Guideline/guidance Comparison on Large Molecule Bioanalysis

6th JBF Symposium 2014.2.26

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MHLW/EMA/FDA BMV guidelines (LBA section)

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
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		

Table: Ishii A. at 7th EBF Open symposium

Scope

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Study • Toxicokinetic studies (GLP) • Clinical trials **Analyte** • Protein • Peptide • Small-molecule Out of scope Non-GLP nonclinical study	Similar to JPN guideline **Analyte** Focused analyte not given	Similar to JPN guideline **Study** • Non-clinical Pharmacology study **Analyte** • Endogenous compounds • Biomarkers • Diagnostic kit • Applicable to veterinary drug

Reference Standard

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
• **CoA** **(or alternative document)** Lot number Content (Purity, Amount, Potency etc..) Storage conditions **Preferable** Expiration date	Similar to JPN guideline Additional cautions: • Calibration/QC Lot = Dosing Lot • Change of Lot (bioanalytical evaluation)	Similar to JPN guideline Detailed requirement is not given.

MWLW LBA Guideline allows to use any lot as reference standard as long as the it conforms to the same quality specifications based on information available from a CoA.

Reference Standard

MWLW LBA Guideline Q&A

Q3. What procedure should be flowed in renewing the reference standard lot?
A3. Confirm comparability of the current and new reference standard lots by referring to the relevant CoA or any appropriate documentation….

Q4. Does the reference standard lot have to be the same as the drug substance lot used for dosing in the non-clinical or clinical studies?
A4. Any lot may be used as the reference standard as long as it conforms to the same quality specifications based on information available from a CoA or other appropriate document….

Full Validation

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
• **Validation is required…** Each Species Each Matrix Commercial kit • **Parameters** Specificity Selectivity Calibration curve Accuracy Precision Dilutional Linearity Stability	Similar to JPN guideline Carryover, MRD and parallelism are stated separately.	Similar to JPN guideline Selectivity and dilution linearity are not included. Less detailed definition, leaving judgement to scientists

MRD (Minimum required dilution)

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
MRD should be defined a priori. (Chapter of MRD is not given)	Listed as a validation parameter	No description given

MRD

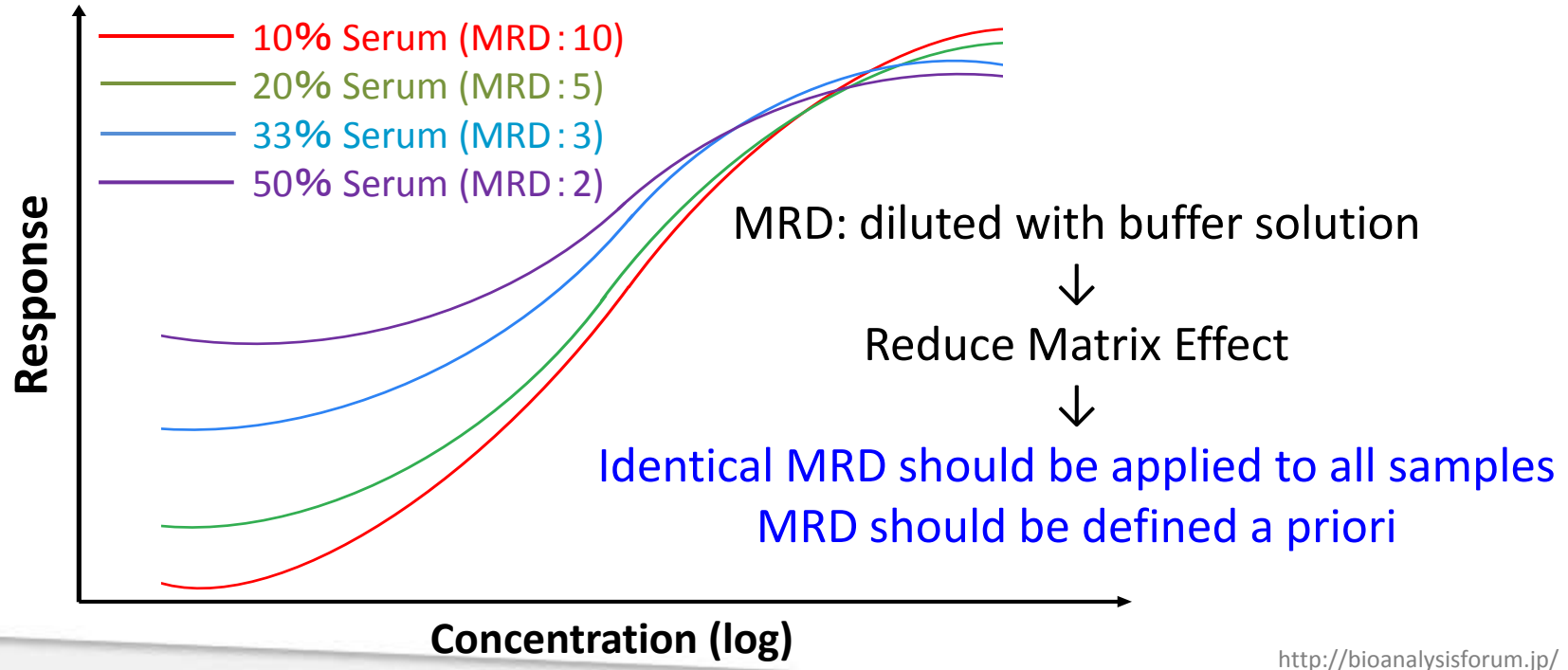
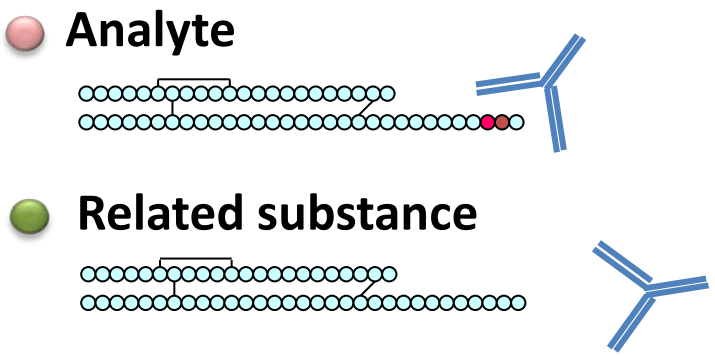


Fig: Nakamura T. at 5th JBF symposium modified

Specificity (definition)

Specificity

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



No differences in definition between MHLW and EMA guideline.

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Details of related substance are not given. Specificity may be evaluated in the course of method development.	• **Related substance** Endogenous compounds, isoforms, variants forms of the analyte, or physico-chemically similar compounds, anticipated concomitant medication.	Contained in selectivity section.

Fig: Ishii A. at 7th EBF Open symposium

Specificity (evaluation)

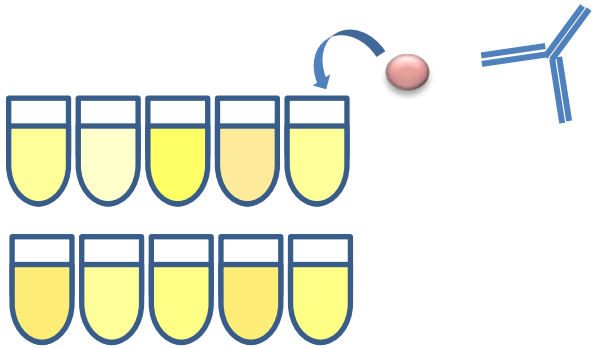
MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Sample Blank + related compound QC: near LLOQ and near ULOQ (+related compound) **Criteria** Blank: <LLOQ. QC: within ±20% (±25% at LLOQ or ULOQ)	Similar to JPN guideline **Sample** QC: LLOQ and ULOQ (+related compound) **Criteria** QC: within ±25% Using blank sample is not addressed.	No details given

Selectivity

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>



No differences in definition between MHLW and EMA guideline.

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Using lipemic, hemolysed and relevant disease sample is not addressed.	Lipemic and Hemolysed matrix should be included. Including relevant disease population is recommended.	No details given

These evaluations are **not** mandatory.

Fig: Ishii A. at 7th EBF Open symposium

Selectivity

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
QC: 10 individuals at or near LLOQ samples. Blank: <LLOQ. QC: within ±20% of nominal (±25% at LLOQ)	Similar to JPN guideline	No details given

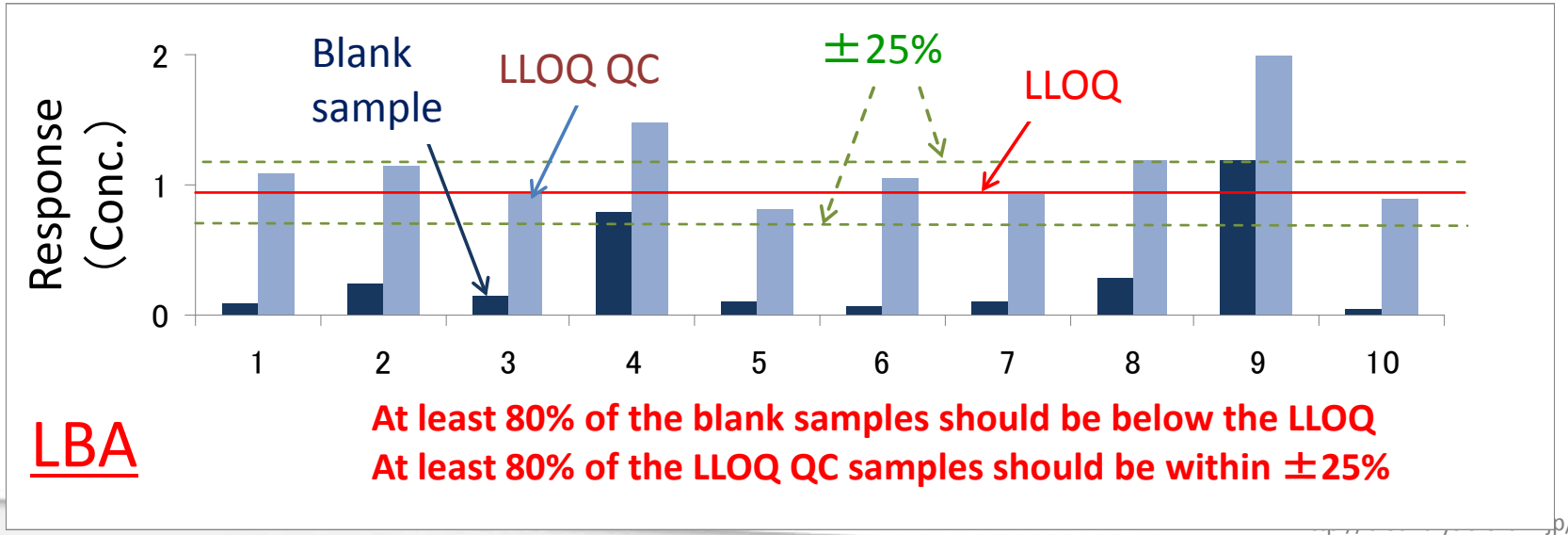


Fig: Ishii A. at 7th EBF Open symposium

Matrix Selection & Matrix Effect

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
None	When alternative matrix is used, the accuracy should be calculated to demonstrate the absence of matrix effect.	Matrix Effect should be evaluated. • Compared with calibrators in buffer. • Parallelism of diluted sample

But in the full validation section:
The use of a surrogate matrix should be rigorously justified in the course of establishing the analytical method.

Calibration curve

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
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.	Similar to JPN guideline **Back-calculated concentrations** : within ±20% of nominal conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration standards meet the criteria.	Similar to JPN guideline **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%**

Table: Ishii A. at 7th EBF Open symposium

Accuracy and Precision

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Accuracy : within **±20%** （±25% at LLOQ and ULOQ）	Similar to JPN guideline **Accuracy** : within **±20%** （±25% at LLOQ and ULOQ）	Similar to JPN guideline **Accuracy** : within **±20%** （±25% at LLOQ ）
Precision : not exceed **20%** （25% at LLOQ and ULOQ）	**Precision** : not exceed **20%** （25% at LLOQ and ULOQ）	**Precision** : not exceed **20%** （25% at LLOQ）
Total error : not exceed **30%** （40% at LLOQ and ULOQ）	**Total error**: not exceed **30%** （40% at LLOQ and ULOQ）	
High QC conc.: **At least 1/3 of ULOQ**	**High QC conc.:** **At least 75% of ULOQ**	

Table: Ishii A. at 7th EBF Open symposium modified

Accuracy and Precision

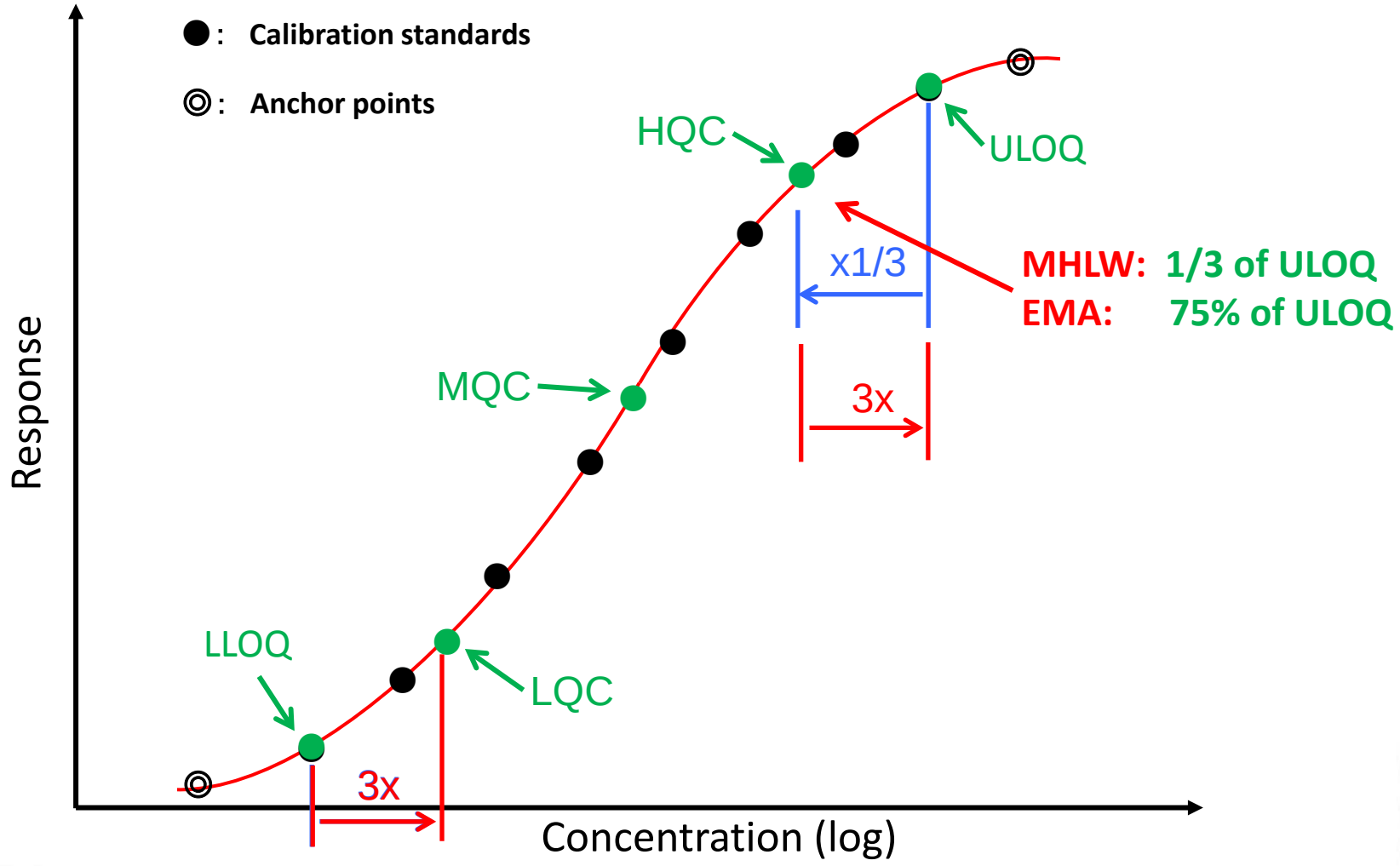


Fig: Nakamura T. at 5th JBF symposium

Dilutional Linearity

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
Sample: QC: Above ULOQ sample → Serially diluted **Accuracy**: within **±20%** **Precision**: not exceed **20%**	Similar to JPN guideline	Included in A&P section. But acceptance criteria is not clear.

Parallelism

Parallelism is evaluated by diluting **study sample** to detect possible matrix effect.

MHLW (LBA) 2014	EMA (7. LBA) 2011	FDA (IV. LBA) draft 2013
None	**Sample:** Study sample should be diluted at least 3concentrations **Precision**: not exceed **30%**	Similar to **EMA** guideline (stated in selectivity section) Acceptance criteria is not clear.

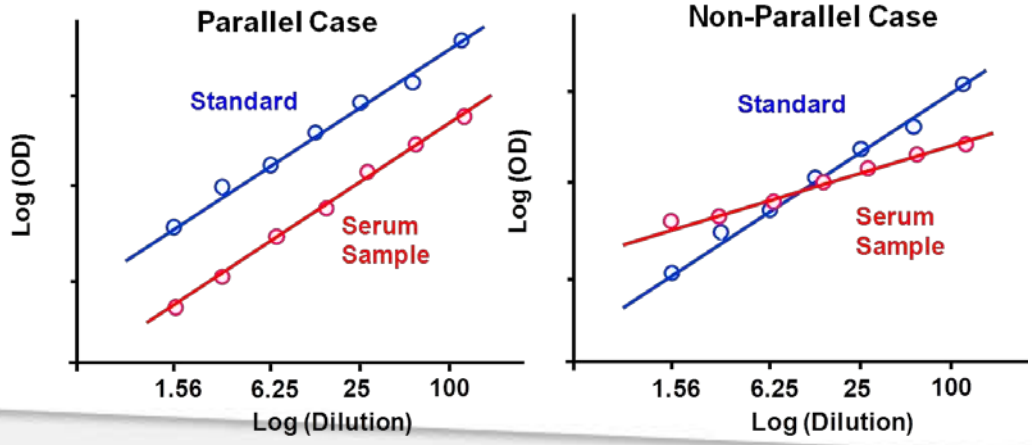


Fig: Nakamura T. at 5th JBF symposium

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

Parallelism

Parallelism evaluation is **not** mandatory.

MWLW LBA Guideline Q&A

Q17. Is it not necessary to evaluate parallelism?

A17. ….. 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.

Stability

	MHLW (LBA) 2014	EMA 2011	FDA draft 2013
Sample	Freeze/Thaw Short-term Long-term Stock Solution High QC and Low QC n=3 at each conc.	Similar to JPN guideline (ISS may be used. Whole blood stability is not routinely required). No definition of No. of sample.	Similar to JPN guideline + processed sample stability High QC and Low QC n=3 at each conc.
Criteria	within ±20% of nominal	within ±20% of nominal	within ±15% of nominal

Partial Validation

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
	Similar to JPN guideline	Similar to JPN guideline
Analytical method transfer	Analytical method transfer	Analytical method transfer
Analytical instruments	Equipment	Instruments
Critical reagent lot		Software
Calibration range	Calibration range	**Analytical methodology**
MRD		
Anticoagulant	Anticoagulant	Anticoagulant
Analytical conditions	Sample processing procedure	**Matrix within species**
Sample storage conditions	Limited sample volume	**Species within matrix**
Concomitant drugs	Another matrix or species	Sample processing procedure
Rare matrices	Storage conditions	Relevant concentration range
		Limited sample volume
		Rare matrices

FDA draft guidance is OK with Partial Validation for change in analytical methodology, matrix within species and species within matrix.

Cross Validation

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
QC : within ±30% of nominal Study sample: variability should be within ±30% for at least two-thirds of the samples	QC : within ±15% of nominal or may be wider Study sample: variability should be within ±20% for at least 67% of the samples	No Criteria given. Require to use both spiked QCs and subject samples.

MHLW guideline accepts wider criteria (±30%).

ISR

	MHLW (LBA) 2014	EMA (6. ISR) 2011	FDA (V. ISR) draft 2013
sample	approximately 10% of the samples (total samples ≦ 1000) approximately 5% of the samples (total samples > 1000)	Similar to JPN guideline 10% of the samples (total samples ≦ 1000) 5% of the samples (total samples > 1000)	7% of the samples
criteria	assay variability should be within ±30% for at least two-thirds of the samples analyzed in ISR	Similar to JPN guideline assay variability should be within ±30% for at least 67% of the samples analyzed in ISR	Similar to JPN guideline assay variability should be within ±30% for at least two-thirds (67%) of the samples analyzed in ISR

Table: Ishii A. at 7th EBF Open symposium

Points to Note

Critical Reagent

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
A critical reagent has a direct impact on the results. The quality of critical reagent should be appropriately maintained. Partial validation is in principle required when the critical reagent lot is changed.	Similar to JPN guideline	Similar to JPN guideline

Summary

- **MHLW LBA Guideline is fundamentally similar to the EMA BMV guideline (2011)**
  - ✓ LBA in the scope if used for small molecules.
  - ✓ Not have to be Reference standard lot = Dosing lot.
  - ✓ MRD should be defined a priori.
  - ✓ High QC = At least 1/3 of ULOQ.
  - ✓ Parallelism is not routinely required.
  - ✓ Wider criteria for cross-validation.
- **MHLW LBA Guideline is not different from the FDA draft guidance (2013) in the basic concepts.**
  - ✓ But need to wait for the finalized FDA guidance for complete comparison.

Acknowledgement

Working Group Members for LBA Guideline

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Japan Pharmaceutical Manufacturers Association <JPMA>

Masataka Katashima (Astellas Pharma)
Kotaro Maekawa (Hisamitsu Pharmaceutical)

Japan Bioanalysis Forum <JBF> – LBA Task Force

Members of LBA taskforce and steering committee

Thank you for your attention!

KYOWA KIRIN

Validation – Calibration curve

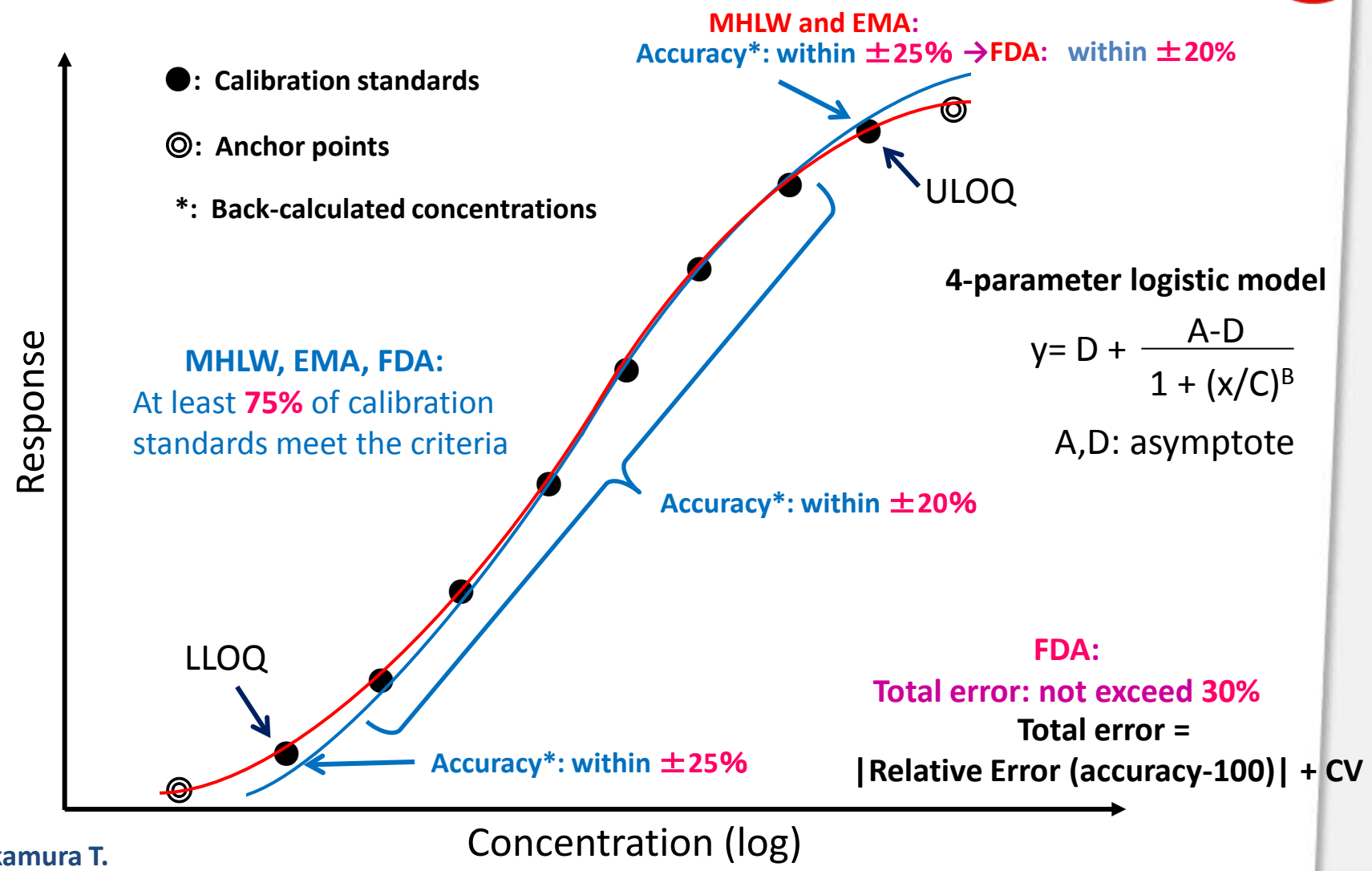

Fig: Nakamura T.
at 5th JBF symposium

Accuracy and Precision

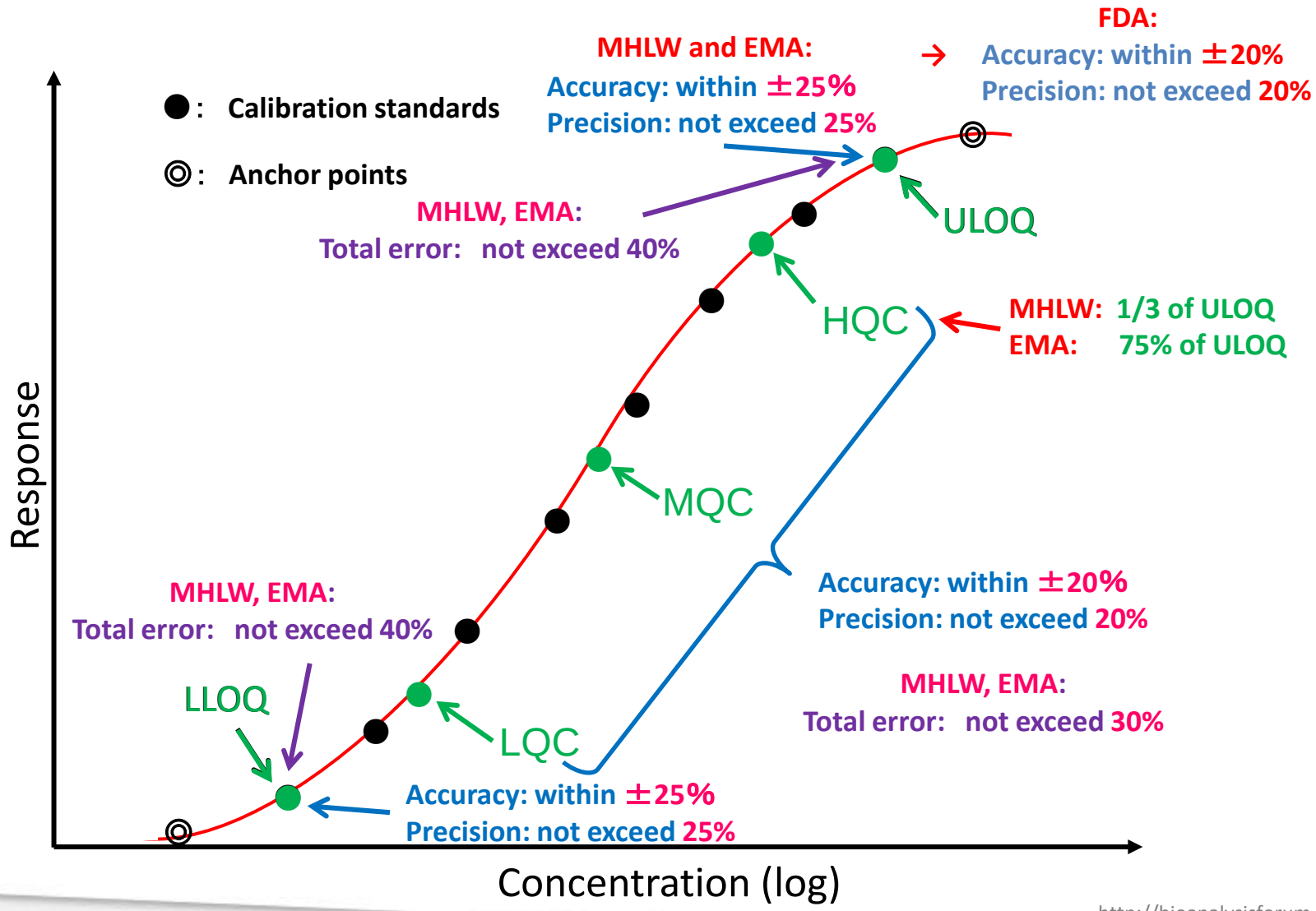

Fig: Nakamura T. at 5th JBF symposium

Run Acceptance Criteria

	MHLW (LBA) 2014	EMA 2011	FDA draft 2013
Calibration curve	**Back-calculated concentrations** : within **±20%** conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration standards and **6** conc. meet the criteria.	**Back-calculated concentrations** : within **±20%** conc. (±25% at LLOQ and ULOQ) At least **75%** of calibration sample and **6** conc. meet the criteria	**Back-calculated concentrations** : within **±20%** conc. (±25% at LLOQ) At least **75%** of calibration standards meet the criteria. **Total error** : not exceed **30%**
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.

Table: Ishii A. at 7th EBF Open symposium

Reporting

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
Study sample analysis report • Summary of the study sample analysis • Information on the reference standards • Information on the blank matrices • Information on receipt and storage of study samples • Analytical method • Parameters, acceptance criteria, and results of the validity evaluation • Results and discussion of study sample analysis • Rejected runs together with the reason for rejection • Information on reanalysis • Deviations from the protocol and/or SOP, along with impact on study results • Information on reference study, protocol, and literature • Representative chromatograms, as needed	Similar to JPN guideline	Similar to JPN guideline + More detailed requirement for summary table.

Points to Note

Reanalysis

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
Possible reason, procedure and criteria should be defined a priori.	Similar to JPN guideline	Similar to JPN guideline Clearly mentioned the number of replicates for reassays. No special description about safety concerns.

Carryover

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
If carry-over is inevitable, its impact needs to be examined.	Similar to JPN guideline	None

Crosstalk

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
If crosstalk is inevitable, its impact needs to be examined.	Similar to JPN guideline	None

Points to Note

Interfering substance

MHLW (LBA) 2014	EMA 2011	FDA draft 2013
If interfering substances (e.g. soluble ligand, ADAs) are potentially present in study samples, it is advisable to examine the impact.	None Included in selectivity section.	None Included in selectivity section.