from: **Reinjection reproducibility**

Current text in M10	Suggested changes
Section 3.2.8 4) Processed sample stability: The stability of processed samples, including the time until completion of analysis (in the autosampler/instrument), should be determined. For example (i) Stability of the processed sample at the storage conditions to be used during the analysis of study samples (dry extract or in the injection phase) (ii) On-instrument/ autosampler stability of the processed sample at injector or autosampler temperature.	remove entire paragraph from section 3.2.8 See 3.2.9
3.2.9 Reinjection reproducibility Reproducibility of the method is assessed by replicate measurements of the QCs and is usually included in the assessment of precision and accuracy. However, if samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated and included in the Validation Report or provided in the Bioanalytical Report of the study where it was conducted.	3.2.9 ~~reinjection reproducibility~~ Processed sample Viability The viability of processed samples supporting the storage of processed samples before analysis and re-analysis in the event of an analytical run failing to complete or the entire run needing to be reinjected due to technical error, including the time until completion of analysis (in the autosampler/instrument), should be determined. Re-inject a stored run, comprising of calibration and QC samples, if sufficient processed sample volume permits. The re-injected run should include a minimum n=5 replicates of the low and high QCs. Calculate the QC results from the re-injected calibration curve regression and assess the assay accuracy and precision criteria.

Recommendations from:
paragraphs we didn't discuss in detail LBA

Current text in draft M10	Suggested changes
Lines 748-751 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	Within-run accuracy and precision data should be reported for each run, unless there was an obvious documented error to justify the rejection of a run(s). An overall calculation of within-run accuracy and precision for each QC level should be determined. Within-run accuracy or precision criteria do not need to be met in all runs for the assessment to be successful. Between-run precision and accuracy should be calculated by combining the data from all runs.

Recommendations from: **Key Reagents, LBA - 1/2**

Current text in draft M10	Suggested changes
Line 626 If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out prior to use to ensure that the performance characteristics of the method are within the acceptance criteria.	If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out prior to use to ensure that the performance characteristics of the method are within the acceptance criteria and to ensure consistency of results between batches in case of change during bioanalysis of samples from a given nonclinical or clinical study

Recommendations from: **Key Reagents, LBA - 2/2**

Current text in draft M10	Suggested changes
Line 633 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	~~Critical reagents bind the analyte and, upon interaction, lead to an instrument signal corresponding to the analyte concentration.~~ Critical reagents, including binding reagents (e.g. binding proteins, aptamers, antibodies or conjugated antibodies), have direct impact on the results of the assay and therefore their quality must be assured. The critical reagents should be identified and defined in the assay method
Line 636 Reliable procurement of critical reagents, whether manufactured in-house or purchased commercially, should be considered early in method development.	~~Reliable procurement of critical reagents, whether manufactured in-house or purchased commercially, should be considered early in method development~~.

Recommendations from:
Scientific aspects for the use of surrogate matrix in calibration, dilution and QC

Current text in draft M10	Suggested changes
Line 705 Calibration standards should be prepared in the same biological matrix as the study samples	Please add text: “Calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed“ Also needs to be added in sections “4.2.4.1 Preparation of Quality Control Samples 4.2.6. Dilution Linearity and Hook Effect”

Recommendations from:
Analytes that are also Endogenous Compounds – 1/6

- Industry consolidated comment = industry requests to rewrite this section, as it is felt to be too detailed, focussed on a single type of assay. There was broad consensus that the section should be shorter and more details/emphasis should be placed in training
- See next 5 slides for suggestions for edits

Recommendations from:
Analytes that are also Endogenous Compounds – 2/6

Current text in draft M10	Suggested changes
Line 1024 Title “Endogenous compounds”	Change Title to ““Endogenous homologue compounds”
To the full section 7.1	General comment to the section: Rather detailed and should be reworked. There are several approaches that may be used, add additional and add text that the sponsor should justify their approach.
Line 1025 For analytes that are also endogenous compounds, the accuracy of the measurement of the analytes poses a challenge when the assay cannot distinguish between the therapeutic agent and the endogenous counterpart.	For analytes that are identical or have a structural similarity to endogenous compounds, the accuracy of the measurement of the analytes poses a challenge when the assay cannot distinguish between the therapeutic agent and the endogenous counterpart.

Recommendations from:
Analytes that are also Endogenous Compounds – 3/6

Current text in draft M10	Suggested changes
Line 1040 **1) Standard Addition Approach:** Every study sample is divided into aliquots of equal volume. All aliquots, but one, are separately spiked with known and varying amounts of the analyte standards to construct a calibration curve for every study sample. The study sample concentration is then determined as the negative x-intercept of the standard calibration curve prepared in that particular study sample.	• Please state that it mainly applies to linear responses/LC-MS - applicability limited for LBA
Line 1046 **2) Background Subtraction Approach:** The endogenous background concentrations of analytes in a pooled/representative matrix are subtracted from the concentrations of the added standards, subsequently the subtracted concentrations are used to construct the calibration curve.	• It should be defined what endogenous concentration level is acceptable – eg. maximally up to desired LLOQ levels • State that endogenous background concentration should be determined using a surrogate matrix in order to avoid bias

Recommendations from:
Analytes that are also Endogenous Compounds – 4/6

Current text in draft M10	Suggested changes
Line 1054 **4) Surrogate Analyte Approach:** Stable-isotope labelled analytes are used as surrogate standards	• Please state that it is mainly applicable for LC-MS • Erase acceptance criteria for LBA
Line 1066 **7.1.1 Quality Control Samples** The QCs used for validation should be aliquots of the authentic biological matrix unspiked and spiked with known amounts of the authentic analyte. In spiked samples, the added amount ***should be enough*** to provide concentrations that are ***statistically different*** from the endogenous concentration.	• In spiked samples, the added amount **should be at least the same amount as endogenous** to provide concentrations that are ~~*statistically*~~ different from the endogenous concentration. • Suggest to include the sentence ”**Dilution of authentic blank matrix with the surrogate matrix is acceptable to prepare LOW QCs”**

Recommendations from:
Analytes that are also Endogenous Compounds – 5/6

Current text in draft M10	Suggested changes
Line 1076 **7.1.2 Calibration Standards** In the Standard Addition and Background Subtraction Approaches the same biological matrix and analyte as the study samples is used to prepare the calibration standards. ~~However, when the background concentrations are lowered by dilution of the blank matrices before spiking with the standards (e.g., if a lower LLOQ is required in the Background Subtraction Approach) the composition of the matrices in the study samples and the calibration standards is different, which may cause different recoveries and matrix effects~~	Delete ”*However, when the background concentrations are lowered by dilution of the blank matrices before spiking with the standards (e.g., if a lower LLOQ is required in the Background Subtraction Approach) the composition of the matrices in the study samples and the calibration standards is different, which may cause different recoveries and matrix effects*”

Recommendations from:
Analytes that are also Endogenous Compounds – 6/6

Current text in draft M10	Suggested changes
Line 1109-1110 **7.1.4 Parellelism** Parallelism should be evaluated in the Surrogate Matrix and Surrogate Analyte Approaches by means of the Standard Addition approach, spike recovery or dilutional linearity.	Delete

Recommendations from:
Dilution Linearity, Hook Effect & Parallelism – 1/3

Dilutional Linearity

Current text in Draft ICH M10	Proposed changes
Line #776-778 …and diluting this sample (to at least 3 different dilution factors) with blank matrix to a concentration within the calibration range. For each dilution factor tested, at least 3 runs should be performed...	Add: Dilution of samples in a matrix different from the study samples should be justified and appropriate experiments should be performed. A single dilution series (including 3 dilutions within the calibration range) should be assessed once.

Recommendations from:
Dilution Linearity, Hook Effect & Parallelism – 2/3

Hook Effect

Current text in Draft ICH M10	Proposed changes
Line #778-779 The absence or presence of response reduction (hook effect) is checked in the dilution QCs and, if observed, measures should be taken to eliminate response reduction during the analysis of study samples.	The absence or presence of response reduction (hook effect) is checked in the dilution QCs and, if observed, measures should be **implemented to exclude any erroneous result** during the analysis of study samples.

Recommendations from:
Dilution Linearity, Hook Effect & Parallelism – 3/3

Parallelism

Current text in Draft ICH M10	Proposed changes
Line #1133-1134 Parallelism investigation or the justification for its absence should be included in the Bioanalytical Report. Line #1144 In the case that the sample does not dilute linearly (i.e., in a non-parallel manner), a procedure for reporting a result should be defined ~~a priori~~	**Where assessed, parallelism investigation should** be reported. ~~**be included in the Bioanalytical Report**~~. In the case that the sample does not dilute linearly (i.e., in a non-parallel manner), a procedure for reporting a result should be defined.

Recommendations from: **stability for LBA – 1/2**

Current text in Draft ICH M10	Proposed changes
Line 797… A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint....	A minimum of three stability QCs should be ~~prepared and~~ analysed per concentration level/storage condition /timepoint (preparation of a single QC aliquot should suffice)....
Lines 799# , it is recognised that in some cases, for macromolecules… In such cases, valid justification should be provided …	Remove *in some cases* and *in such cases valid justification should be used*

Recommendations from: **stability for LBA – 2/2**

Current text in Draft ICH M10	Proposed changes
Line 813… For chemical drugs, it is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C). For biological drugs, it is acceptable to apply a bracketing approach, e.g., in the case that the stability has been demonstrated at -70°C and at -20°C, then it is not necessary to investigate the stability at temperatures in between those two points at which study samples will be stored.	It is considered acceptable to extrapolate the stability at one temperature (e.g., -20°C) to lower temperatures (e.g., -70°C).
Line 806-809 …Since sample dilution may be required for many LBA assays 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 stability QCs should be adjusted, considering the applied sample dilution, to represent the actual sample concentration range…	Delete this section

Recommendations from:
considerations on calibration range LBA – 1/3
(during validation)

Current text in draft M10	Suggested changes
703 Calibration standards should be prepared in the same biological matrix as the study samples	calibration standards prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed.
717 A minimum of 6 independent runs should be evaluated over several days considering the factors that may contribute to between-run variability.	A minimum of 6 independent runs should be evaluated over 2 or more days considering the factors that may contribute to between-run variability.
726 If freshly spiked calibration standards are not used, the frozen calibration standards can be used within their defined period of stability.	If freshly spiked calibration standards are not used, the frozen calibration standards can be used under the condition that freeze-thaw stability and a defined period of stability has been proven for the calibrator standards.

Recommendations from:
considerations on calibration range LBA – 1/3
(during validation)

Current text in draft M10	Suggested changes
Lines 711 The blank sample should not be included in the calculation of calibration curve parameters.	Clarify “calibration curve parameters” to ensure it means don’t use in curve fit or calculate stats- follow up for wording
Line 717-718 A minimum of 6 independent runs should be evaluated over several days considering the factors that may contribute to between-run variability.	Harmonise with QC’s “in at least 6 runs over 2 or more days”
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	Please clarify “preparation from a single stock”. Please add definition of stock solution for LBA assays. Please add information regarding possibility to use “working solution” as in the chromatography section

Recommendations from:
considerations on calibration range LBA – 3/3
(during sample analysis)

Current text in draft M10	Suggested changes
Lines 825. - 4.3.1 Analytical Run An analytical run consists of a blank sample, calibration standards at a minimum of 6 concentration levels, at least 3 levels of QCs (low, medium and high) applied as two sets (or at least 5% of the number of study samples, whichever is higher) and the study samples to be analysed. The blank sample should not be included in the calculation of calibration curve parameters. 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 Text in draft ICH M10	Harmonise wording of blank sample with comment on 711
874. - 4.3.3 Calibration Range At least 2 QC sample levels should fall within the range of concentrations measured in study samples. At the intended therapeutic dose(s), if an unanticipated clustering of study samples at one end of the calibration curve is encountered after the start of sample analysis, the analysis should be stopped and either the standard calibration range narrowed (i.e., partial validation), existing QC concentrations revised, or QCs at additional concentrations added to the original curve within the observed range before continuing with study sample analysis.	Please delete - Paragraph not applicable for LBA *Justification* *During method validation it is demonstrated that samples can be diluted into the validated range.* *Assay range is narrow, fixed and validated for LBA For chromatography methods no change of method is needed when adjusting calibration range, however, for LBA it would mean a new method needs to be established (e.g. titration of reagents, change of reagents etc.*

Recommendations from: **specificity & selectivity LBA – 1/4**

Current text in draft M10	Suggested changes
Lines 668-669: Specificity is evaluated by spiking blank matrix samples with related molecules at the maximal concentration(s) of the structurally related molecule anticipated in study samples.	Insert definition of structurally related molecules
Lines 670 The accuracy of the target analyte **at the LLOQ and at the ULOQ** should be investigated in the presence of related molecules at the maximal concentration(s) anticipated in study samples. The response of blank samples spiked with related molecules should be below the LLOQ. The accuracy of the target analyte in presence of related molecules should be within ±25% of the nominal values.	The accuracy of the target analyte **at the LLOQ and at the High QC** should be investigated in the presence of related molecules at the maximal concentration(s) anticipated in study samples. The response of blank samples spiked with related molecules should be below the LLOQ. The accuracy of the target analyte in presence of related molecules should be within ±25% and ±20% of the nominal values for the LLOQ and High QC spike respectively

Recommendations from: **specificity & selectivity LBA – 2/4**

Current text in draft M10	Suggested changes
Lines 677-680 In the event of non-specificity, the impact on the method should be evaluated by spiking increasing concentrations of interfering molecules in blank matrix and measuring the accuracy of the target analyte at the LLOQ and ULOQ*. **It is essential to determine the minimum concentration of the related molecule where interference occurs.** Appropriate mitigation during sample analysis should be employed, e.g., it may be necessary to adjust the LLOQ/ULOQ accordingly or consider a new method.	Re-phrase to **If relevant**, a minimum concentration of the related molecule causing interference should be determined. * at the maximal concentration of the target analyte
Selectivity is the ability of the method to detect and differentiate the analyte of interest in the presence of other "unrelated compounds" (non-specific interference) in the sample matrix**.** The matrix can contain non-specific matrix component such as degrading enzymes, heterophilic antibodies or rheumatoid factor which may interfere with the analyte of interest.	Delete: presence of ~~**other**~~ "unrelated compounds"

Recommendations from: **specificity & selectivity LBA – 3/4**

Current text in draft M10	Suggested changes
Line 697 Selectivity should be evaluated in lipaemic samples and haemolysed samples (Refer to Section 3.2.1). For lipaemic and haemolysed samples, tests can be evaluated once using a single source of matrix.	re-phrase: Selectivity should be evaluated in lipaemic samples and haemolysed samples **if relevant**. For lipaemic and haemolysed samples, tests can be evaluated once using a single source of matrix **as part of the 10 individuals**. Add text from 3.2.1 rather than a reference

Recommendations from: **specificity & selectivity LBA – 4/4**

Current text in draft M10	Suggested changes
Selectivity should be assessed in samples from relevant patient populations. **In the case of relevant patient populations there should be at least five individual patients.**	Re-phrase to: In the case of relevant patient populations, **when available**, there should be at least five individual patients.

Recommendations from: **ISR – 1/2**

Current text in draft M10	Suggested changes
Line 926-934 ISR should be performed at least in the following situations: For preclinical studies, ISR should, in general, be performed for the main nonclinical TK studies once per species. However, ISR in a PK study instead of a TK study might also be acceptable, as long as the respective study has been conducted as a pivotal study, used to make regulatory decisions. All pivotal comparative BA/BE studies First clinical trial in subjects Pivotal early patient trial(s), once per patient population First or pivotal trial in patients with impaired hepatic and/or renal function	Use GLP toxicity studies in stead of "*main nonclinical TK studies*" Avoid the term "*Pivotal*" Define which Phase I, II and III and studies are in scope.
Line 935-936 ISR is conducted by repeating the analysis of a subset of samples from a given study in separate (i.e., different to the original) runs on different days using the same bioanalytical method. Line 949-950 ISR should be performed within the stability window of the analyte, but not on the same day as the original analysis.	It should be allowed to conduct ISR analysis on the same day, but in separate runs for methods with eg. stability related issues or with short runtime. Rephrase line 935 and 948 to "*Separate runs*" and "*Extracted or processed separately and within the stability window*".

Recommendations from: **ISR – 2/2**

Current text in draft M10	Suggested changes
Line 938-941 However, as a minimum, if the total number of study samples is less than 1000, then 10% of the samples should be reanalysed; if the total number of samples is greater than 1000, then 10% of the first 1000 samples (100) 940 plus 5% of the number of samples that exceed 1000 samples should be assessed.	Reduce ISR to 5% and to add cap for both minimum and maximum number of samples (e.g 21-xxx, 2/3 = integer). Or Consider to allow Fixed number as an alternative approach to the current fixed ratio approach.
Line 960-962 If ISR meets the acceptance criteria yet shows large or systemic differences between results for multiple samples, this may indicate analytical issues and it is advisable to investigate this further.	The paragraph is considered vague and needs clarification Eg. It is stated that it is advisable to investigate yet ISR did not fail criteria.

From the discussion at the meeting: ISR = post validation = OK
During production however, ISR something else, i.e. process control, and for this you have QCs. Don't need to have multiple process controls (QC, ISR, Dil QC…) in all studies

Recommendations from: **repeat analysis – 1/11**

ICH M10 - 3.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Possible reasons for reanalysis of study samples, the number of replicates and the decision criteria to select the value to be reported should be predefined in the protocol, study plan or SOP, before the actual start of the analysis of the study samples. The number of samples (and percentage of total number of samples) that have been reanalysed should be reported and discussed in the Bioanalytical Report.	• <clarification>: acceptance criteria a priori defined < specify where> • Needs clarification that reanalysis calculations should be based on a valid result (i.e. concentration in range, ULOQ or <LLOQ). If not not acceptable, there is no valid result (batch QC and/or QC fail, poor chromatography or anomalous ISTD response)
Rejection of an analytical run because the run failed the acceptance criteria with regard to accuracy of the calibration standards and/or the precision and accuracy of the QCs	• This requirement causes technical issues: Samples of a rejected run are not considered to be reassayed samples in Watson LIMS. They can not be listed as requested in Table 1 • it is not clear if reanalysed sample should be discussed as required at line 130 • Only place where IS acceptance criteria are mentioned

Recommendations from: **repeat analysis – 2/11**

ICH M10 - 3.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Improper sample injection or malfunction of equipment	Improper sample injection or malfunction of equipment in case samples can not be reinjected
Identification of quantifiable analyte levels in pre-dose samples, control or placebo samples	• <recommendation>: replace “identification” with “re-confirmation” • naïve pre-dose samples. You might expect drug in a pre-dose sample after repeated administration • clarify if allowed for comparative BA/BE • predose samples could contain detectable concentrations. So they should be removed from the text. E.g.: "*pre-dose samples prior to first drug administration”*

Recommendations from: **repeat analysis – 3/11**

ICH M10 - 3.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Poor chromatography (as pre-defined in an SOP)	Pre-specifying the poor chromatography in SOP may not cover each <reason> of poor chromatography that may occur. Pointing out between brackets "(as predefined in and SOP)" is not necessary in the guidance Please rephrase to: *Poor Chromatography* change "as pre-defined in an SOP" to "if such criteria have been pre-defined in an SOP". The IS response may be significantly different from artificial samples although all QC samples are OK
For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result	Add: “laboratory error can occur and can be investigated”

Recommendations from: **repeat analysis – 4/11**

ICH M10 - 3.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Any reanalysed samples should be identified in the Bioanalytical Report and the initial value, the reason for reanalysis, the values obtained in the reanalyses, the final accepted value and a justification for the acceptance should be provided. Further, a summary table of the total number of samples that have been reanalysed for each reason should be provided. In cases where the first analysis yields a non-reportable result, a single reanalysis is considered sufficient (e.g., concentration above the ULOQ or equipment malfunction). In cases where the value needs to be confirmed (e.g., pre-dose sample with measurable concentrations) replicate determinations are required if sample volume allows.	• <clarification>: Re-analysis calculations should be based on a valid result (i.e. concentration in range, ULOQ or <LLOQ). If not acceptable, there is no valid result • <clarify>: initial value may be presented – what if no valid result from the initial analysis? • <placebo sample with measurable concentration>: analysis of entire placebo profile should be performed vs. a replicate analysis of single sample due to potential mislabeling / misdosing. If all samples in the placebo profile produce measurable concentration the correctness of the analysis is demonstrated. Having as mandatory the reanalysis in duplicate should be removed to allow better scientific approaches • Pre-dose samples may contain measurable drug. Remove statement • There is no value in reporting original value if run failed for reasons identified. Stating the reason for re-analysis is sufficient • There is no value in reporting original value if it was >ULOQ and re-tested at greater dilution

Recommendations from: **repeat analysis – 5/11**

ICH M10 - 3.3.4 Reanalysis of Study Samples	Comments / Suggested changes
The safety of trial subjects should take precedence over any other aspect of the trial. Consequently, there may be other circumstances when it is necessary to reanalyse specific study samples for the purpose of an investigation	• <Clarify>: Who identifies whether the samples should be reanalyzed or not? • Statement on “reanalysis is not acceptable for BA/BE studies” is inconsistent with lines 590-592 • Later the document mentions BE studies and relative BA studies should not have sample reanalysis. Suggested to clarify text, making it consistent throughout the document.

Recommendations from: **repeat analysis – 6/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
4.3.4 Reanalysis of Study Samples	need clear and unambiguous definition of what a Reanalysis is. Differentiate between a) do not have yet a final, reportable result (due to run rejection, sample is AQL), need to analyze the sample again b) have final, reportable result but <sample is reanalyzed> by mistake or for TK/PK reasons. Whereas a) and b) should be defined in SOPs, only b) should be discussed in the report.

Recommendations from: **repeat analysis – 7/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Some examples of reasons for study sample reanalysis are:	<Suggestion>: Include the reason - Where the analyte has an observed concentration that is >ULOQ, but yields an observed concentration of greater than 25% below the ULOQ after repeat analysis with dilution
Rejection of an analytical run because the run failed the acceptance criteria with regard to accuracy of the calibration standards and/or the precision and accuracy of the QCs	• <Rephrase>: "*Rejection of an analytical run because the run failed the acceptance criteria with regard to the precision and accuracy of the calibration standards or of the QCs"* Rationale: not "and/or" but only "or" because if the standards fail, then QC results are not exploitable • Reinjection normally not applicable for LBA • No results can be assigned for the samples from a rejected run and there are no original results to be presented in the re-nanalysis table. The reanalysis table becomes unnecessarily large and might obscure sample specific reanalysis which is more relevant. Suggested to remove. Failed run is identified anyway in the summary table of all runs.
Identification of quantifiable analyte levels in pre-dose samples, control or placebo samples.	pre-dose might be expected to contain drug "naïve pre-dose samples". Rather than reason for re-analysis, can be a reason for an investigation

Recommendations from: **repeat analysis – 8/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
Malfunction of equipment	In this case the run should be rejected as a technical error, in which case there are no results for the samples. We suggest to remove this from the examples. The failed run is identified anyway in the summary table of all runs.
The diluted sample is below the LLOQ	Sample may generate below LLOQ value while analyzed at the MRD dilution. A clarification needs to be added that in this case, sample will not be re-analyzed. We suggest editing the text to read: The diluted sample is below the LLOQ while tested at the assay MRD

Recommendations from: **repeat analysis – 9/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
• When samples are analysed in more than one well and non-reportable values are obtained due to one replicate failing the pre-defined acceptance criteria (e.g., excessive variability between wells, one replicate being above the ULOQ or below the LLOQ).	• Acceptance criteria for replicate well measurements should be given • <Clarify>: What regarding samples with reproducible variability (one replicate still <LLOQ in primary and repeat analysis): should be considered as not reportable after x repeats? • Values near the LLOQ are likely to have one replicate reportable and one replicate non-reportable. Does this mean the sample should be re-analysed until 2 reportable or non-reportable vales are obtained within the same run? Consider how industry standard LIMS (Watson) treats this scenario. Alternatively the industry standard should be that replicate detector responses are “meaned”, and the mean response is used to determine a single sample concentration which is then reported (either as an in-range value or <LLOQ/>ULOQ) • Requirement to re-analyze based on performance of one replicate failing the pre-defined acceptance criteria is confusing. Appears related to inter-well with <20%? If multiple replicates are tested and mean of assay signal is used to calculate reported concentration, the concentration per individual replicate will not be known. <Suggested change>: When individual replicate concentrations are calculated... and inter-well %CV is acceptable

Recommendations from: **repeat analysis – 10/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
For comparative BA/BE studies, reanalysis of study samples for a PK reason (e.g., a sample concentration does not fit with the expected profile) is not acceptable, as it may bias the study result.	• <Suggestion>: Revise text to allow the reanalysis of certain samples (e.g. single timepoint). • Need to resolve the aspect of pre-dose samples with <measurable> concentration - allow or not

Recommendations from: **repeat analysis – 11/11**

ICH M10 - 4.3.4 Reanalysis of Study Samples	Comments / Suggested changes
• The reanalysed samples should be identified in the Bioanalytical Report and the initial value, the reason for reanalysis, the values obtained in the reanalyses, the final accepted value and a justification for the acceptance should be provided. • Further, a summary table of the total number of samples that have been reanalysed due to each reason should be provided. In cases where the first analysis yields a non-reportable result, a single reanalysis is considered sufficient (e.g., concentration above the ULOQ or excessive variability between wells). • The analysis of the samples should be based on the same number of wells per study sample as in the initial analysis. In cases where the value needs to be confirmed, (e.g., pre-dose sample with measurable concentrations) multiple determinations are required where sample volume allows.	• <Clarify>: Naïve pre-dose samples. A pre-dose on multiple dosing may have quantifiable drug • <Clarify>: Does this include reanalysis due to rejected runs • <Clarify>: What is the merit in reporting the first analysis where the result is above the ULOQ? There is no "result", so after the sample has been reanalysed at a (greater) dilution factor to give a reportable result there is no justification required for the reported result • <Suggestion>: Only samples that were reanalyzed due to PK reasons should have to be identified in the BA report. Samples that yield non-reportable result due to analytical reasons shouldn't be identified or explained reasons for reanalysis. The guideline should state that a procedure for reasons and numbers of reanalysis needs to be in place, but not that multiple determinations are necessarily required. Proposed text: *The reanalysed samples due to pharmacokinetic reasons should be identified in the bioanalytical report and the initial value, the reason for reanalysis, the values obtained in the reanalyses, the final accepted value and a justification for the acceptance should be provided.* <remove>: *"Further, a summary table …where sample volume allows."* • One table including all re-analysed samples, reasons, initial values and values obtained from reanalysis should be sufficient

Recommendations from: **Dried Matrix Methods**

Current text in M10	Suggested changes (CLEAN COPY)

Section 7.6.1 (lines 1207 to 1225)
Dried matrix methods (DMM) is a sampling methodology that offers benefits such as collection of reduced blood sample volumes ~~as a microsampling technique~~ for drug analysis and ease of collection, storage and transportation. In addition to the typical methodological validation for LC-MS or LBA, use of DMM necessitates further validation of this sampling approach before using DMM in studies that support a regulatory application, such as:
• Haematocrit (~~especially for spotting of whole blood into cards~~)
• Sample homogeneity (~~especially for sub-punch of the sample on the card/device~~)
• Reconstitution of the sample
• DMM sample collection for ISR
  ~~• Care should be taken to ensure sufficient sample volumes or numbers of replicates are retained for ISR~~
  ~~• Should be assessed by multiple punches of the sample or samples should be taken in duplicate~~
When DMM is used for clinical or nonclinical studies in addition to typical liquid approaches (e.g., liquid plasma samples) in the same studies, these two methods should be cross validated as described (Refer to Section 6.2). For nonclinical TK studies, refer to Section 4.1 of ICH S3A Q&A. Feedback from the appropriate regulatory authorities is encouraged in early drug development.
	Section 7.6.1 (lines 1207 to 1225)
	Dried matrix methods (DMM) is a sampling methodology that offers benefits such as collection of reduced blood sample volumes for drug analysis and ease of collection, storage and transportation. In addition to the typical methodological validation for LC-MS or LBA, use of DMM necessitates further assessment of this sampling approach before using DMM in studies that support a regulatory application, such as:
	• Haematocrit
	• Sample homogeneity
	• Reconstitution of the sample
	• Consideration for being able to conduct ISR
	When DMM is used for clinical or nonclinical studies in addition to typical liquid approaches (e.g., liquid plasma samples), the comparability of the two methods should be determined using a priori defined correlation approach. In addition, for nonclinical TK studies, refer to ICH S3A Q&A. Feedback from the appropriate regulatory authorities is encouraged in early drug development.

Recommendations from: **Commercial and Diagnostic Kits – 1/2**

Current text in draft M10	Suggested changes
Lines # 1169-1172 If an applicant repurposes a kit (instead of developing a new assay) or utilises “research use only” kits to measure chemical or biological drug concentrations during the development of a novel drug, the applicant should assess the kit to ensure….	If an applicant uses a kit, repurposes a kit (instead of developing a new assay) or utilises “research use only” kits to measure chemical or biological drug concentrations during the development of a novel drug, the applicant should perform a validation to ensure …..
Line # 1173 Validation considerations for kit assays include, but are not limited to, the following:	Specific considerations for kit assays include, but are not limited to, the following:
Add at Line 1174 Added text	Kits components should be considered as a source of critical reagents (refer to Section 4.1.2)
Line #1174-1178 If the reference standard in the kit differs from that of the study samples, testing should evaluate differences in the assay performance of the kit reagents. The specificity, accuracy, precision and stability of the assay validation should be demonstrated under actual conditions of use in the facility conducting the sample analysis. Modifications from kit processing instructions should be completely validated.	As described in this guideline, the same principles of validation apply to the use of kits and validation should be performed under actual conditions of use in the facility conducting the sample analysis.

Recommendations from: **Commercial and Diagnostic Kits – 1/2**

Current text in draft M10	Suggested changes
Line # 1179-1181 Kits that use sparse calibration standards (e.g. one or two point calibration curves) ……	Remove this bullet
Line # 1185-1187 Calibration standards and QCs should be prepared in the same matrix as the study samples. Kits with calibration standards and QCs prepared in a matrix different from the study samples should be justified and appropriate experiments should be performed.	Calibration standards should adhere to the requirements in Section 4.2.3 and QCs should be prepared in the same matrix as the study samples. Kits with calibration standards and QCs prepared in a surrogate matrix different from the study samples should be justified and appropriate experiments should be performed. If an endogenous counterpart is present refer to Section 7.1
Line # 1188-1189 If multiple kit lots are used within a study, lot to lot variability and comparability should be addressed for any critical reagents included in the kits.	Remove this bullet
Line # 1190-1192 If a kit using multiple assay plates is employed, sufficient replicate QCs should be used on each plate to monitor the accuracy of the assay. Acceptance criteria should be established for the individual plates and for the overall analytical run.	Remove this bullet

Recommendations from: **new or alternative technologies**

- Industry is comfortable with the current text in that it will allow for the development of new or alternative technologies, however:
  - There were different interpretations of the text → Is the text too bland?
    - New and alternative are not defined and can mean different things to different people (BA scientist, auditor, assessor).

<u>Recommendation and discussion point:</u>

- Define what we mean by new and alternative technologies
  - **Alternative technology:** In use within multiple labs, acceptance criteria are externally referenceable and contemporary scientific principles are accepted within the bioanalytical community
  - **New Technology:** ??
- Is anything but the last sentence in the paragraph *(The use of new technology in regulated bioanalysis should be supported by acceptance criteria established a priori based on method development and verified in validation)* truly relevant to new technologies

Recommendations from:
documentation & glossary

General recommendation	Add additional column for BE only Simplify the list for reports for other studies (validations)
General comment	Detailed discussion on the tab le 1 from the draft ICH Guideline in a conference setting is does not allow to capture some of the important but minor edits. More and more detailed feedback will be provided to EMA (EWG) to fully reflect the comments given in the different surveys

Recommendations from:
documentation & glossary CHROM

Current text in draft M10 Table 1	Suggested changes
List of SOPs in BA report	Remove
Analysis: Instrument ID for each run in BA/BE studies for validation report	Need clarification: copy/paste error or is this required for validations used for BE studies?
Chromatograms and Reintegration: 100% chromatograms and summary sheets BA/BE studies for validation report	Need clarification: copy/paste error or is this required for validations used for BE studies?
Chromatograms and Reintegration: BA/BE studies 100 % of run summary sheets for BA report	Remove: does not belong under chromatograms Create separate section “8.3: Documentation to be provided upon request” dealing with summary sheets and what they should look like with a clear example.
Summary sheet information	Still needed if all the info is on the chromatograms?

Recommendations from:
Documentation and Glossary – LBA – 1/6

Current text in draft M10	Suggested changes
Sample Tracking – “Sample inventory and reasons for missing samples”	Sample Tracking – “Sample inventory and, where available, reasons for missing samples”
Line 1264-1267 - Validation Report Stock Solutions – “Stock solution stability”	 Change to – “Stored working solution stability”
Blank Matrix – “Description, lot number, receipt dates”	Change to – “Description”
Calibration Standards and QCs – “Batch number, preparation dates and stability period”	Change to – “Batch number and stability period”
Calibration Standards and QCs – “Storage conditions (temperatures, dates, duration, etc.)”	Change to – “Storage conditions (temperatures, duration, etc.)”
Analysis – “Table of all runs (including failed runs), and analysis dates”	Change to – “Table of all runs (including failed runs and reasons for failure) and analysis dates”
Analysis – “Instrument ID for each run in comparative BA/BE studies”	Delete – not applicable

Recommendations from:
Documentation and Glossary – LBA – 2/6

Current text in draft M10	Suggested changes
Line 1264-1267 Validation Report Analysis – “Table of calibration standard concentration and response functions results (calibration curve parameters) of all accepted runs with accuracy and precision”	Change to – “Table of calibration standard concentration and response functions results (~~all~~ applicable calibration curve parameters) of all runs, with accuracy and precision of accepted runs”
Analysis	Add – “Table including ~~of~~ run acceptance QCs results”

Recommendations from:
Documentation and Glossary – LBA – 3/6

Current text in draft M10	Suggested changes
Line 1264-1267 Bioanalysis Report	
Blank Matrix – “Description, lot number, receipt dates”	Change to – “Description”
Calibration Standards and QCs – “Preparation dates and stability period”	Change to – “Stability period”
SOPs – “A list of SOPs/analytical protocols used for the assay procedure”	Change to – “A list of analytical procedure(s)”
Sample Tracking – “Dates of receipt of shipments number of samples, and for comparative BA/BE studies the subject ID”	Change to – “For comparative BA/BE studies, dates of receipt of shipments, number of samples and subject ID”

Recommendations from:
Documentation and Glossary – LBA – 4/6

Current text in draft M10	Suggested changes
Line 1264-1267 Bioanalysis Report Sample Tracking – “Analytical site storage condition and location”	Change to – “Analytical site storage condition”
Sample Tracking – “List of any deviations from planned storage conditions, and potential impact”	Change to – “List of any deviations from planned storage conditions that impacted on study results”
Analysis – “Table of calibration standard concentration and response functions results (calibration curve parameters) of all accepted runs with accuracy and precision”	Change to – “Table of calibration standard concentration and response functions results (all calibration curve parameters) of all runs, with accuracy and precision of accepted runs”
Line 1264-1267 Bioanalysis Report Analysis – “Table of QCs results of all accepted runs with accuracy and precision results of the QCs and between-run accuracy and precision results from accepted runs.”	Change to – “Table of QCs results of all runs with accuracy and precision results of the QCs and between-run accuracy and precision results from accepted runs.”

Recommendations from:
Documentation and Glossary – LBA – 5/6

Current text in draft M10	Suggested changes
Line 1340-1343 Critical Reagent: Critical reagents for LBAs include binding reagents (e.g., antibodies, binding proteins, peptides) and those containing enzymatic moieties that have a direct impact on the results of the assay.	Change to: Reagents, including binding reagents (e.g. binding proteins, aptamers, antibodies or conjugated antibodies), that have direct impact on the results of the assay and therefore their quality must be assured.
Line 1352 Dilution Linearity: A parameter demonstrating that the method can appropriately analyse samples at a concentration exceeding the ULOQ of the calibration curve without influence of hook effect or prozone effect and that the measured concentrations are not affected by dilution within the calibration range in LBAs.	Change to: A parameter demonstrating that the method can appropriately analyse samples at a concentration exceeding the ULOQ of the calibration curve when diluted within the calibration range in LBAs.

Recommendations from:
Documentation and Glossary – LBA – 6/6

Current text in draft M10	Suggested changes
Glossary - Line 1277 Accuracy (%) = ((Measured Value-Nominal Value)/Nominal Value) × 100	Change to: Accuracy (%) as defined by relative error = Measured Value/Nominal Value x 100
Glossary - Line 1272-1483 Not currently in Guideline	Add definitions: Geometric mean: Pivotal Study: Primary Matrix: Reference Standard:
Table 1 Log records	records
Table 1 Deviations	Include only deviations that impacted on study results
Line 1264-1267 - Validation Report Stock Solutions	Stock solutions (Chromatography)

Recommendations from:
Documentation and Glossary – general

Current text in draft M10	Suggested changes
Table 1: List of SOPs in BA report	Concern that this may significantly increase reporting burden for report which never become part of a filing (cfr scope)
Table 1 general comments	Addition of column for BA/BE only to add clarity Simplify the list for reports for other studies (& validations) Clarity required re. validation report expectations in support of BE studies – since validation report is prepared before use for a BE studies (may not be known at time of reporting)
Glossary	Consider additional definitions for: • Sample/aliquot/replicate • Fresh(ly) spiked/prepared • Re-integration (per GBC S1-3 recommendation paper) • Dilution integrity : ”The assessment of the sample dilution procedure to confirm that it does not impact the accuracy & precision of the measured concentration of the analyte”) • Dilution ratio (factor) : The ratio of sample to diluent used to dilute the sample”

Additional and more detailed comments were given on all the paragraphs as part of EBF/delegate pre-meeting surveys.

When considered providing more context to the summary recommendations from the meeting, and not in conflict with those recommendations, also these comments will be considered for FB to to EWG via EMA

Acknowledgment and questions

- The EBF community and meeting delegates for survey data and feedback
- AAPS, JBF and CBF
- Note takers at the meeting
- Further questions to info@e-b-f.eu