7th EBF Open Symposium "Beyond the Horizon"
Nov 21st, 2014 Hotel Hesperia Tower Hotel, Barcelona, Spain

The Japanese BMV Guideline for Ligand Binding Assay

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Contents

1. Overview of the new Japanese BMV guideline for LBA

2. Topics
    1) Minimum Required Dilution
    2) Total error
    3) Critical reagents
    4) Parallelism

The Japanese BMV guidelines

BMV guideline (Chromatography)

Released on 11th Jul, 2013

BMV guideline (Ligand Binding Assay)

Draft version was released on 10th Jan, 2014.

↓

Public comments ～10th Feb, 2014

↓

Final version was released on Apr 1st, 2014

Public comments to the new draft Japanese BMV guidelines for LBA

from Companies

- Thirteen pharmaceutical companies including domestic and foreign capital companies

from Organizations outside of Japan

- European Bioanalysis Forum
- AAPS LBA Bioanalytical Focus Group

In total, 181 comments were submitted.

“Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development”

Notification No. 0401 of the Evaluation and Licensing Division, PFSB dated April1, 2014

1. Introduction
2. Scope
3. Reference Standard
4. Analytical Method Validation
5. Analysis of Study Samples
6. Points to Note
7. Documentation and Archives

Glossary

Scope of Japanese BMV guideline for LBA

Analyte:

- Drugs analyzed by LBA
  - peptides
  - proteins
  - others

Study:

- non-clinical TK (GLP)
- clinical PK

Out of scope

- ✓ Biomarker
- ✓ Anti-drug antibody

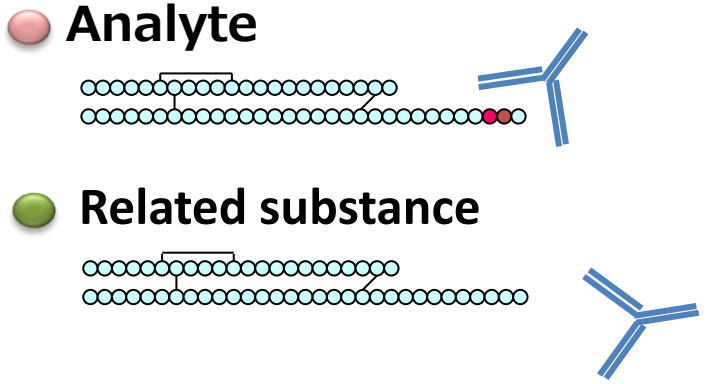
Japanese BMV guidelines for Chromatography and LBA

BMV guideline(Chromatography)2013	BMV guideline (LBA) 2014
1. Introduction 2. Scope 3. Reference Standard	1. Introduction 2. Scope 3. Reference Standard
4. Analytical Method Validation 4.1. Full validation 4.1.1. Selectivity 4.1.2. Lower limit of quantification 4.1.3. Calibration curve 4.1.4. Accuracy and precision 4.1.5. Matrix effect 4.1.6. Carry-over 4.1.7. Dilution integrity 4.1.8. Stability 4.2. Partial validation 4.3. Cross validation	**4. Analytical Method Validation** 4.1. Full validation 4.1.1. Specificity 4.1.2. Selectivity 4.1.3. Calibration curve 4.1.4. Accuracy and precision 4.1.5. Dilutional linearity 4.1.6. Stability 4.2. Partial validation 4.3. Cross validation
5. Analysis of Study Samples 5.1. Calibration curve 5.2. QC samples 5.3. ISR 5.4. Carry-over	**5. Analysis of Study Samples** 5.1. Calibration curve 5.2. QC samples 5.3. ISR
6. Points to Note 6.1. Calibration range 6.2. Reanalysis 6.3. Chromatogram Integration 6.4. System suitability 6.5. Recovery	**6. Points to Note** 6.1. Calibration range 6.2. Reanalysis 6.3. Carry-over 6.4. Cross-talk 6.5. Critical reagents 6.6. Interfering substances
7. Documentation and Archives	7. Documentation and Archives

Specificity and Selectivity in LBA method validation

Specificity

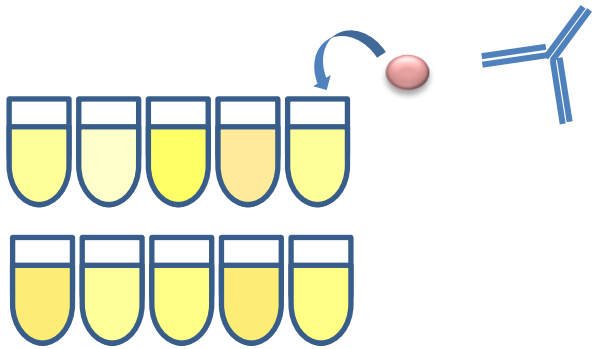
Specificity is the ability of an analytical method to detect and distinguish the analyte from other related substances.



Selectivity

Selectivity is the ability of an analytical method to measure the analyte in the presence of other unrelated substances in the **matrix**.

<at least 10 sources of blank matrix>



MHLW / EMA / FDA BMV guidelines (LBA section)

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
1. Introduction **2. Scope** **3. Reference Standard**		
4. Analytical Method Validation 4.1. Full validation 4.1.1. **Specificity** 4.1.2. **Selectivity** 4.1.3. Calibration curve 4.1.4. Accuracy and precision 4.1.5. **Dilutional linearity** 4.1.6. Stability 4.2. Partial validation 4.3. Cross validation **5. Analysis of Study Samples** 5.1. Calibration curve 5.2. QC samples 5.3. ISR **6. Points to Note** 6.1. Calibration range 6.2. Reanalysis 6.3. **Carry-over** 6.4. **Cross-talk** 6.5. **Critical reagents** 6.6. **Interfering substances**	**7.1 Method Validation** 7.1.1 Full validation 7.1.1.1 Reference standards 7.1.1.2 **Specificity** 7.1.1.3 **Selectivity** 7.1.1.4 **Carry-over effect** 7.1.1.5 Matrix selection 7.1.1.6 **Minimum required dilution** 7.1.1.7 Calibration curve 7.1.1.8 Precision and accuracy 7.1.1.9 **Dilution linearity** 7.1.1.10 **Parallelism** 7.1.1.11 Stability of the sample 7.1.1.12 **Reagents** 7.1.1.13 Commercial kits **7.2 Partial Validation and Cross-validation** **7.3 Analysis of Study Samples** 7.3.1 Analytical run 7.3.2 Acceptance criteria 7.3.3 ISR	**A. Key reagents** **B. Bioanalytical Method Development and Validation** 1. **Selectivity** 2. Accuracy, precision and recovery 3. Calibration curve 4. Sensitivity 5. Reproducibility 6. Stability **C. Validation Method: Use, Data Analysis, and Reporting**
7. Documentation and Archives		

Acceptance Criteria for Validation

	MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Calibration curve	**Back-calculated concentrations** : within **±20%** of nominal conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration standards and a minimum of **6** concentrations including LLOQ and ULOQ meet the criteria.	**Back-calculated concentrations** : within **±20%** of nominal conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration standards meet the criteria.	**Back-calculated concentrations** : within **±20%** of nominal conc. (±25% at LLOQ) At least **75%** of calibration standards including LLOQ meet the criteria. **Total error** : not exceed **30%**
Accuracy and precision (QC samples)	**Accuracy** : within **±20%** (±25% at LLOQ and ULOQ) **Precision** : not exceed **20%** (25% at LLOQ and ULOQ) **Total error** : not exceed **30%** (40% at LLOQ and ULOQ)	**Accuracy** : within **±20%** (±25% at LLOQ and ULOQ) **Precision** : not exceed **20%** (25% at LLOQ and ULOQ) **Total error**: not exceed **30%** (40% at LLOQ and ULOQ)	**Accuracy** : within **±20%** (±25% at LLOQ) **Precision** : not exceed **20%** (25% at LLOQ)

Validation – Calibration Curve

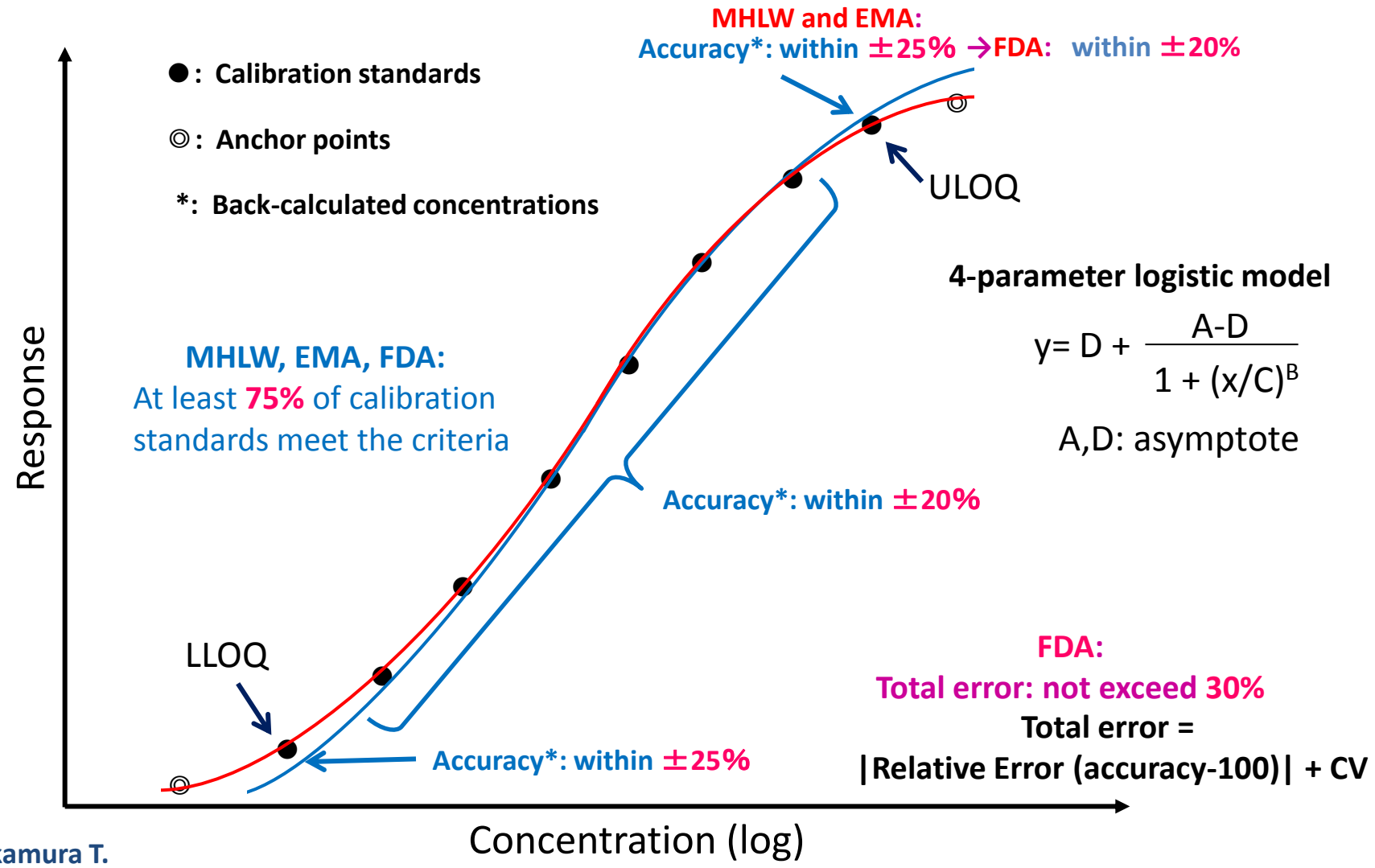


Fig: Nakamura T.
at 5th JBF symposium

Validation – Accuracy and precisions (QCs)

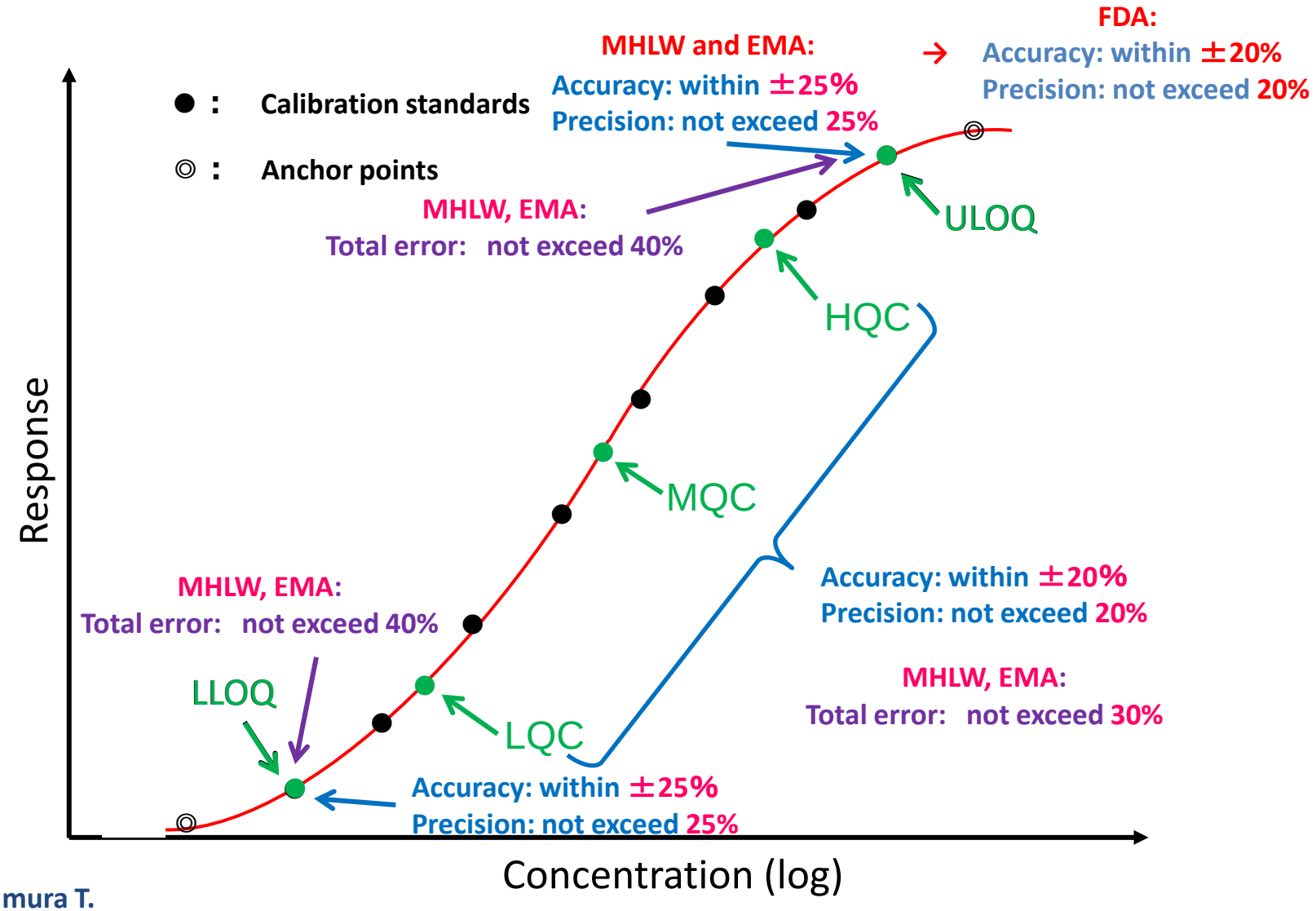


Fig: Nakamura T.
at 5th JBF symposium

Run acceptance criteria for study sample analysis

	MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Calibration curve	**Back-calculated concentrations** : within **±20%** conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration standards and **6** concentrations including LLOQ and ULOQ meet the criteria.	**Back-calculated concentrations** : within **±20%** conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration sample and **6** concentrations including LLOQ and ULOQ meet the criteria	
QC samples	**Accuracy** : within **±20%** At least **two-thirds** of QC samples and at least **50%** at each concentration level meet the criteria.	**Accuracy** : within **±20%** At least **67%** of QC samples and at least **50%** at each concentration level meet the criteria.	**Accuracy** : within **±20%** At least **67%** of QC samples and at least **50%** at each concentration level meet the criteria.

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Topic 1 : Minimum Required Dilution (MRD)

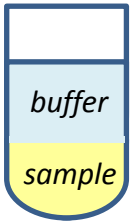
4.1. Full validation

In an LBA validation, full validation should be conducted at a minimum required dilution (MRD), which has been determined in the course of method development.

4.1. Full validation

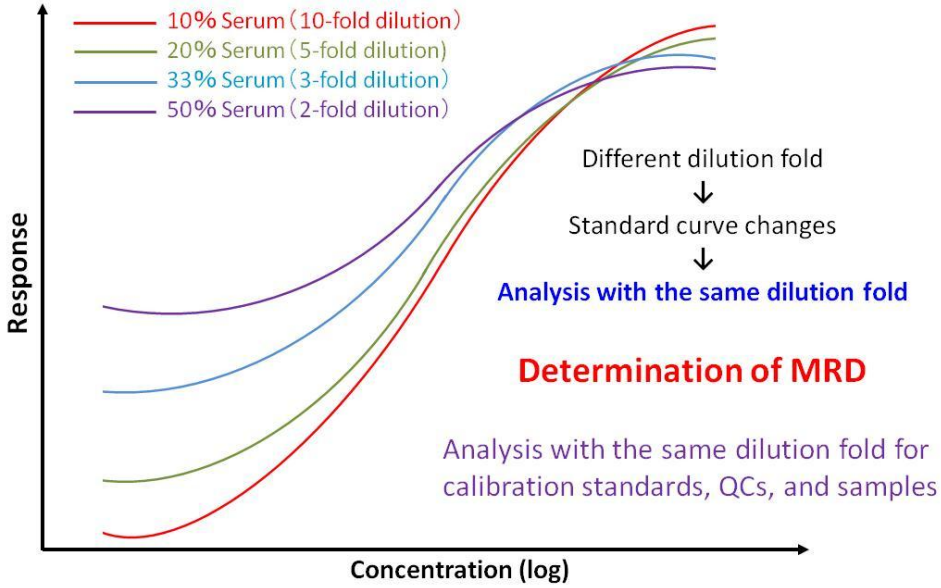
- 4.1.1. Specificity
- 4.1.2. Selectivity
- 4.1.3. Calibration curve
- 4.1.4. Accuracy and precision
- 4.1.5. Dilution linearity
- 4.1.6. Stability

MRD is not included in the validation parameter.



MRD

- MRD should be identical for all samples, however, it is not necessarily the minimum dilution.
- The MRD is regarded as a component of an analytical method, not as a validation parameter.
- Validity of the MRD is assessed through the full validation.
- When MRD is changed, partial validation is necessary.



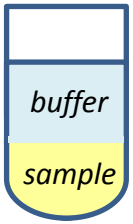


Fig: Nakamura T. at 5th JBF symposium

Topic 2: Total Error

Acceptance criteria for accuracy and precision for full validation in Japanese BMV guideline

Chromatography

Accuracy : **within ±15%**
(within ±20% at LLOQ)

Precision : **not exceed 15%**
(not exceed 20% at LLOQ)

Ligand Binding Assay

Accuracy : **within ±20%**
(within ±25% at LLOQ and ULOQ)

Precision : **not exceed 20%**
(not exceed 25% at LLOQ and ULOQ)

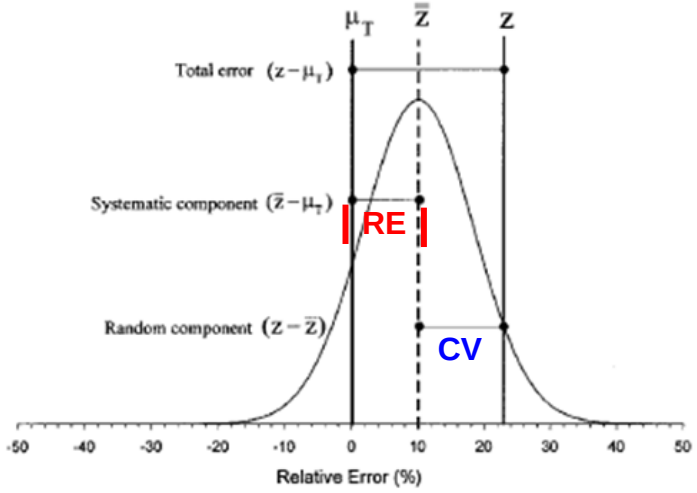
Total Error : **not exceed 30%**
(not exceed 40% at LLOQ and ULOQ)

Total error = |Relative Error (accuracy-100)| + CV

eg. accuracy : 115%, precision : 15% → total error = (115-100) + 15 =30 (%)

Total Error

- Statisitical feature of total error is not clear, however, setting the total error in the criteria leads to omit less reliable methods with both high systematic error and variability.
- Practically, total error fits for purpose of the guideline.



Modified from B. DeSilva *et al*., Pharm. Res., 20: 1885-1900 (2003)

Total error = |RE| + CV

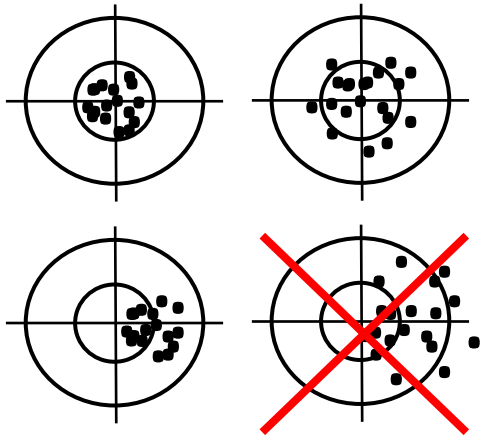


Fig: Nakamura T. at 5th JBF symposium

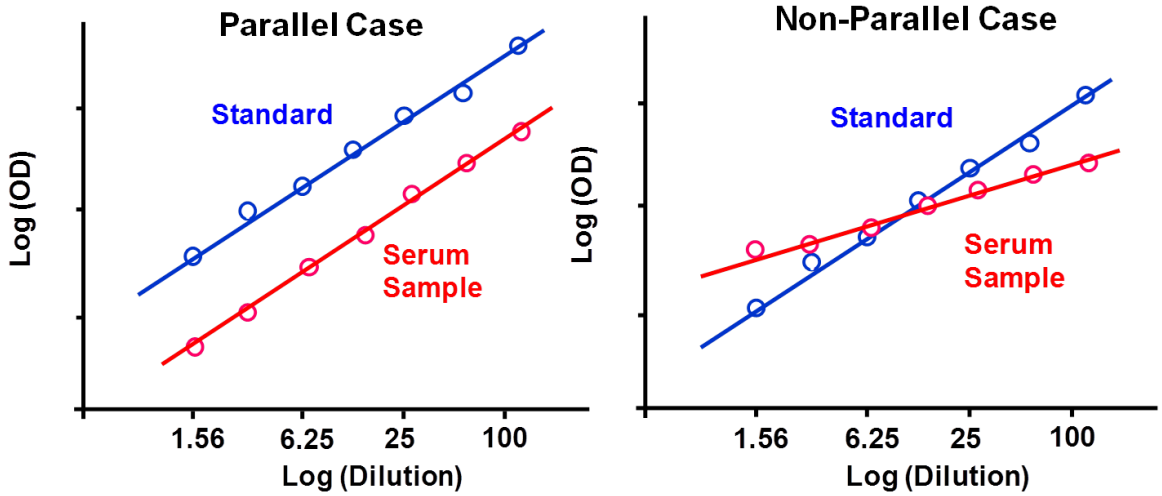
Topic 3 : Critical Reagents

Critical reagent
is typically a binding reagent etc. including labeled or unlabeled antibody that has a direct impact on the results of LBA.

- A critical reagent should be selected by considering the specificity for the analyte, and stored under conditions that can ensure its quality.
- The quality of critical reagent should be appropriately maintained throughout the period of use in analytical method validation and study sample analyses.
- Partial validation is required when the critical reagent lot is changed.

Topic 4 : Parallelism

Parallelism is evaluated to assess the possible matrix effect of the study samples.



Modified from Plikaytis BD et al. J Clin Mictobiol. 32, 2441, 1994

Fig: Nakamura T. at 5th JBF symposium

- Parallelism may be important for some biomarkers.
- However, there were not enough examples to show the needs of parallelism evaluation for PK analysis.

Parallelism

Parallelism is **not** mentioned in the text of the LBA guideline.

⇨ Parallelism evaluation is **not** mandatory.

LBA guideline Q&A

Q17. Is it not necessary to evaluate parallelism?

A17. Parallelism is defined as an established parallel relationship between a dose-response curve from a study sample dilution series and a curve from a calibration standard series, with no difference among back-calculated concentrations for multiple dilutions of a study sample. As of the issuance of this guideline, domestic and international knowledge has neither accumulated nor discussion yet matured regarding cases in which parallelism was not established, causes for failing to establish parallelism, and the extent of impact the failure might have on pharmaceutical development. Therefore, evaluation of parallelism is not necessarily required for all analytical methods. However, if parallelism is an intrinsic issue for an LBA-based bioanalytical method and is likely to cause a problem based on the nature of the analyte or method or data accumulated in the course of pharmaceutical development, scientifically valid evaluation and assessment of the impact on measured concentrations should be considered to the extent possible.

Summary

Japanese BMV guideline for LBA was released in Apr 2014.

It is based on the same concept as the Japanese BMV guideline for chromatography, and includes the specific issues for LBA.

Topics presented:

- Minimum Required Dilution is determined as one of the analytical conditions.
- Total error is included in the requirement items for validation.
- Critical reagent lot change needs the partial validation.
- Parallelism evaluation is not mandatory.

Study group for Japanese BMV guideline development

Bioanalytical Method Validation study group granted by MHLW
(Chair: Dr. Noriko Katori)

LBA Working Group

JBF LBA Task Force

LBA guideline draft preparation

厚生労働省
Ministry of Health, Labour and Welfare

MHLW

Japan Bioanalysis Forum (JBF)

Acknowledgement

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Ministry of Health, Labour and Welfare <MHLW>

Toshinari Mitsuoka (as an observer)

English version of Japan BMV guideline for LBA is available at the NIHS website.

http://www.nihs.go.jp/drug/DrugDiv-E.html

Thank you for your attention!