15th JBF Symposium

ICH M10 Implementation Insights: Experiences and Lessons Learned from Bioanalytical Communities (1)

Feb 6, 2024　MIYAKO MESSE, Kyoto

Toward Implementation of ICH M10 Guideline

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Contents

1. ICH M10 and related documents

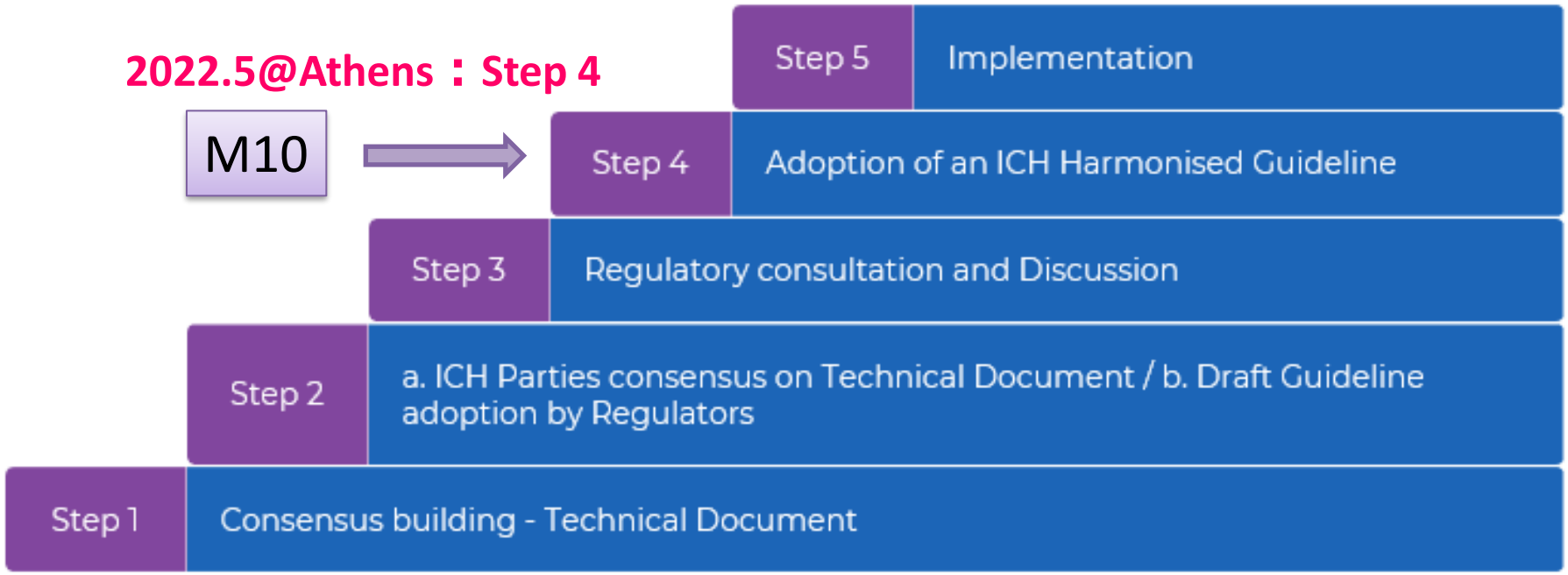
2. Concept of M10: Strategy to ensure the reliability of bioanalytical data

3. Points to consider in implementation of M10

Harmonization steps of ICH M10

Formal ICH Procedure

The Formal ICH Procedure is a step-wise procedure consisting of 5 steps (see below, click to have information on a particular step). This procedure is followed for the harmonisation of all new ICH topics.



https://www.ich.org/page/formal-ich-procedure

coming soon!

MHLW Notification

2019 Public consultation

2019.1
after Charlotte meeting

2016.11
1st F2F EWG meeting in Osaka

2016.6 “Bioanalytical Method Validation” proposed by MHLW was adopted as a new topic M10

M10 Guideline and related official documents

M10 EWG **Bioanalytical Method Validation and Study Sample Analysis**

This Guideline is intended to provide recommendations for the validation of bioanalytical methods for chemical and biological drug quantification and their application in the analysis of study samples. Adherence to the principles presented in this Guideline will ensure the quality and consistency of the bioanalytical data in support of the development and market approval of both chemical and biological drugs.

The objective of the validation of a bioanalytical method is to demonstrate that it is suitable for its intended purpose. Changes from the recommendations in this Guideline may be acceptable if appropriate scientific justification is provided. Applicants are encouraged to consult the regulatory authority(ies) regarding significant changes in method validation approaches when an alternate approach is proposed or taken.

Rapporteur: Dr. Akiko Ishii-Watabe (MHLW/PMDA, Japan)

Regulatory Chair: Dr. Brian Booth (FDA, United States)

Date of *Step 4*: 24 May 2022

Status: *Step 5*

Guideline
- M10 Guideline

Endorsed Documents
- M10 Concept Paper
- M10 Business Plan
- M10 Work Plan

WG Presentations / Trainings
- M10 Step 4 Presentation

Other documents
- M10 FAQs

WG list

Guideline

→ MHLW Notification

Step 4 presentation

FAQ

Training Slides

M10 Q&As **Questions and Answers: Bioanalytical Method Validation and Study Sample Analysis**

ICH M10 Q&As have been devised to provide clarity around some of the bioanalytical issues covered in the ICH M10 Guideline. The ICH M10 Q&As are intended to provide additional clarification and to promote convergence and improve harmonisation of the bioanalytical method validation and study sample analysis. The scope and organisation of this Q&A document follow that of ICH M10 Guideline.

Date of *Step 4*: 16 November 2022

Status: *Step 5*

Questions and Answers
- M10 Q&As

Q&A

→ MHLW Office Memorandum

https://www.ich.org/page/multidisciplinary-guidelines

Implementation status of M10

EC, Europe - Implemented; Date: 21 January 2023
FDA, United States - Implemented; Date: 7 November 2022
Health Canada, Canada - Implemented; Date: 20 January 2023
NMPA, China - Implemented; Date: 29 July 2023
SFDA, Saudi Arabia - Implemented; Date: 10 August 2023
Swissmedic, Switzerland - Implemented; Date: 25 May 2022
TFDA, Chinese Taipei - Implemented; Date: 30 May 2023

ANVISA, Brazil - In the process of implementation
EDA, Egypt - In the process of implementation
HSA, Singapore - In the process of implementation
MFDS, Republic of Korea - In the process of implementation; Date: 31 October 2023
MHRA, UK - In the process of implementation

COFEPRIS, Mexico - Not yet implemented
MHLW/PMDA, Japan - Not yet implemented
TITCK, Türkiye - Not yet implemented

https://www.ich.org/page/multidisciplinary-guidelines

Contents

Table of contents of M10 guideline

Section 1: Scope

Category	Contents
Analyte	・Chemical and biological drugs (incl. Drugs that are also endogenous molecules) ・Metabolites
Matrix	・Biological samples (e.g., blood, plasma, serum, other body fluids or tissues)
Method	・LC or GC typically used combination with MS ・Ligand binding assay
Study	・ **Nonclinical PK studies** conducted as surrogates for clinical studies ・ **Nonclinical TK studies** conducted under GLP ・ **All phases of clinical trials** including BA, BE studies

Out of scope：
Biomarker assay
Anti-drug antibody assay

Example which includes nonclinical PK data to support human dosing:

✓rescue agents for acute radiation syndromes or anthrax

急性放射線症候群や炭疽菌等に対するレスキュー薬

Structure of M10 to ensure the reliability of bioanalytical data of study samples

Purpose of the analysis：Quantitation of drug concentration in biological matrices

Section 2

Method Development

- Chromatography
- Ligand Binding Assay (LBA)

Section 3&4

Method Validation

Evaluate the analytical performance

- ✓ Selectivity
- ✓ Specificity
- ✓ Matrix effect
- ✓ Calibration curve and Range
- ✓ Accuracy and Precision
- ✓ Carry over
- ✓ Dilution Integrity/Linearity
- ✓ Stability
- ✓ Reinjection reproducibility

Section 3&4

Study Sample Analysis

Check the validity of each run

- ✓ Calibration curve
- ✓ QCs

Section 5

Incurred Sample Reanalysis (ISR)

Confirm the reproducibility of study sample analysis

Section 6

- ➢ Modification of method
- ➢ Change of matrix

Partial Validation

- ➢ Multiple bioanalytical methods
- ➢ Multiple bioanalytical laboratories

Cross Validation

Section 8

Documentation

Conform to GLP, GCP requirements

Section 3&4 Performance characteristics to be evaluated in method validation

Section	Chromatography	LBA
Sections 3.2 and 4.2 Full validation	➢ Selectivity ➢ Specificity ➢ Matrix effect ➢ Calibration curve and Range ➢ Accuracy and Precision ➢ Carry over ➢ Dilution Integrity ➢ Stability ➢ Reinjection reproducibility	➢ Specificity ➢ Selectivity ➢ Calibration curve and Range ➢ Accuracy and Precision ➢ Carry over ➢ Dilution linearity and Hook effect ➢ Stability
7. Additional considerations		7.2 Parallelism

These items were established by considering the sources of variability in bioanalysis (e.g., biological matrix) and characteristics of the technologies (Chromatography or LBA).

In M10, **experimental procedure** and **acceptance criteria** are described for each validation test.

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2. GENERAL PRINCIPLES

3. CHROMATOGRAPHY
- 3.2 Validation
- 3.3 Study Sample Analysis

4. LIGAND BINDING ASSAY
- 4.1 Key Reagents
- 4.2 Validation
- 4.3 Study Sample Analysis

5. INCURRED SAMPLE REANALYSIS

7. ADDITIONAL CONSIDERATIONS
- 7.1 Methods for Analytes that are also Endogenous Compounds
- 7.6 New or Alternative Technologies

Surrogate matrix

Question

In situations where a matrix is unavailable (e.g., shortage, 3Rs - Reduce, Refine, Replace) can a similar surrogate matrix (e.g., human plasma) be used to dilute samples?

マトリックスの入手が困難な場合（例えば供給不足や3Rs (Reduce, Refine, Replace) の観点で）、類似の**代替マトリックス**（例えば、ヒト血漿）を試料の希釈に使用することは可能か。

Answer

Yes, as long as the use of the surrogate matrix meets the recommendations of the guideline, including accuracy and precision, lack of interferences, etc. and the dilution quality control samples (QCs) are processed in the same way. The rationale needs to be well justified because the approach might be questioned.

代替マトリックスの使用が真度及び精度、妨害物質の影響がないこと等を含むガイドラインの推奨事項を満たしてしており、希釈Quality Control (QC) 試料が他の試料と同様に処理されるのであれば、代替マトリックスを試料の希釈に使用することが可能である。このアプローチは疑問視される可能性があるので、十分に根拠の妥当性を示す必要がある。

3.2.2 Specificity

Question

Can the physicochemical properties of the related substances be used to justify that the related substances do not co-elute or interfere with the analyte measurement during mass spectrometry (MS) analysis?

質量分析装置での分析時に、類似物質が分析対象物質と共溶出しないことや分析対象物質の測定を干渉しないことの妥当性を、類似物質の物理化学的性質に基づいて説明することは可能か。

Answer

Yes, but if co-elution of the related substance and the analyte is not excluded, additional investigations are needed to demonstrate chromatographic separation (e.g., for isomers). If the analyte and the related substance co-elute, matrix effect (ion suppression/ enhancement) and back-conversion should be evaluated.

説明可能である。ただし、分析対象物質と類似物質との共溶出が否定できない場合（例：異性体）、追加の検討を実施してクロマトグラム上の分離を示す必要がある。分析対象物質と類似物質が共溶出する場合、マトリックス効果（イオン化抑制／増強）と逆変換について評価すべきである。

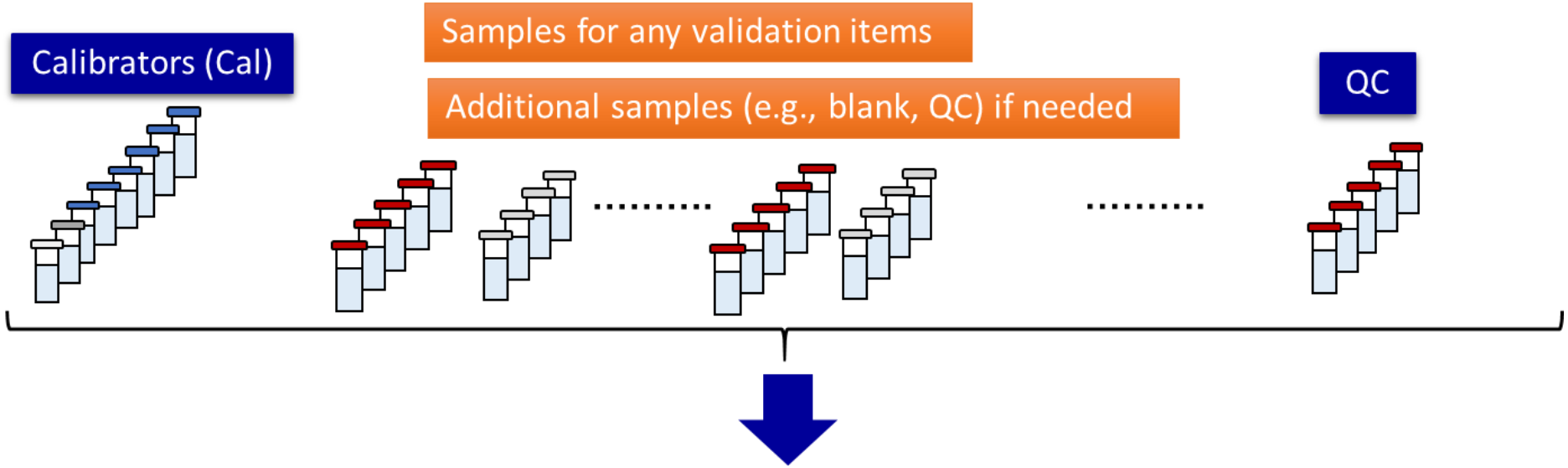
3.2.5.2 Evaluation of Accuracy and Precision

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

Example of run size evaluation:

Samples for any validation run can be used for run size evaluation.

Additional samples (e.g., blank, QC) can be added to reach a prospective run size.



Evaluate accuracy & precision using all QC samples interspersed in a whole run

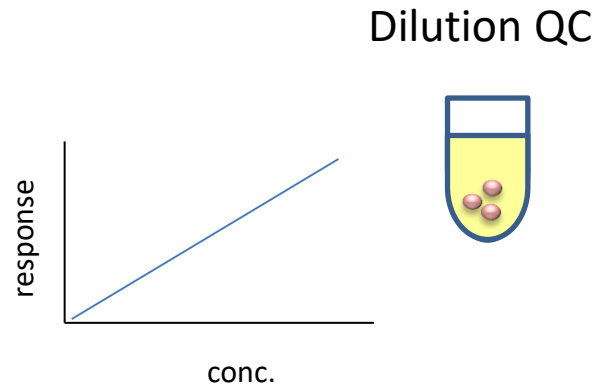
3.2.8 Stability

Dilution QC

"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."

Is proven stability for dilution QC samples required ?

- It is recommended as best practice to include stability testing of dilution QCs in method validation.
- The importance of this stability assessment depends on the relevance of the study with respect to making regulatory decisions, and the number of samples in the concentration range above the ULOQ.
- Although this stability assessment might not be feasible for an early dose escalation study, it is expected for other types of studies, e.g., BA/BE



3.2.8 1) Long-term stability in matrix

Question

For long-term stability, does a failed time-point mean you should not continue with longer time-points?

長期保存安定性の評価において、基準を満たさない時点が生じた場合には、それ以降の時点については評価を中止すべきか。

Answer

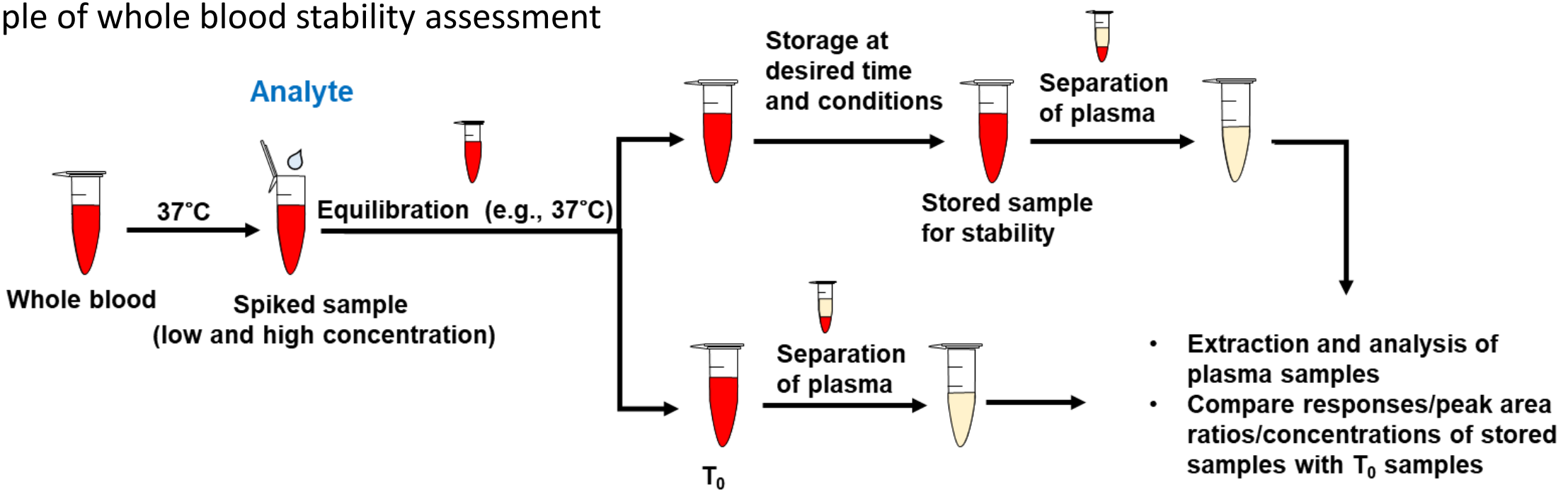
Additional time-points can be evaluated. Any failure should be investigated to identify the root cause and the impact on the stability assessment.

追加の時点を評価することができる。長期保存安定性が基準を満たさない事例については、その根本原因や安定性評価におけるインパクトを明らかにするために調査すべきである。

3.2.8 4) Stability of the Analyte in Whole Blood

Stability of the analyte in blood should be evaluated to ensure the stability of the analyte in sampled matrix (blood) directly after collection from subjects and prior to preparation for storage. Whole blood stability can 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.

Example of whole blood stability assessment



3.2.9 Reinjection Reproducibility

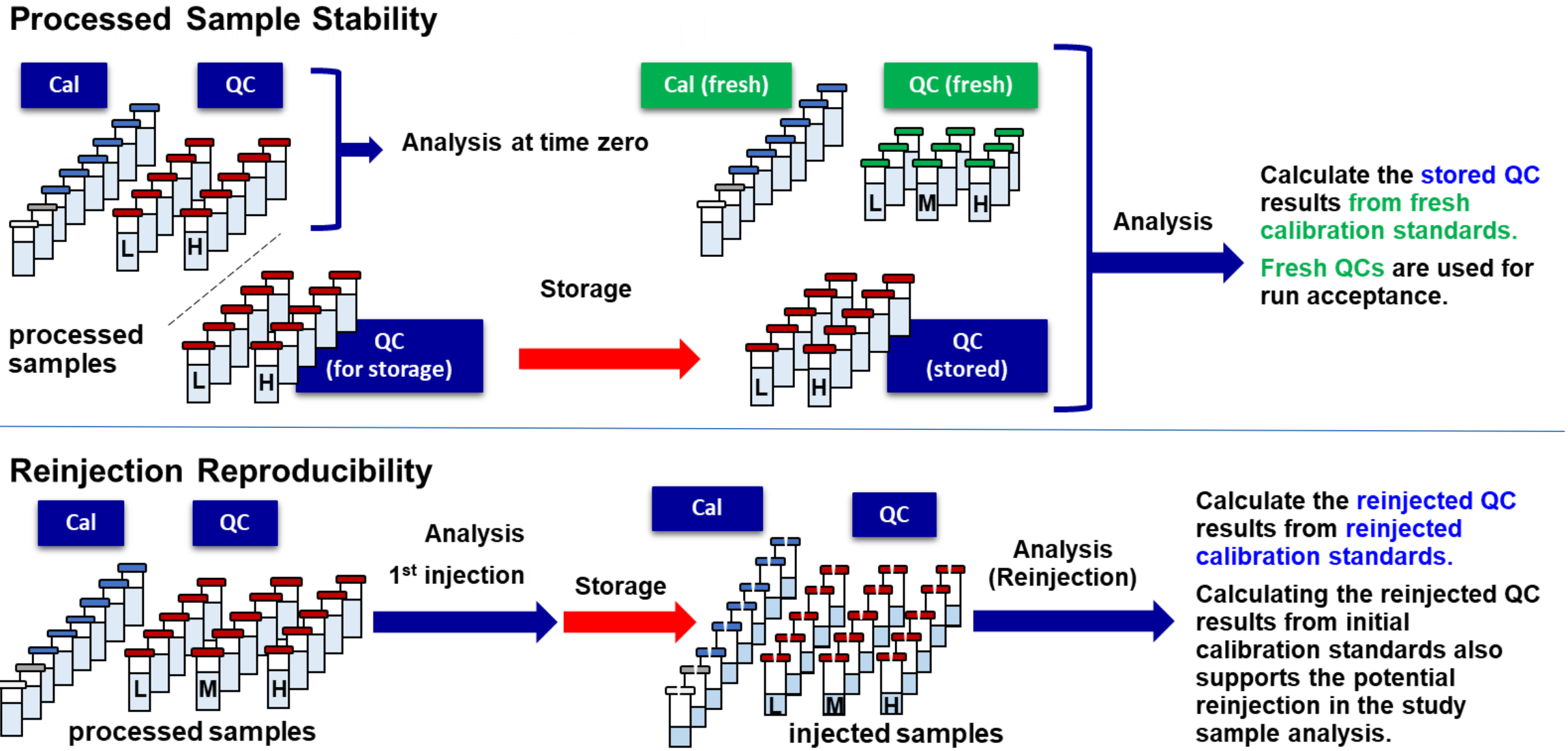
"If samples could be reinjected (e.g., in the case of instrument interruptions or other reasons such as equipment failure), reinjection reproducibility should be evaluated to establish the viability of the processed samples and to support their storage prior to reinjection."

What is the difference between **reinjection reproducibility** and **processed sample stability**?

	Processed Sample stability	Reinjection Reproducibility
Purpose	Determine how long samples are stable post processing and prior to injection	Demonstrate the ability to reinject an analytical run entirely or in part.
QCs for evaluation	• Low and High QCs • Stored under the conditions to be evaluated • Minimum of 3 replicates • Fresh QCs required for run acceptance	• Low, Medium and High QCs • Stored under relevant conditions prior to reinjection • Minimum of 5 replicates
Calibration standards	Freshly prepared	Stored with and then reinjected with QCs

3.2.9 Reinjection Reproducibility

An Example of a Possible Comparison Between **Reinjection Reproducibility** and **Processed Samples Stability** Assessment



New QC concentration

3.3.3 Calibdation Range
At least 2 QC levels should fall within the range of concentrations measured in study samples.

- ✓ narrow the calibration curve range
- ✓ adapt the concentrations of the QCs
- ✓ add new QCs at different concentration levels as appropriate

Question

When adding a new QC concentration level during study sample analysis without changing the calibration curve range in either chromatographic assays or ligand binding assays, is it necessary to validate the new QC concentration level with a partial validation?

クロマトグラフィーあるいはLBAを用いた実試料分析において、定量範囲を変えることなく新たな**QC試料濃度**を追加するとき、その新たなQC試料濃度についてパーシャルバリデーションが必要か。

Answer

The precision and accuracy of the new QC concentration level should be demonstrated before use in study sample analysis. This can be documented either as a partial validation or as a note to the bioanalytical report.

新たなQC試料濃度の真度及び精度を実試料分析で使用する前に示すべきである。
この検討についてはパーシャルバリデーションとして文書化するか、あるいは実試料分析の報告書に記載することができる。

4.1.2 Critical Reagents

When changing a critical reagent what are the expectations for documentation and reporting performance?

- The level of assessment to ensure the performance and quality when changing a critical reagent will depend on whether the change is considered major or minor.

Examples of Minor and Major Changes to Critical Reagents

Minor change	Major change
A new purification derived from a previous qualified batch	A change in production method of antibodies
Source/Supplier is changed but the reagent is the same (e.g., same clone)	A new clone from monoclonal antibody production
A new affinity purification of polyclonal sera from the same animals	A new bleed of animals for polyclonal antibodies
A new conjugation using the same protein lot	A new cell line for the generation of recombinant material

4.2.1 Specificity

Question

Is there a requirement to test specificity in validation with an irrelevant immunoglobulin molecule when the analyte is an immunoglobulin and the assay contains analyte specific reagents (e.g., use of anti-idiotypic antibody(ies) as capture and/or detection reagents)?

分析対象物質が免疫グロブリンであり、分析時に分析対象物質に特異的な試薬（例えば、捕捉試薬及び／または検出試薬として抗イディオタイプ抗体）が用いられる場合、分析法バリデーション時に分析対象物質と関連のない免疫グロブリン分子を用いて特異性の評価を実施する必要はあるか。

Answer

There is no requirement to assess specificity in validation with an irrelevant immunoglobulin as long as the specificity of the reagent(s) has been evaluated during reagent characterisation.

試薬の特性評価時に、その試薬の特異性が評価されている場合には、バリデーションにおいて分析対象物質に関連のない免疫グロブリンを用いた特異性の評価は必要ない。

4.2.6 Dilution Linearity-Sample Analysis

If during sample analysis, a required dilution QC is outside the dilution factor range tested in validation, how do you address this?

- If the dilution is within the range tested but not the exact dilution factor, one solution may be that the dilution factor may be used without any additional validation, assuming that the dilution factors used within that range passed acceptance criteria.

Example 1:

The dilutions tested were 1:10, 1:100, 1:1000 and 1:10000, then a dilution factor of 1:500 may be used.

Example 2:

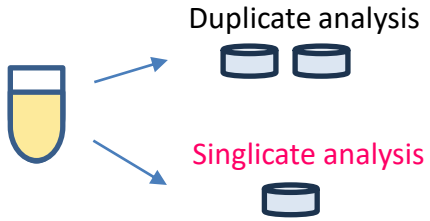
If the dilution range tested was 1:10 to 1:10000 yet samples do not fall in the validated assay range and a 1:2 dilution would be more appropriate, then you could:

Option1: Conduct a partial validation. This approach is recommended when having a large number of samples to be tested with this dilution.

Option 2: Add a dilution QC (1:2) in the sample analysis run and test in the same number of replicates as any other QC concentration if only a few samples require this dilution factor.

4.2 Validation- singlet vs duplicate wells

"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."



Is comparison between single well and duplicate wells necessary?

No, it can be assessed in method development but it is not a pre-requisite.

If a single well is used in the method validation, can it be used in the study sample analysis directly?

Yes, as per text in guideline.

What if you have done validation in duplicate and want to perform sample analysis in singlicate well?

Here the validation data can be used but it needs to be calculated with the first replicate for Calibration Standards and QCs to mimic the conditions of sample analysis in singlicate.

If performing analysis with a single well, should Calibrator Standards always be analysed in duplicates?

No, this is not necessary

4.3.1 Analytical Run-Combined Calibration Curve Over Several Plates

There are 2 options when using multiple plates or CDs within an analytical run:

Option 1:

Calibration standards and QCs on each plate/CD

- Each plate is considered individually and acceptance criteria for the calibration curve and the QCs will be applied to the individual plate/CD

Option 2:

Calibration standards on the first and last plate/CD and QCs on each plate/CD

- The first and last calibration curves will be combined to make one calibration curve that will be used for regression of data from all plates/CDs. This approach is only used when there are not calibration curves on all plates/CDs.
- The QCs on each plate/CD will be used for that individual plate/CD

Option 1

Plate/CD Number	Curve Required	Calibration Standard Placement	QCs Required
1	Yes	Yes	Yes
2	Yes	Yes	Yes
3	Yes	Yes	Yes
4	Yes	Yes	Yes
5	Yes	Yes	Yes

Option 2

Plate/CD Number	Curve Required	Calibration Standard Placement	QCs Required
1	Yes	Yes	Yes
2	No	No	Yes
3	No	No	Yes
4	No	No	Yes
5	Yes	Yes	Yes

Example 1
Calibration Curve on Plate 1 Fails

No.	Calibration Curve Passes	QCs Pass	Result
1	**No**	Yes	**Whole assay run fails**
2	NA	Yes	
3	NA	Yes	
4	NA	Yes	
5	Yes	Yes	

The calibration standards on the first plate fails; therefore the combined calibration curve fails, and the whole run fails

Example 2
QCs on Plate 1 Fails

No.	Calibration Curve Passes	QCs Pass	Result
1	Yes	**No**	**Whole assay run fails**
2	NA	Yes	
3	NA	Yes	
4	NA	Yes	
5	Yes	Yes	

The QCs on the first plate with the calibration curve fail; the first plate fails; the combined calibration curve fails and the whole run fails

Example 3
QCs on Plate 3 Fail

No.	Calibration Curve Pass	QCs Pass	Result
1	Yes	Yes	Plate/CD passes
2	NA	Yes	Plate/CD passes
3	NA	**No**	Plate/CD **fails**
4	NA	Yes	Plate/CD passes
5	Yes	Yes	Plate/CD passes

The QCs on one plate/CD with samples fails, then that individual plate/CD would fail, but the remaining plates would pass

Number of ISR samples

How do you calculate the number of ISR samples from a **NONCLINICAL** study?

The total number of control samples should be excluded when calculating the number of ISR samples.

Example

4-week GLP Tox/TK study in rats:
Number of study samples analysed = 335
Number of control group samples included = 12

Number of ISR samples: (335-12) x 10% = 32 samples

How do you calculate the number of ISR samples from a **CLINICAL** study?

Placebo samples should be excluded when calculating the number of ISR samples.

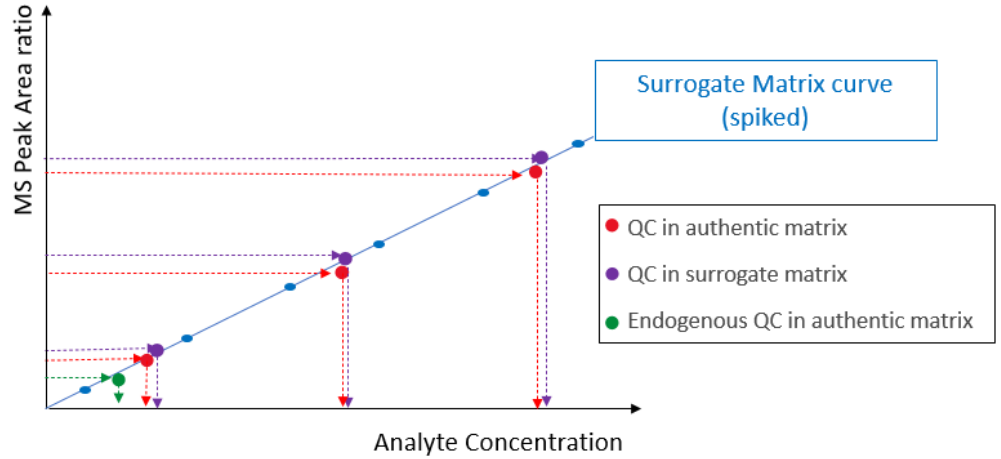
Example

Clinical study (simple 2:1 design):
Total number of samples analysed = 7500
Number of samples from “test” group = 5000
Number of samples from placebo group = 2500

Number of ISR samples, excluding placebo samples: (1000 x 10%) + (4000 x 5%) = 300 samples

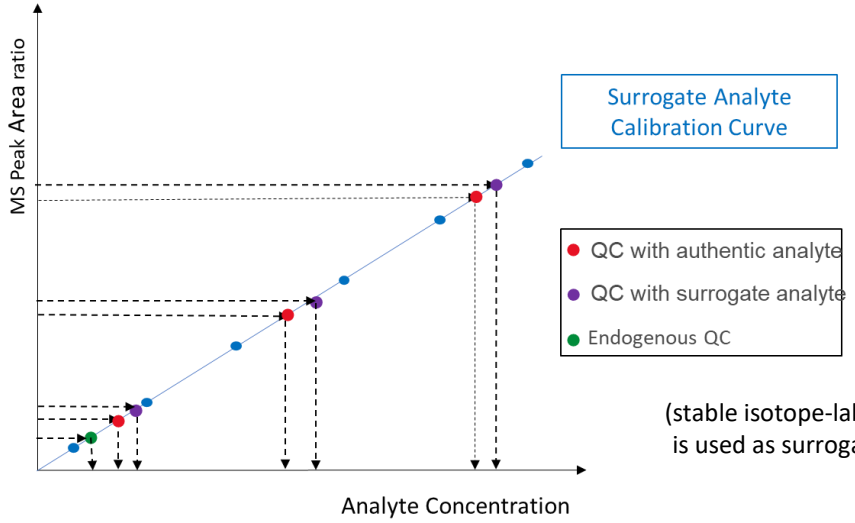
7.1 Approaches for Bioanalysis of Analytes that are also Endogenous Molecules

(1) Surrogate Matrix Approach



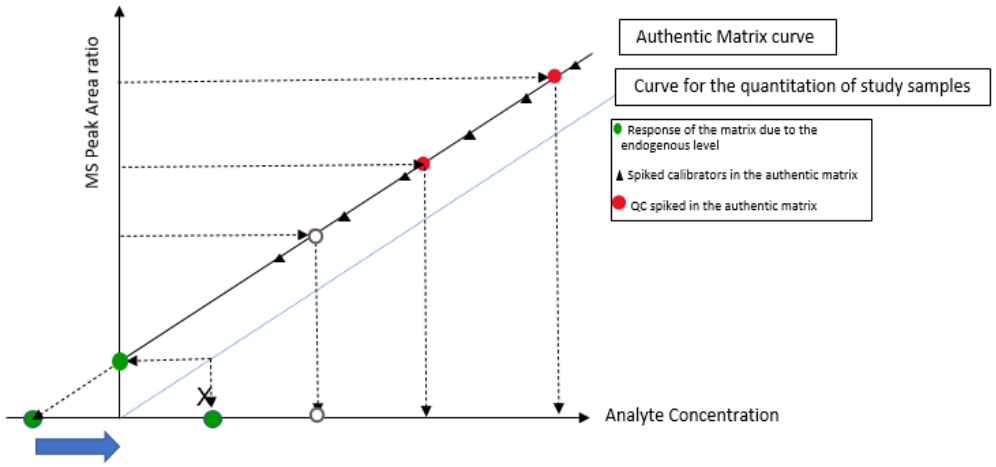
(2) Surrogate Analyte Approach

LC/MS



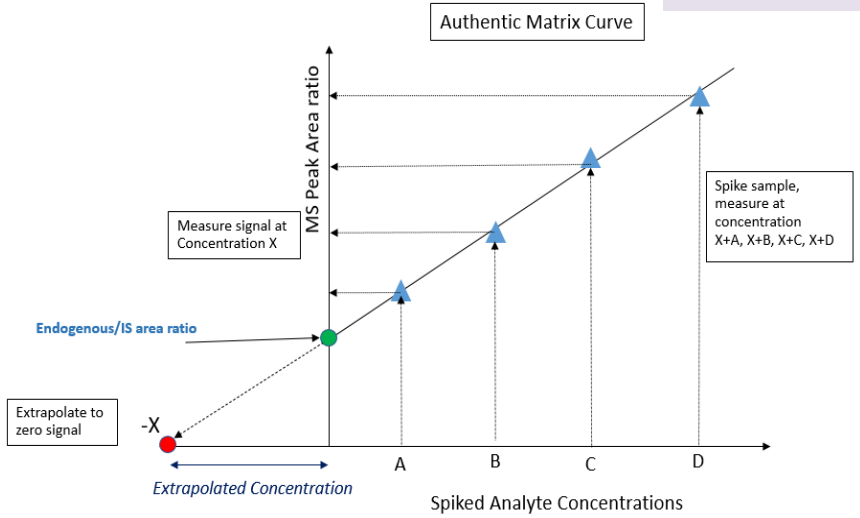
(stable isotope-labelled analyte is used as surrogate standard)

(3) Background Subtraction Approach



(4) Standard Addition Approach

Assay with linear response



7.1 Surrogate Matrix Approach

How do you assess whether you can use the surrogate matrix approach?

For both chromatographic and LBA:

- Spike QCs in authentic matrix and in surrogate matrix.
- Use Endogenous QCs in authentic matrix.
- Analyse them on the calibration curve prepared in surrogate matrix.
- The recovery/accuracy of the QCs should be within acceptance criteria.

Accuracy can be calculated using this formula:

$$Accuracy\ (\%) = 100\ \times \frac{(\text{Measured concentration of spiked sample} - \text{endogenous concentration}\ )}{\text{Spiked concentration}}$$

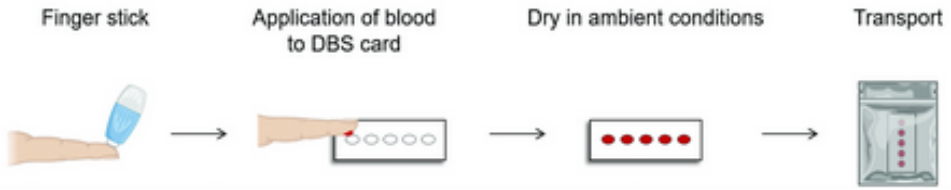
Section 7.6 New or alternative technologies

When a new or alternative technology is used as the **sole** bioanalytical technology from the onset of drug development, cross validation with an existing technology is **not** required.

Section 7.6.1 Dried Matrix Methods (DMM)

Benefits of DMM

- ✓ Collection of reduced blood sample volumes as a microsampling technique
- ✓ Ease of collection, storage and transportation



https://www.aacc.org/cln/articles/2022/september/dried-blood-spots-and-beyond

Further validation required for DMM:

- Haematocrit (especially for spotting of whole blood into cards)
- Sample homogeneity (especially for sub-punch of the sample on the card/device)
- Extraction of the sample from the dried matrix

➢ DMM sample collection for ISR is required. (multiple punches)

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.

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Conclusion

- Basic principles of regulated bioanalysis were harmonised by M10.
- The implementation in details should be done by science- and risk-based approaches.

Future perspectives on regulated bioanalysis

- The principles of M10 may be applied for new modalities those are analysed by other technologies and other analytes outside M10 (e.g., biomarker, anti-drug antibody).
- Critical issues for regulated bioanalysis beyond M10 would be:

Well understanding of

- ✓ Characteristics of the drug product
- ✓ Bioanalytical methods
- ✓ Pharmacokinetic profiles of the drugs in relation to efficacy and safety

⇨ Reliable bioanalytical data fitting for the intended purpose will be obtained.

Acknowledgement

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MHLW/PMDA : Daisuke Iwata, Taishi Horiuchi
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M10 EWG Nov. 18, 2019 @Singapore

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JPMA
Takashi Miyayama, Yosuke Kawai, Shinya Hosaka, Seiji Tanaka, Masataka Katashima

JBF
Akira Nakayama, Masaaki Kakehi

JGA
Hidenao Tachiki

Thank you for your attention!

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