Feedback from the JBF workshop for ICH-M10 guideline in 2023

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15th JBF Symposium

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JBF ICH M10 Workshop 2023

- Date: October 2-3, 2023
- Place: Vision Center Yokohama, Kanagawa
- Participants: 78 people, total
  - 56 people from 33 Industries and CROs
  - 22 JBF steering committee members

Day 1	
12:00~	Entry
12:30~14:30	**Opening & Chrom/LBA**
14:30~15:00	Break
15:00~17:00	**Chrom/LBA**
17:00~17:15	Break
17:15~17:50	**Chrom: Discussion sharing between 2 groups LBA：Discussion**
18:00～20:00	**Information sharing party**

Day 2	
8:40~	Entry
9:00~10:00	**Other chapters**
10:00~10:15	Break
10:15~11:15	**Other chapters**
11:15~11:30	Break
11:30~12:00	**Discussion sharing among 3 groups & Closing remarks**

http://bioanalysisforum.jp/

Discussion points

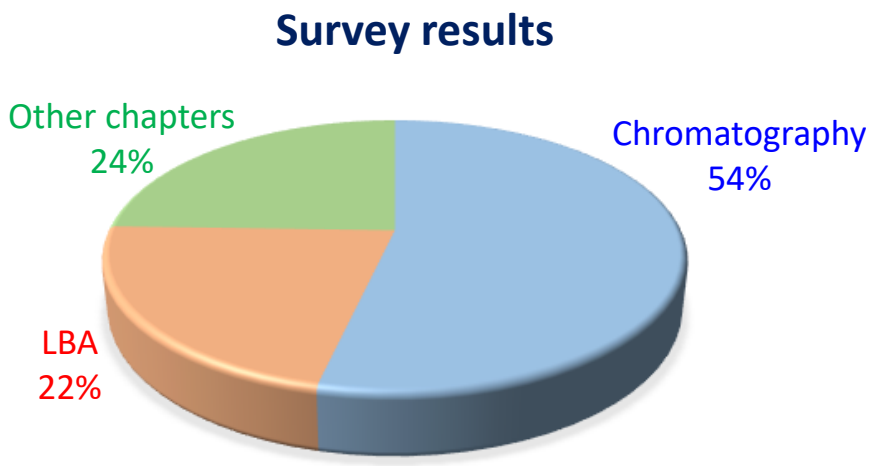
Survey results:

- **306** discussion points/questions were collected. (164 for Chromatography, 67 for LBA, 75 for other chapters)

- JBF SC categorized and prioritized issues (High, Middle, Low), and the issues of high importance and attention were discussed at the Workshop.
  - **Chromatography** **High: 16**, Middle: 7, Low: >20
  - **LBA** **High: 9**, Middle: 10, Low: 8
  - **Other chapters** **High: 4**, Middle: 7, Low: 12

ICH M10 Guideline

1. INTRODUCTION
2. GENERAL PRINCIPLES
3. CHROMATOGRAPHY
4. LIGAND BINDING ASSAYS
5. INCURRED SAMPLE REANALYSIS (ISR)
6. PARTIAL AND CROSS VALIDATION
7. ADDITIONAL CONSIDERATIONS
8. DOCUMENTATION



Note

These are excerpts from the discussion at JBF ICH-M10 workshop 2023. Please note that these do not represent the official position of JBF.

General

1.3 Scope

This guideline describes the validation of bioanalytical methods and study sample analysis that are expected to support regulatory decisions.

- ***Do we conduct additional validation for analytical method which was fully validated before ICH-M10 implementation.***
  ➡ ***Many participants are NOT thinking about additional validation***. *But many of the opinions were personal, and many participants considered company policy to be determined after domestic notifications and training material were released.*

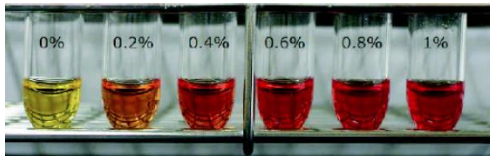
Chromatography

3.2.1 Selectivity

For the investigation of **selectivity in lipaemic matrices at least one source of matrix** should be used. To be scientifically meaningful, the matrix used for these tests should be representative as much as possible of the expected study samples. A naturally lipaemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipaemic matrix from donors, if this is difficult to obtain, matrix can be spiked with triglycerides even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidaemic, the use of spiked samples is discouraged. **This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism** or is administered in a particular animal strain that is hyperlipidaemic.

- ***How do we prepare lipaemic matrices? (Patients plasma? or Spiking triglyceride?)***
- ***How many lipaemic matrices should we evaluate?***
  ➡ *In clinical cases, lipaemic plasma (TG 150-300 mg/mL) is commercially available, so* ***N=1 will be evaluated using purchased lipaemic patient plasma****.*
- ***Regarding drugs that have a pharmacological mechanism that improves lipid metabolism, even if only normal animals are used in non-clinical studies, lipaemic plasma evaluation is necessary?***
  ➡ ***No***

3.2.1 Selectivity



http://www.chiringi.or.jp/k_library/kaishi/kaishi2012_3/index.html

Chromatography

For the investigation of **selectivity in haemolysed matrices** at least one source of matrix should be used. Haemolysed matrices should be obtained by spiking matrix with haemolysed whole blood (**at least 2% V/V**) to generate a visibly detectable haemolysed sample.

- ***Are there any difference for haemolysed matrix evaluation between clinical and non-clinical?***
  ➡ ***No*** *(Since the possibility of hemolysis cannot be denied even in non-clinical studies,* ***hemolysed matrix evaluation should be performed both non-clinical and clinical****.)*

- ***In addition to 2% haemolysed plasma, should 5% or 10% heamolysed plasma be evaluated?***
  ➡ ***No*** *(It is difficult to determine haemolyse degree in actual sample. We should evaluate the potential effect of haemolyse using 2% haemolysed plasma.)*

- ***Are there any experience that selectivity was out of criteria when using lipaemic/haemolysed matrices?***
  ➡ *Some participants have experiences.*
  - *For arginine determination,* ***arginase*** *was released due to haemolysis ana affected the quantitative value.*
  - *For SPE pretreatment,* ***clogging*** *of the solid phase when using lipaemic plasma.*
  - ***If the blood cell transfer rate is high****, the quantitative value was affected by haemolysis.*

- ***What should we do in that case?***
  ➡ *If the selectivity criteria was not met, basically* ***start over from the method development****. Although the quantitative range changes,* ***sample concentration determination with dilution*** *might be possible as the one of the option.*

Chromatography

3.2.3 Matrix effect

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. **For each individual matrix sources/lots evaluated, 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%**. Use of fewer sources/lots may be acceptable in the case of rare matrices.
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.

- ***Considering the accuracy and precision of only each individual or each lot? Is the accuracy and precision of all samples (6 lots x 3 repeats) necessary?***
  ➡ *A&P evaluation for each individual is essential.* ***A&P evaluation for all samples is optional.***

- ***Can 6 individuals or 6 lots contain haemolysed or lipaemic matrix? Or should we add it as the 7th individual?***
  ➡ ***Special matrix should exclude 6 individual evaluation*** *(add as the 7th individual).*

- ***At what stage should matrix effects be verified for special populations and patient populations?***
  ➡ *Evaluation of patient population and special population basically be performed* ***using pre-dose samples****. Since* ***plasma from patients with liver and kidney disorders can be purchased****, it is also possible to conduct a preliminary evaluation.*

- ***If the matrix effect criteria was not met using special population matrix, what action should we take?***
  ➡ ***The first choice is to change the analytical method****. One option is to* ***dilute the analytical samples****.*

Chromatography

3.2.5.1 Preparation of Quality Control Samples

During method validation the QCs for accuracy and precision runs 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).

- ***In the case of a calibration curve range of 1 to 1000 ng/mL, conventionally the medium concentration QC would have been set around 50 ng/mL, but according to ICH M10 it would have been set at 300 to 500 ng/mL. In that case, should we put traditional medium QC (50 ng/mL) additionally?***
  ➡ ***Some participants are putting traditional medium QC additionally*** *(Others are not putting).*

Chromatography

3.2.5.2 Evaluation of Accuracy and Precision

To enable the **evaluation of any trends over time within one run**, it is recommended to demonstrate accuracy and precision of the QCs over at least one of the runs in a **size equivalent to a prospective analytical run of study samples**.

- ***How do we evaluate batch-size?***

  ➡ *Major evaluation method is below.*

  - *Inject **matrix containing samples** (e.g., blank samples, QC samples, zero samples. etc.) until batch size evaluation sample injected.*
  - *Conduct at **4 concentrations including LLOQ**.*
  - ***The last 20 QC samples** (LL, L, M, H × 5 each) are sufficient for setting the criteria.*
  - *The sample to be measured mid-way may be used for the purpose of evaluating only the trend (exclude from evaluating accuracy and precision).*

- ***If the sample number exceeds validated batch-size, the results should be rejected?***

  ➡ *Most participants commented that **it is acceptable** even if the number of actual sample measurements exceeds the number of evaluation samples for validation.*

Chromatography

3.2.7 Dilution Integrity

Dilution QCs should be prepared with analyte concentrations in matrix that are greater than the ULOQ and then diluted with blank matrix. **At least 5 replicates per dilution factor should be tested in one run to determine** if concentrations are accurately and precisely measured within the calibration range. The dilution factor(s) and concentrations applied during study sample analysis **should be within the range of the dilution factors and concentrations evaluated during validation**.

- ***How do we prepare dilution QC? (N=5 preparation? Or N=1 preparation → N=5 dilution?)***
  ➡ ***N=1 preparation*** → *N=5 dilution.*

- ***If the actual sample concentration exceeds dilution QC concentration, is the additional validation needed?***
  ➡ ***Yes*** *and* ***No***

Comments form Yes

- ***There is no problem*** *even if the actual sample exceeds the diluton QC concentration as long as it is* ***within the validated dilution ratio****.*

Chromatography

3.2.8 Stability

For **fixed dose combination products and specifically labelled drug regimens**, the freeze-thaw, bench-top and long-term stability tests of an analyte in matrix should be conducted with the matrix spiked with all of the dosed compounds.

- ***How do we interpret "specifically labeled drug regimens"?***
  - *Many companies interpreted the concomitant drugs that require developing as **combination drugs and fixed regimens** in actual clinical practice.*
  - *There are many opinions that they **do not follow up with the DDI drugs**. However, there is an opinion that **the drugs evaluated in the standard DDI study are planned to evaluate** mixed stability.*
  - *"Specifically labeled drug regimens" are interpreted as **drugs listed in the package insert**, and there was an opinion that it would be better to implement them during Phase III.*
  - *Many companies interpreted that evaluation is necessary **even if the concomitant drugs are biologic drugs**. In the case of ADC, an example was introduced in which the stability of a payload was evaluated under the coexistence of ADC.*

3.2.8 Stability

If the concentrations of the study samples are **consistently higher than the ULOQ** of the calibration range, the concentration of the high QC should be adjusted to reflect these higher concentrations.

- ***How do we interpret "consistently"?***
  - ➡ ***All samples except for pre-dose and placebo samples*** *exceed the ULOQ.*
  - ➡ ***All samlples around C_{max} in all subjects*** *exceed the ULOQ .*

- ***Is stability of dilution QC needed?***
  - ➡ *Stability of dilution QC is necessary* ***if a large number of samples exceed the ULOQ*** *or* ***if analyte aggregation is a concern****.*

Chromatography

3.2.9 Reinjection Reproducibility

Reinjection reproducibility is assessed by reinjecting a run that is comprised of calibration standards and a minimum of 5 replicates of the low, middle and high QCs after storage. The precision and accuracy of the reinjected QCs establish the viability of the processed samples.

- ***What is the difference between processed sample stability and reinjection reproducibility?***
  ➡ ***Processed sample stability***: ***Use freshly prepared calibration curve samples***, *evaluate* ***2 conc. QCs (Low/High)***.
  ➡ ***Reinjection reproducibility***: ***Reinject including calibration curve samples*** *(NOT prepared freshly), evaluate* ***4 conc. QCs (LLOQ-High QC)***.

- ***How do we evaluate reinjection reproducibility? (number of reinjection, storage duration before reinjection)***
  ➡ *All participants evaluate* ***1 time reinjection*** *(There are no participants who evaluate >2 times reinjection).*
  ➡ *There is an opinion that reinjection reproducibility should be evaluate* ***within processed sample stable duration***.

Chromatography

3.3.2 Acceptance Criteria for Analytical Run

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**. If multiple dilution factors are used in one analytical run, then dilution QCs need only be diluted by the highest and lowest dilution factors.

- ***How do we put and evaluate dilution QC? (number of determination, criteria)***
  ➡ *Many participants put dilution QC at each dilution factor, N=3, and evaluate those accuracy and precision.*
- ***Should we use same dilution factor in validation study?***
  ➡ ***NOT necessary***.
  - *In validation study, the dilution factor and concentration (preparation of dilution QC) are evaluated.*
  - *In study sample analysis, **the dilution operation** is evaluated.*

Chromatography

3.3.2 Acceptance Criteria for Analytical Run

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- ***If one of the highest and lowest dilution factor fails the criteria, how do we treat intermediate dilution factor?***
  ➡ *Intermediate dilution factor should be rejected.* ***Only dilution factor that meet the criteria should be adopted.***
- ***When the concentration of study samples exceed the dilution QC concentration, should it be reanalyzed?***
  ➡ *If the concentration falls within the concentration range of the calibration curve after dilution,* ***it can be adopted****.*

LBA

4.1.1 Reference Standard

If the reference standard batch used for bioanalysis is changed, bioanalytical evaluation should be carried out with QCs from the original material and the new material prior to use to ensure that the performance characteristics of the method are within the acceptance criteria.

- ***If the reference standard lot is changed, what kind of evaluation should be done?***

  ➡ *Many participants evaluate **QCs (accuracy & precision) prepared from old lot and new lot.***

  ➡ *Confirm the validity **using bioanalysis**, regardless of the details of the lot change.*

- ***Is the evaluation contents different between chemically synthetic compounds and biologically synthetic compounds?***

  ➡ ***No** (same contents)*

LBA

4.1.2 Critical Reagents

The data sheet for the critical reagent should include at a minimum identity, source, batch/lot number, purity (if applicable), concentration (if applicable) and stability/retest date/storage conditions (Refer to Table 1). Additional characteristics may be warranted.

- ***Some items that listed in guideline may not be available.***
  ➡ *All participants agreed that these items are "**nice to have**". (Not required if there is a reason why data could not be obtained).*

- ***What is "additional characteristics"?***
  ➡ *One of the example is **the labeling rate** of labeled reagent used in LBA analysis, but only a few companies listed it.*

LBA

4.2 Validation

Most often microtitre plates are used for LBAs and **study samples can be analysed using an assay format of 1 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 1 or more well(s) per sample, then study sample analysis should also be performed using 1 or more well(s) per sample, respectively.

- ***How do we decide singlicate or duplicate?***
- ***In the case of singlicate, what is the criteria of data adoption?***

  ➡ *Although there are discussions about singlicate measurement,* ***there is no company that actually performs singlicate measurement****.*

  ➡ *Many participants understand the benefits of singlicate mesurement, but there are hurdles in how to guarantee the data, so it's basically* ***a "waiting" state****.*

LBA

4.2.3 Calibration Curve and Range

If freshly spiked calibration standards are not used, **the frozen calibration standards can be used within their defined period of stability.**

- ***Are there any experiences using the frozen calibration standards?***
  ➡ *5 companies has the experiences using frozen calibration standards. In the validation study, frozen stability was confirmed using QC samples.*

LBA

4.2.4.2 Evaluation of Accuracy and Precision

Within-run accuracy and precision data should be reported for each run. If the within-run accuracy or precision criteria are not met in all runs, **an overall estimate of within-run accuracy and precision for each QC level should be calculated.** Between-run (intermediate) precision and accuracy should be calculated by combining the data from all runs.

- ***Regarding the "overall estimate", are there any examples of implementation in each companies?***
  Shared examples and discussions
  - *If the batch fails, the batch will be rejected, but **the failed data included in the report.***
  - *"Overall estimate" is positioned as a supporting role.*
  - *To see overall trends and judge reliability, it is important **to report all data openly.***

LBA

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 the LBA analysis, should stability evaluate at actual sample concentration range?***

  ➡ *Many participants consider the stability evaluation* ***at actual sample concentration range.***

- ***For the LBA analysis, is dilution QC analysis needed in study sample analysis?***

  ➡ *Put dilution QC: 4 companies (minor)*

LBA

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.

- ***How do each company respond to "at least 2 QC sample levels should fall within the range of concentrations measured in study samples"?***

  ➡ ***No company has actively considered QC concentration*** *to accommodate study sample clustering.*

  ➡ *If clustering occurs,* ***consider adding the concentration of the QC sample (3 conc⇒4 conc)****, but* ***not consider changing the concentration range of the calibration curve*** *(majority opinion).*

General

6.2 Cross validation

Cross validation is required to demonstrate how the reported data are related when multiple bioanalytical methods and/or multiple bioanalytical laboratories are involved.
Cross validation is required under the following situations:

- Data are obtained from different fully validated methods within a study.
- Data are obtained within a study from different laboratories with the same bioanalytical method.
- Data are obtained from **different fully validated methods** across studies that are going to be combined or compared to support special dosing regimens, or regulatory decisions regarding safety, efficacy and labelling.

- ***How do we interpret "different fully validated method"?*** *(methods with different analytical platform such as LC/MS and LBA? Or the methods at another facility at different time?)*
  ➡ *There was no disagreement that **cross-validation is necessary for analytical methods with different analytical platform**. **No conclusion could be reached** regarding when the analytical methods are slightly different using the same analytical platform.*
- ***When third facility is added, how do we conduct cross validation?***
  ➡ *The theory of inter-facility cross validation is **comparison with original facility** (When A is the original, confirm A/B and A/C). However, in the case of disappearing the original facility, consider B/C comparison.*
- ***If there is big difference in calibration curve range, how do we conduct cross validation?***
  ➡ *There is an opinion that **best practice should be needed** (Prepare high concentration QC and analyze with dilution when lower calibration range method is used.).*

General

6.2 Cross validation

Cross validation should be performed in advance of study samples being analysed, if possible.
Cross validation should be assessed by measuring the same set of QCs (low, medium and high) at least in triplicate and **study samples (if available)** that span the study sample concentration range (n≥30) with both methods, or in both laboratories.

- ***For actual Ph1 samples, it is possible to select ``span the study sample concentration range," but the concentration may be biased depending on the clinical trial. Should we still give priority to using real samples?***
  ➡ ***Use actual samples whenever possible*** *(even if they cannot cover a wide concentration range)*

- ***Even if the validated stability period has elapsed, should cross-validation using actual samples be performed?***
  ➡ ***Yes and No.***
  Comments
  - ***Evaluation using QC only is allowed*** *because "if available" is described in guideline.*
  - ***Additional validation of stability*** *is possible.*

- ***How do we evaluate QC samples?***
  ➡ ***Set criteria (accuracy & precision)*** *based on theoretical values and* ***include into statistical analysis*** *in the same way as actual samples.*

General

6.2 Cross validation

Bias can be assessed by Bland-Altman plots or Deming regression. **Other methods appropriate** for assessing agreement between two methods (e.g., concordance correlation coefficient) may be used too. Alternatively, the concentration vs. time curves for study samples could be plotted for samples analysed by each method to assess bias.

- ***Do you have experiences using Bland-Altman plots or Deming regression?***
  ➡ *A few participants had experiences using Bland-Altman plots in studies conducted in the foreign country.*

- ***What is criteria?***
  ➡ *It is **difficult to set criteria** for statistical evaluation. There were no participants who has the experience that large bias was detected.*
  ➡ *Use **ISR criteria** with statistical evaluation.*
  ➡ *One aspect is that it is important to **understand the distribution** (importance is "creating plot").*

Summary

- *The 306 discussion points/questions were provided by 33 Industries and CROs.*
- *The issues of high importance and attention which JBF SC categorized and prioritized were discussed at the JBF ICH M10 Workshop.*
- *Many opinions were exchanged at the workshop, and we were able to deepen our understanding of the points of discussion.*
- *As for some points of discussion that could not be concluded, it is desirable that specific views be presented through training materials etc.*

Detail contents are exhibited as a poster at the Poster & Booth Venue (1F)

Acknowledgement

- *All participants from Industries and CROs*
- *JBF steering committee members*

Thank you for your attention!