Feedback from Workshop

Towards harmonised implementation of the ICH M10 Guideline

ICH M10 team

15 November 2022, Barcelona

The roadmap

- Two EBF internal meetings (Zoom) in September/October
  - We selected items for discussion this week considering EBF survey and survey to workshop delegates
- Face to face Internal EBF members meeting (BCN, 14NOV)
  - Deeper dive chapter per chapter which generated draft suggestions for harmonised implementation and identified additional actions
- The workshop on 15 Nov to discuss and further calibrate draft suggestions for harmonised implementation or action items

Next slides:

Key areas of consensus / proposal for harmonised implementation / additional EBF actions

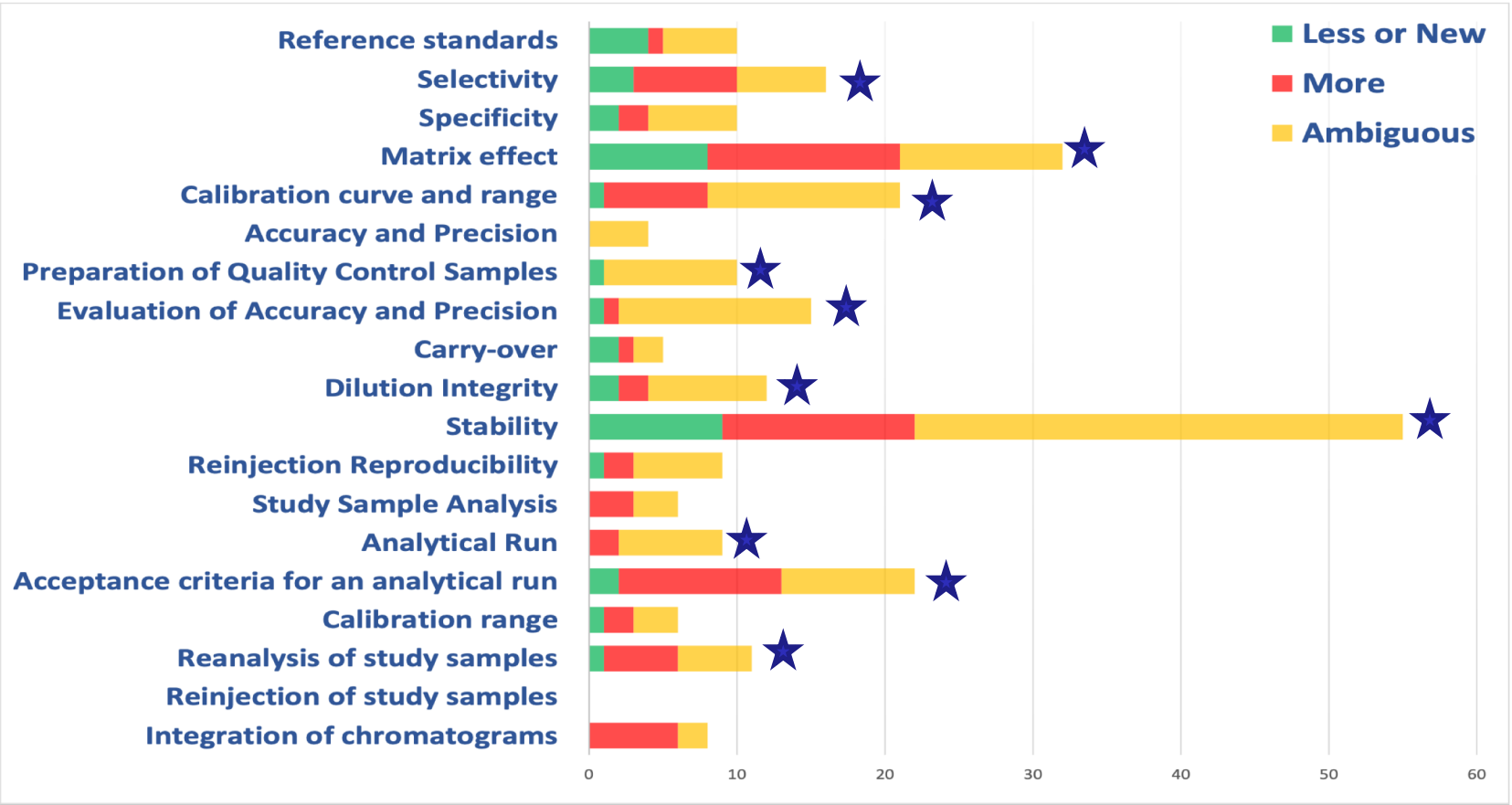
Presented by the Workshop moderators during the 15th OS (Regulatory Updates)

Chapters 1&2

- **Metabolites in scope**
  - → A good start is to concentrate full validations to late stage molecules e.g. Ph3
- **Non Clinical PK Studies – examples of what this means would be useful**
  - → When you can't dose to humans e.g. Anthrax
- **Method development - What is minimally needed for record keeping?**
  - → Very high level, capture the lifecycle of the assay (if an assay changes after validation capture the why and what has changed)
- **Why is parallelism not listed as a must have?**
  - → Use scientific judgement – If it adds real scientific value do it, if it doesn't don't
- **Primary *vs.* secondary matrices – what is primary? What is secondary?**
  - → Comes down to what the data is going to be used for – a good dialogue is required with stakeholders

Chapter 3

Themes/questions discussed



(3.2.1 Selectivity)

- Majority understand design
- Majority support using fewer lots for ‘rare matrices’ (3Rs).
- Need EBF definition of ‘rare matrix’ (i.e. small animal species & destructive sampling?)
- If selectivity fails, investigate failing batch and/or increase to n=10

(3.2.3 Matrix Effect)

- Majority understand design
- Majority support using fewer lots/batches for ‘rare matrices’ (3Rs)
- Majority agree that we are more likely to receive haemolysed samples rather than lipemic samples (but should assess both haemolysed and lipemic in man)

(3.2.4 Calibration Curve and Range)

- Majority understand design
- For a ‘simple’ model, may be sufficient to state linear < quadratic and $1/x < 1/x^2$ in SOP (depends on dynamic range/instrument)

(3.2.5.1 Preparation of Quality Control Samples)

- Majority understand design
- MID QC placement (30-50% arithmetic or geometric; your choice)
- Majority need clarity on how FAQ links to ICH-M10

(3.2.5.2 Evaluation of Accuracy and Precision)

- Majority understand design and that fresh calibration is used for at least one A&P run
- Majority agree that QCs don't need to be fresh (if frozen, then we need to document stability)
- Majority understand design and value of the 'full size' robustness run
- EBF need to provide examples of A&P calculations (within-day, between day, ANOVA)

(3.2.7 Dilution Integrity)

- Majority support using ‘surrogate matrix’ for ‘rare matrices’ (3Rs)
- Majority understand design; 1 bulk, 5 diluted aliquots per DF and 1 replicate per diluted aliquot
- Majority interpret 3.2.7 to mean validation bracketing lowest and highest dilution factors (e.g. **1:5** 1:10, 1:50, **1:100**)
- Majority interpret 3.2.7 to mean highest [DQC] ≥ [in-vivo]
- Majority understand potential implications if [in-vivo] ≥ [DQC]

(3.2.8 Stability – Part 1)

- Majority interpret 3.2.8 to indicate stability does not need repeating if we have documented evidence (i.e. an approved & traceable BMV-compliant report)
- Majority agree stability is performed for each analyte and species
- We understand design; **1 bulk, 3 aliquots and 1 replicate** (vs independent variables i.e. time and temperature) and including ‘time zero’
- Majority agree that frozen run QC may be used (stability proven / will be proven)
- Majority agree that stability [DQC] ≥ [in-vivo] (or as high as possible, if [in-vivo] is TBD)
- Majority agree that stability of FDC is ‘special’ and does not mean ‘general’ concomitant medication (co-med)

(3.2.8 Stability – Part 2)

- Majority agree that **'freshly prepared'** means 'fresh in matrix' not from freshly prepared primary solutions (SS & WS)
- Majority accept design of 3 **replicates** for **Stock And Working Solution Stability** (acceptance criteria TBD based on measurement method i.e. UV-VIS, MS or other)
- Majority understand **Blood Stability** is only applicable for plasma assay
- Majority accept design of 3 **replicates** (not aliquots) for **Blood Stability** (acceptance criteria based on scientific judgment)
- Majority agree to limit scope to human **Blood Stability** (or use human blood as surrogate of animal blood stability; 3Rs)
- Recommendation to update EBF position publication on blood stability (Bioanalysis(2011) 3(12) 1333-1336))

(3.2.9 Reinjection Reproducibility)

- Majority agree this' is different and separate to 'Processed Sample Stability' (time between original and reinjection on autosampler)
- Majority understand the design of ≥ 5 **replicates** at LOW, MID and HIGH vs within-run CAL (i.e. reinjection and quantification as 'self-contained' run)
- Majority agree acceptance criteria of ≤ ±15% accuracy and precision

(3.3.1 Analytical Run)

- Majority interpret bracketing to mean at a minimum 'QC – Samples – QC'?
- Majority agree that several 'batches' can form a 'run' and that QCs (duplicate at three levels) are needed in each 'batch'
- Majority understand carry-over impact and how to mitigate it (note; carry-over only assessed for 'Run' not per 'Batch')

(3.3.2 Acceptance Criteria for Analytical Run – Dilution QC)

- Majority understand this to mean minimum of ≥ 2 **aliquots** per DF
- Majority accept acceptance criteria; ≥ 50% pass & A @ ≤ ± 15%
- Majority agree to bracket the lowest and highest Dilution Factors with DQCs
- Majority agree and accept that process control [DQC] ≥ [ULOQ]
- Majority agree that if a Dilution Factor (DQC) 'bracket' fails, only samples diluted with the passing Dilution Factor (DQC) are accepted

(3.3.3 Calibration Range)

- Majority agree and interpret '**at intended therapeutic dose**' to limit scope of any changes to Phase II/III and Pivotal BA/BE studies

(3.3.4 Reanalysis of Study Samples)

- Majority agree that there are no bioanalytically 'valid' study sample concentrations obtained from a failed run

Chapter 4

- **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 using in formal validation assessment
- 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.

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.
  - Need for assessment should be driven by modality and mechanism of action.
- 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).

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 hemolysed 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.

Chapter 5

Not much is new in ICH M10 related to ISR, see next slide

Recommended to use ICH M10 to reset your compass and not overdo ISR – not all studies are in scope for ISR, they never were and still aren't

- Understand pivotal as where the primary endpoint of the clinical trial is related to PK.
- Know your project and understand the purpose of each and every study you support.
- Be clear how to distinguish between ISR conduct pr. guidance requirements and how to used ISR to mitigate business risks for pivotal studies.
- For guidance on for how, when and where to conduct and document investigation for failed ISR, and for selection of samples for ISR, see Bioanalysis, (2009) 1(6) 1049-1056

Evolution of ISR

Requirement	ICH M10	FDA	EMA
Studies in scope	TK species FIH Pivotal BA/BE (Pivotal) FIP (Pivotal) hepatic/renal	All Pivotal for labelling or approval: TK/species All BE, pivotal PK and PD	TK/species BE, FIH, FIP, hepatic/renal
When to execute	Not on the same day as the original analysis	In separate runs	In separate run, at different days
How many samples	10% first 1000	10% first 1000	10% first 1000
	5% > 1000	5% > 1000	5% > 1000
Which samples	Near C_{max} and elimination phase - representative for the whole study	Near C_{max} and elimination phase	Near C_{max} and elimination phase
Acceptance Criteria	2/3 (67%) within 20% (LC/MS), 30% (LBA)	2/3 (67%) within 20% (LC/MS), 30% (LBA)	2/3 (67%) within 20% (LC/MS), 30% (LBA)
If it fails…	SOP based investigation	SOP based investigation	Investigation

Chapter 6

Focus on WHEN and HOW to X-validate

Areas of consensus:

- Consultation with stakeholder (clin pharm, regulatory) is key:
  - Before X-val occurs:
    - BA to explain why X-val is needed
    - Clin pharm to explain which studies will be combined
    - Decide on what method will be used to assess bias between assays (no acceptance criteria)
  - After X-val has occurred
    - Clin pharm to assess the impact of any bias between methods

Potential EBF actions:

- Is there value in a cybermeeting with stakeholders (e.g. clinical PK/statisticians) on how to “manage” Cross-validation

Chapter 7

7.1 Methods for Endogenous Compounds

Good to remined → Biomarkers are not in scope.

- Modern drugs are often slightly modified versions of endogenous molecules and thus specific bioanalysis is possible: chapter 3 or chapter 4.
- The approach adopted (1 of 4 for Chrom, 1 of 2 applicable for LBA) selected based on the needs of the project.
- Approach adopted for each program should be scientifically and technology driven.
- Further dialogue required in the situation where the dosed compound is not the pharmacologically active entity.

7.2 Parallelism

- You know your drug program, do what makes sense.
- When assessed include sufficient samples to understand any limitations.

7.3 Recovery

- Consistency of recovery may be assessed in method development.
- EBF propose that the well understood extract/post-spike is a valid means of recovery determination.
- Most often not relevant for LBA assays: For large molecules recovery may be considered for dried matrix methods/tissue.

7.4 Dried matrix methods

- It is welcomed that microsampling is supported to allow consideration of 3Rs and reduce patient burden.

7.5 Commercial and Diagnostic Kits

- Where commercial kits are used, validation should fulfil expectations in section 4 and relevant parts of section 7.

7.6. New or alternative technologies

- When technologies not mentioned in the scope of the guideline are used, validation design and acceptance criteria should be science driven.

Chapter 8

- Not recommended as general practice, but it can be a business decision to include a brief history/evolution of the methods in the validation reports when changes have been made to the assay to facilitate the compilation of the CTD modules.
- For BA/BE studies, include the additional requested information (e.g. 100% chroms, 100% summary tables of all runs) to the validation reports of methods used to support BA/BE. This information can be added retrospectively by amending the reports ahead of the submission.
- Reinjected runs:
  - include tables of reinjected runs (including results) to all BA reports.
- Rejected runs: for all BA/BE studies, include the tables containing the results from rejected runs and document the reason. Results could be marked as NR in case no reportable data is available.

- SOPs: it was proposed to include the procedures used for the method in the form of a bioanalytical method description (and not to include a list of all SOPs followed to conduct the analysis, as this is information that should be kept at the site).
- Instrument I.D.'s: it was recommended to include only the equipment used for data acquisition (i.e. no ancillary equipment).
- Sample tracking: it was proposed to indicate only the storage temperature of samples (i.e. no freezer i.d. or location).

- Templates (e.g. Summary Tables) would be greatly appreciated (EBF can help)

From here….

- Provide FB to global BA community
  - meeting report
  - Via EBF website →these slides
- Follow up on actions
  - Allow workshop discussion dust to settle and from there, take it further
  - Continue to share and evaluate across regions and companies
  - EBF commits to include ICH M10 experience discussions in 2023 activities.

Acknowledgements

EBF community
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