Guideline/Guidance Comparison on Ligand Binding Assays (LBA)

MHLW LBA Guideline (2014) vs EMA Guideline (2011, updated 2014) vs FDA Guidance (2018)

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Scope

MHLW LBA Guideline (2014)
This guideline is applicable to the validation of LBAs as analytical methods for the measurement of drugs in biological samples obtained in toxicokinetic studies and clinical trials, as well as to the analysis of study samples using such methods. The information in this guideline generally applies to the quantification of peptides and proteins as well as low-molecular-weight drugs that are analyzed by LBAs. A typical example of an LBA is an immunological assay based on antigen-antibody reaction, such as enzyme immunoassay (EIA).
This guideline is not mandatory for analytical methods used in non-clinical studies that are beyond the scope of "Ministerial Ordinance Concerning the Standards for the Conduct of Non-clinical Studies on the Safety of Drugs (Ministry of Health and Welfare ordinance No. 21, dated March 26, 1997)," but could be used as a reference for conducting the required validation of such methods.

EMA Guideline (2011)
Similar to Japanese Guideline, but focused analytes are not given.

FDA Guidance (2018)
Similar to Japanese Guideline.
Description about chromatography assay is given.
Biomarker concentration evaluation is mentioned.
Applicability to veterinary drug is mentioned.
Fit-for-purpose concept is given.

Underline: key words in the item

Reference standards

MHLW LBA Guideline (2014)
A reference standard serves as the scale in quantifying an analyte, and is mainly used to prepare calibration standards and quality control (QC) samples, which are relevant blank matrix spiked with a known concentration of the analyte of interest. The quality of the reference standard is critical, as the quality affect measurement data. A certificate of analysis or an alternative statement that provides information on lot number, content (amount, purity, or potency) and storage conditions should accompany the standard. Also, the expiration date or its equivalent is preferably clarified. As for a reference standard, it is important that the material is procured from an authenticated source and is of well-controlled quality.

EMA Guideline (2011)
Similar to Japanese Guideline, but additional cautions about the consistent use of the same lot of standard and the change of lot of standard are mentioned.

FDA Guidance (2018)
Similar to Japanese Guideline.
If the reference standard expires, this lot of standard should not be used to make stock solutions unless the standard's purity is re-established.

Underline: key words in the item

Full validation

MHLW LBA Guideline (2014)
A full validation should be performed when establishing a new bioanalytical method for quantification of an analyte/analytes. A full validation is also required when implementing an analytical method that is disclosed in literature or commercialized as a kit product.
The objective of a full validation is to demonstrate the assay performance of the method, e.g., specificity, selectivity, calibration curve, accuracy, precision, dilutional linearity, and stability. Generally, a full validation should be performed for each species or matrix (mainly plasma or serum) to be analyzed.
The matrix used in analytical validation should be as close as possible to the target study samples, including anticoagulants and additives. When an analytical method is to be established for a matrix of limited availability (rare matrix, e.g., tissue, cerebrospinal fluid, bile) and a sufficient amount of matrix cannot be obtained from sufficient number of sources (subjects or animals), a surrogate matrix may be used to prepare calibration standards and QC samples. However, the use of a surrogate matrix should be justified as much as possible in the course of establishing the analytical method.
In an LBA full validation, the minimum required dilution (MRD) should be defined a priori (i.e., in the course of method development) to dilute samples with buffer solution.
When using a plate-based LBA, analysis should generally be performed in at least 2 wells per sample; a sample concentration should then be determined either by calculating a mean of responses from the wells or by averaging the concentrations calculated from each response.

EMA Guideline (2011)
Similar to Japanese Guideline, but carryover, MRD and parallelism are stated separately.

FDA Guidance (2018)
Similar to Japanese Guideline, but MRD is not included.
Performance QCs are included in validation runs to determine if analytical runs are acceptable.

Underline: key words in the item

Full validation: MRD

MHLW LBA Guideline (2014)
(Addressed in full validation)
In an LBA full validation, the minimum required dilution (MRD) should be defined *a priori* (i.e., in the course of method development) to dilute samples with buffer solution.
(Addressed in glossary)
A dilution factor where biological samples are diluted with buffer solution for the analysis by LBAs. The MRD may not necessarily be the ultimate minimum dilution but should be identical for all samples including calibration standards and QC samples.

EMA Guideline (2011)
Similar to Japanese Guideline, but MRD means the smallest dilution.

FDA Guidance (2018)
Not addressed.

Underline: key words in the item

Full validation: Specificity

MHLW LBA Guideline (2014)
Specificity is the ability of an analytical method to detect and differentiate the analyte from other substances, including its related substances (i.e., substances that are structurally similar to the analyte). For an LBA, it is important that the binding reagent specifically binds to the target analyte but does not cross-react with coexisting related substances. If presence of related substances is anticipated in biological samples of interest, the extent of the impact of such substances should be evaluated. Specificity may be evaluated in the course of method development. In some cases, an additional specificity testing may have to be conducted after a method validation is completed.
Specificity is evaluated using blank samples (matrix samples without analyte addition) and blank samples spiked with the related substance at concentration(s) anticipated in study samples; in addition, QC samples with the analyte concentrations near the lower limit of quantification (LLOQ) and near the upper limit of the quantification (ULOQ) of calibration curve should be evaluated after spiking with the related substance at anticipated concentration(s).
Assay results for the “neat” blank sample and blank samples spiked with the related substance should be below the LLOQ; and accuracy in the measurements of the QC samples spiked with the related substance should demonstrate an accuracy of within ±20% of the theoretical concentration (or within ±25% of the theoretical concentration at the LLOQ and ULOQ).

EMA Guideline (2011)
Similar to Japanese Guideline.
Accuracy of QC samples is within ±25%.
Using blank sample is not addressed.

FDA Guidance (2018)
Similar to Japanese Guideline.
Concomitant medications are included as exogenous interference.

Underline: key words in the item

Full validation: Selectivity

MHLW LBA Guideline (2014)
Selectivity is the ability of an analytical method to detect and differentiate the analyte in the presence of other components in the samples.
Selectivity is evaluated using blank samples obtained from at least 10 individual sources and near-LLOQ QC samples (i.e., QC samples at or near the LLOQ) prepared using the individual blank samples. In the case of a matrix with limited availability, it may be acceptable to use matrix samples obtained from less than 10 sources.
Assay results for at least 80% of the blank samples should be below the LLOQ; at least 80% of the near-LLOQ QC samples should demonstrate an accuracy of within ±20% of the theoretical concentration (or within ±25% at the LLOQ).

EMA Guideline (2011)
Examples of unrelated compounds present in matrix are described; degrading enzymes, heterophilic antibodies or rheumatoid factor. Matrix sources should include lipemic and haemolysed samples. It is also strongly recommended that sources from relevant disease population be included. It may be prudent also to evaluate selectivity at higher analyte concentrations.

FDA Guidance (2018)
Similar to Japanese Guideline.
Depending on the intended use of the assay, the impact of hemolyzed samples, lipemic samples, or samples from special populations can be included in the selectivity assessment.
In addition to blank and LLOQ QC samples, HQC samples should be evaluated. The acceptance criteria for LLOQ and HQC samples are ±25% and ±20% accuracy, respectively.

Underline: key words in the item

Full validation: Matrix selection or effect

MHLW LBA Guideline (2014)
The sponsor should prepare the calibration standards and QCs in the same biological matrix as the samples in the intended study. When surrogate matrices are necessary, the sponsor should justify the use of the surrogate matrices.

EMA Guideline (2011)
(Addressed in matrix selection)
The measurement of some macromolecules may not be possible in complex matrices without extraction due to high interferences with high levels of structurally related endogenous compounds. Although the use of extracted matrix (e.g. charcoal, immuno-affinity) or alternative matrix (e.g. protein buffers, dialysed serum) is not recommended, the use of such matrices may be necessary when there is no other strategy to quantify the analyte of interest. The calibration standard curve may be prepared in these surrogate matrices. QC samples should be prepared in the actual sample matrix and the accuracy should be calculated to demonstrate the absence of matrix effect.

FDA Guidance (2018)
(Matrix effect)
Matrix effects evaluation involves comparing calibration curves in multiple sources of the biological matrix against a calibration curve in the matrix for parallelism (serial dilution of incurred samples) and nonspecific binding.

(Matrix selection)
Similar to Japanese Guideline.
In addition, for analytes that are also endogenous compounds, the accuracy of the measurement of the analytes poses a challenge when the assay cannot distinguish between the therapeutic agent and the endogenous counterpart. In such situations, the following approaches are recommended to validate and monitor assay performance. Other approaches, if justified by scientific principles, can also be considered.

- The biological matrix used to prepare calibration standards should be the same as the study samples and free of the endogenous analyte.
- Parallelism should be evaluated for assays for endogenous compounds.

Underline: key words in the item

Full validation: Calibration curve

MHLW LBA Guideline (2014)

The calibration curve demonstrates the relationship between a theoretical analyte concentration and its resulting response variable. A calibration curve should be prepared by using the same matrix as the intended study samples, whenever possible, by spiking the blank matrix with known concentrations of the analyte. A calibration curve should be generated with at least 6 concentration levels. Anchor point samples at concentrations below the LLOQ and above ULOQ of the calibration curve may also be used to improve curve fitting. A 4- or 5-parameter logistic model is generally used for the regression equation of a calibration curve. The validation report should include the regression equation and weighting conditions used.

The accuracy of back-calculated concentration of each calibration standard should be within ±25% deviation of the theoretical concentration at the LLOQ and ULOQ, and within ±20% deviation at all other levels. At least 75% of the calibration standards excluding anchor points, and a minimum of 6 levels of calibration standards, including the LLOQ and ULOQ, should meet the above criteria.

EMA Guideline (2011)

Similar to Japanese Guideline.
General recommendation for spacing calibration standards is provided.

FDA Guidance (2018)

Similar to Japanese Guideline.
(Compared to the draft FDA Guidance (2013), the accuracy criterion for ULOQ was changed from ± 20% to ± 25% and the evaluation of total error was eliminated).

Underline: key words in the item

Full validation: Accuracy and precision

MHLW LBA Guideline (2014)
Accuracy of an analytical method describes the degree of closeness between the analyte concentration determined by the method and its theoretical concentration. Precision of an analytical method describes variation between individual concentrations determined in repeated measurements. Accuracy and precision are assessed by performing analysis with QC samples, i.e., samples spiked with known amounts of the analyte. In the validation, QC samples with a minimum of 5 different concentrations (LLOQ, low-, mid-, high-levels, and ULOQ) within the calibration range are prepared. The low-level should be within 3 times the LLOQ, the mid-level is near the midpoint on the calibration curve, and the high-level should be at least one-third of the ULOQ of the calibration curve. Accuracy and precision should be evaluated by repeating the analysis in at least 6 analytical runs. The mean within-run and between-run accuracy at each concentration level should be within ±20% deviation of the theoretical concentration, except at the LLOQ and ULOQ, where it should be within ±25%. Within-run and between-run precision of concentrations determined at each level should not exceed 20%, except at the LLOQ and ULOQ, where it should not exceed 25%. Furthermore, a total error (sum of the absolute value of the relative error [i.e., accuracy minus 100%] and precision) at each level should not exceed 30%, except at the LLOQ and ULOQ, where it should not exceed 40%.

EMA Guideline (2011)
Similar to Japanese Guideline, however, high QC should be at least 75% of ULOQ. The use of frozen QCs is recommended.

FDA Guidance (2018)
Similar to Japanese Guideline, however, target concentrations for LQC/MQC/HQC are not specified. The number of determination per each QC levels is provided (3 replicates) and freshly prepared QC is recommended. Also, it is recommended that calibrators and QC samples are prepared from separate stock solutions.
No QC acceptance criteria is necessary as run acceptance.

Underline: key words in the item

Full validation: Dilutional linearity

MHLW LBA Guideline (2014)
Dilutional linearity is assessed to confirm the following: (i) the method can appropriately analyze samples at concentrations exceeding the ULOQ of a calibration curve without influence of a hook effect or prozone; (ii) measured concentrations are not affected by dilution within the calibration range. Dilutional linearity is evaluated by analyzing a QC sample exceeding the ULOQ of a calibration curve and its serial dilutions at multiple concentrations. The absence or presence of response reduction (hook effect or prozone) is checked in the analyzed samples, and if discovered, measures should be taken to eliminate response reduction in study sample analysis. Accuracy and precision in the measurements corrected for the dilution factor should be within ±20% deviation of the theoretical concentration and not more than 20%, respectively

（Comment）
Need to evaluate dilution linearity by diluting QC sample exceeding ULOQ serially at multiple concentration with blank matrix. Accuracy: within ±20%, Precision: within 20% deviation of theoretical concentration

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline. The number of replicates per dilution (5 replicates) is provided.

Underline: key words in the item

Full validation: Parallelism

MHLW LBA Guideline (2014)
Not addressed

（Comment）
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.

EMA Guideline (2011)
If study samples are available, parallelism between the calibration standard curve and serially diluted study samples should be assessed to detect possible matrix effect or differing affinities for metabolites. A high concentration study sample (preferably close to Cmax) should be diluted to at least three concentrations with blank matrix. The precision between samples in a dilution series should not exceed 30%. In case the sample does not dilute linearly (i.e. in a non parallel manner), a procedure for reporting a result should be defined a priori. If study samples are not available during the validation of the method, parallelism should be evaluated as soon as study samples become available.

（Comment）
Need to assess parallelism in method validation.

FDA Guidance (2018)
Parallelism should be evaluated for assays for endogenous compounds. The acceptance criteria are not provided.

Underline: key words in the item

Full validation: Stability

MHLW LBA Guideline (2014)

Analyte stability should be evaluated to ensure that the concentration is not affected through each step of the process from the sample collection to the analysis. The stability of the analyte should be assessed under conditions that are as close as possible to the actual circumstances, e.g. sample storage and sample analysis. Careful consideration should be given to the solvent or matrix type, container materials, and storage conditions used in the stability-determination process. Validation studies should determine analyte stability after freeze and thaw cycles, and after short-term (e.g., at room temperature, on ice, or under refrigeration) and long-term storage. All stability experiments should be performed on samples that have been stored for a time that is longer than the actual storage period. Stability of the analyte in the stock and working solutions is evaluated using solutions at or near the highest and lowest concentration levels for the actual solution storage situation. Stability of the analyte in the studied matrix is evaluated using low- and high-level QC samples. The QC samples should be prepared using a matrix that is as close as possible to the actual study samples, including anticoagulant and additives. Stability is evaluated by analysis of at least 3 replicates per QC concentration level before and after stability storage. The mean accuracy in the measurements at each level should be within ±20% deviation of the theoretical concentration, in principle. Other criteria could be used if they are deemed scientifically more appropriate for the evaluation of a specific analyte.

EMA Guideline (2011)

Similar to Japanese Guideline, but no definition on number of repeat per conc. ISS may be used but not considered sufficient. In F/T stability, at each cycle, samples should be frozen for at least 12 hours before they are thawed. Whole blood stability is not required on a routine basis.
(" A bracketing approach may be considered " was deleted in the update version on 19 Sept. 2014)

FDA Guidance (2018)

Similar to Japanese Guideline.

- Stability samples should be prepared from a freshly made stock solution.
- In F/T stability, samples should be frozen for at least 12 hours between cycles and at least three freeze-thaw cycles should be covered.
- Determination of stability at -20ºC would cover stability at colder temperatures.
- Stability in the presence of the other drug should be considered in some cases.
- Whole blood stability is not required on a routine basis.

Underline: key words in the item

Partial validation

MHLW LBA Guideline (2014)
Partial validation may be performed when minor changes are made to an analytical method that has already been fully validated. The items in a partial validation are determined according to the extent and nature of the changes made to the method. Typical bioanalytical method changes subjected to a partial validation are as follows: analytical method transfers between laboratories, changes in analytical instruments, changes of the critical reagent lot, changes in calibration range, changes in the MRD, changes in anticoagulant, changes in analytical conditions, changes in sample storage conditions, confirmation of impact by concomitant drugs, and use of rare matrices. Acceptance criteria used in partial validation should, in principle, be the same as those employed in the full validation.

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline, but OK with partial for matrix change within species & species change within matrix
"Change in analytical methodology" is described and "Changes in sample storage conditions" is not described as one of typical example.

Underline: key words in the item

Cross validation

MHLW LBA Guideline (2014)
Cross validation is primarily conducted when data are generated in multiple laboratories within a study or when comparing analytical methods used in different studies, after a full or partial validation. The same set of QC samples spiked with the analyte or the same set of study samples is analyzed, and the mean accuracy at each concentration level of QC samples or the assay variability in the measurements of study samples is evaluated. In the cross validation among 2 or more laboratories within a study, the mean accuracy of QC samples (low-, mid-, and high-levels) evaluated by at least 3 replicates at each level, should be within ±30% deviation of the theoretical concentration, in principle, considering intra- and inter-laboratory precision. When using a set of study samples, the assay variability should be within ±30% for at least two-thirds of the samples. When conducting cross validation between different analytical methods based on different assay principles, both validation procedure and acceptance criteria (i.e., mean accuracy or assay variability) should be separately defined based on scientific judgment according to the type of the analytical methods.

EMA Guideline (2011)
Similar to Japanese Guideline, but 15% for QCs is acceptable.

FDA Guidance (2018)
Described, but leaving judgment to scientists.
Cross-validation with spiked matrix QCs and non-pooled subject samples should be conducted at each site or laboratory.
Pooled incurred samples can be used when insufficient volume exists.

Underline: key words in the item

Analysis of study samples

MHLW LBA Guideline (2014)
Study samples are biological specimens that are obtained from toxicokinetic studies and clinical trials for analysis. Analysis of study samples should be carried out using a fully validated analytical method. During analysis, study samples should be handled under conditions that are validated for adequate stability, and analyzed within a confirmed stability period, along with a blank sample, calibration standards at a minimum of 6 concentration levels, and QC samples at a minimum of 3 concentration levels. In a plate-based LBA, assay should generally be performed in at least 2 wells per sample prepared. A sample concentration should then be determined either by calculating a concentration from an average of each response or by averaging the concentrations calculated from each response. Validity of the analytical method during study sample analysis should be evaluated in each analytical run by using the calibration curve and QC samples. In a plate-based assay, each plate represents a single analytical run. In studies that serve pharmacokinetic data as a primary endpoint, reproducibility of the analytical method should be confirmed for each representative study per matrix by performing incurred sample reanalysis (ISR: reanalysis of incurred samples in a separate analytical run on a different day to determine whether the original analytical results are reproducible).
（Comment）
Calibration curve, at least 6 levels; QC, at least 3 concentration levels
Assay should be performed at least 2 well per sample

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Following evaluation items or recommendations are added:
- Selectivity and sensitivity in the blank and zero calibrators.
- QC results should be included in the estimation of accuracy and precision.
- Acceptance of separation batch of the analytical run.
- Drift should be monitored and its impact on the accuracy should be addressed, if any.
- All study samples from a subject should be analyzed in a single run, especially for studies designed with repeated measures from individual subjects (e.g., crossover or sequential design required for BE studies).
- If a unique or disproportionately high concentration of a metabolite is discovered in human studies, assay method validation may be needed for the metabolite.
- Parallelism should be conducted if not done during validation

Underline: key words in the item

Analysis of study samples: Calibration curve

MHLW LBA Guideline (2014)
A calibration curve is used to determine the concentration of the analyte of interest in study samples. A calibration curve used in study sample analysis should be generated for each analytical run by using the validated analytical method. The same model as in the bioanalytical method validation should be used for the regression equation and weighting conditions of the calibration curve. The accuracy of back-calculated concentrations of calibration standards at each level should be within ±25% deviation of the theoretical concentration at the LLOQ and ULOQ of the calibration curve, and ±20% deviation at all other levels. At least 75% of the calibration standards excluding anchor points, with a minimum of 6 levels, should meet the above criteria.
If the calibration standard at the LLOQ or ULOQ does not meet the criteria in study sample analysis, the next lowest/highest-level calibration standard may be used as the LLOQ or ULOQ of the calibration curve. Even though narrowed, the modified calibration range should still cover at least 3 different QC sample levels (low-, mid-, and high-levels).

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline.

Underline: key words in the item

Analysis of study samples: QC samples

MHLW LBA Guideline (2014)
QC samples are analyzed to assess the validity of the analytical method used for calibration curve and study sample analysis.
QC samples with a minimum of 3 different concentration levels (low-, mid-, and high-levels) within the calibration range are analyzed in each analytical run. Usually, the low-level is within 3 times the LLOQ, the mid-level is in the midrange of the calibration curve, and the high-level needs to be at least one-third of the ULOQ of the calibration curve. QC samples are processed in the same manner as with study samples. The analysis requires 2 QC samples at each QC level or at least 5% of the total number of study samples in the analytical run, whichever is the greater.
The accuracy of measurement of QC samples should be within ±20% deviation of the theoretical concentrations. At least two-thirds of the QC samples and at least 50% at each concentration level should meet the above criterion.

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline, but FDA Guidance added description as follows:
A distinct batch or batches in an analytical run may be rejected when it fails to meet QC acceptance criteria, but the remaining batches may pass provided that the analytical run meets the overall QC acceptance criteria.

Underline: key words in the item

Analysis of study samples: ISR

MHLW LBA Guideline (2014)

In bioanalysis, it can happen that the results of analyses of study samples are not reproducible, even when the method validation is successfully conducted and the validity of at each analytical run is confirmed by calibration standards and QC samples. Such failures can be attributed to various factors, including inhomogeneity of study samples, contamination and other operational errors, and interference of biological components unique to the study samples or of unknown metabolites. ISR refers to reanalysis of incurred samples in separate analytical runs on different days to check whether the original analytical results are reproducible. Confirmation of the reproducibility by ISR improves the reliability of the analytical data. In addition, a failure to demonstrate the reproducibility of the original data in ISR can trigger a cause investigation and remedial measures for the analytical method. Usually, ISR is performed for representative studies selected for each matrix in studies that use pharmacokinetic data as the primary endpoint. For instance, ISR should be conducted in the following situations: non-clinical toxicokinetic studies for each different species, representative clinical pharmacokinetic studies in healthy volunteers and patients with renal/hepatic impairment, as well as bioequivalence studies. For non-clinical studies, ISR may be performed with samples obtained in a independent non-GLP study, if the study design is similar to the relevant toxicokinetic study in terms of sampling conditions. ISR should be performed with samples from as many subjects or animals as possible, including those near the maximum blood concentration (Cmax) and the elimination phase, within a time window that ensures the analyte stability. As a guide, approximately 10% of the samples should be reanalyzed in cases where the total number of study samples is less than 1000 and approximately 5% of the number of samples exceeding 1000. The results of ISR are evaluated using assay variability. Assay variability can be calculated as the difference between the concentration obtained by ISR and that in the original analysis divided by their mean and multiplied by 100. The assay variability should be within ±30% for at least two-thirds of the samples analyzed in ISR. In case the ISR data failed to meet the above criteria, root cause investigation should be conducted for the analytical method and necessary measures should be taken by considering the potential impact on study sample analysis. It should be noted that ISR is performed to monitor assay variability. The original data should never be discarded or replaced with the reanalysis data even if the assay variability exceeds ±30% in a specific sample.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
Description to usable bulk frozen calibration curve is added.
Incurred samples should not be pooled.
In addition to Japanese Guideline, ISR is expected for all pivotal pharmacodynamic and biomarker studies.

Underline: key words in the item

Points to note: Calibration range

MHLW LBA Guideline (2014)

In LBAs, calibration range is largely dependent on the characteristics of the binding reagent and it may be difficult to arbitrarily determine the range. In addition, because the calibration range of LBA is comparably narrow, dilutional linearity should be appropriately established to bring the concentrations of analyte in diluted study samples within the range of the calibration curve. In case the calibration range is changed, partial validation should be performed. However, it is not necessary to reanalyze the study samples that have been quantified prior to the change in the calibration range.

EMA Guideline (2011)

Not addressed.

FDA Guidance (2018)

Similar to Japanese Guideline.

Underline: key words in the item

Points to note: Reanalysis

MHLW LBA Guideline (2014)

Possible reasons and procedures for reanalysis, as well as criteria for handling of concentration data should be defined a priori in the protocol or standard operating procedure (SOP).
Examples of reasons for reanalysis are as follows: calibration curve or QC samples failed to meet the criteria for validity of the analytical run; the obtained concentration exceeded the ULOQ of the calibration curve or fell below the LLOQ due to excess dilution; the analyte of interest was detected in pre-dose or placebo samples; improper analytical operation or malfunction of analytical instrument; and causal investigation on abnormal values.
Reanalysis of study samples for pharmacokinetic reasons should be avoided, whenever possible. Particularly in bioequivalence studies, it is not acceptable to reanalyze study samples and modify the concentration data only because the initial data were pharmacokinetically questionable. However, reanalysis of specific study samples is acceptable when, for instance, the initial analysis yielded an unexpected or anomalous result that may affect the safety of subject in a clinical trial.
In any case, when reanalysis is performed, the analytical report should provide information of the reanalyzed samples, the reason for reanalysis, the data obtained in the initial analysis, if any, the data obtained in the reanalysis, and the final accepted values and the reason and method of selection.

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Similar to Japanese Guideline.
No special description about reanalysis due to safety concerns.
No confirmatory reanalysis for BE studies is accepted.

Underline: key words in the item

Points to note: Carry-over

MHLW LBA Guideline (2014)

Carry-over is an alteration of a measured concentration due to residual analyte in the analytical equipment. Carry-over is not an issue for analyses performed in plates and tubes, while it should be taken into account in analyses that use a single flow cell, flow path, and/or autosampler.
If carry-over is inevitable, its impact needs to be examined, and appropriate measures should be taken to avoid any impact on the actual study sample analysis. Should there be any concern that carry-over may affect the quantification of analyte in study samples, it should be evaluated during the actual study sample analysis to assess the impact on the concentration data.

EMA Guideline (2011)

If robotic liquid handling systems are used, potential for carry-over should be investigated by placing blank samples after samples with a high analyte concentration or calibration standard at the upper limit of quantification.

FDA Guidance (2018)

Not applicable for LBA.
Carryover, if any, should be monitored, and its impact on the quantitation of study samples should be addressed.

Underline: key words in the item

Points to note: Cross-talk

MHLW LBA Guideline (2014)
Cross-talk is an alteration of a measured concentration due to a leak of fluorescence or luminescence to adjacent wells in plate-based assay. If cross-talk is inevitable, its impact needs to be examined, and appropriate measures should be taken to avoid any impact on the actual study sample analysis. Should there be any concern that cross-talk may affect the quantification of analyte in study samples, this should be evaluated during the actual study sample analysis to assess the impact on the concentration data.

EMA Guideline (2011)
Not addressed.

FDA Guidance (2018)
Not addressed.

Underline: key words in the item

Points to note: Critical reagents

MHLW LBA Guideline (2014)

A critical reagent is the one that has a direct impact on the results of an LBA-based bioanalytical method and usually includes, but is not limited to, binding reagents (e.g., unlabeled or labeled antibodies).

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

EMA Guideline (2011)

Similar to Japanese Guideline.

FDA Guidance (2018)

Handling and operation of critical reagents are the same as those of reference standards.

If there are changes to any critical reagents, the following items should be considered.

- Evaluate binding and re-optimize assays
- Verify performance with a standard curve and QCs
- Evaluate cross-reactivities

Underline: key words in the item

Points to note: Interfering substances

MHLW LBA Guideline (2014)

Interfering substances are those that may affect the concentration data in study sample analysis and may include, but are not limited to, soluble ligands and anti-drug antibodies.
If interfering substances are potentially present in study samples, it is advisable to examine the impact of interfering substances on the concentration data.

EMA Guideline (2011)

Not addressed

(Addressed in selectivity)

The analytical method should be able to differentiate the analyte(s) of interest and IS from endogenous components in the matrix or other components in the sample. Selectivity should be proved using at least 6 individual sources of the appropriate blank matrix, which are individually analysed and evaluated for interference. Use of fewer sources is acceptable in case of rare matrices. Normally, absence of interfering components is accepted where the response is less than 20% of the lower limit of quantification for the analyte and 5% for the internal standard.

FDA Guidance (2018)

Not addressed.

(Addressed in selectivity and specificity)

It is important to investigate any interference originating from structurally or physiologically similar analytes (i.e., exogenous interference) or matrix effects (i.e., endogenous interference). Investigating exogenous interference involves determining the cross-reactivity of molecules that could potentially interfere with the binding interaction, including molecules structurally related to the drug, any metabolites, concomitant medications (and their significant metabolites), or endogenous matrix components.

Underline: key words in the item

Documentation and Archives

MHLW LBA Guideline (2014)
In order to ensure adequate reproducibility and reliability of bioanalysis, results obtained in analytical method validations and study sample analyses should be documented in a validation report and a study sample analysis report as described below. The reports should be stored along with relevant records and raw data in an appropriate manner.
All relevant records and raw data should be kept, including those obtained in rejected analytical runs, specifically record of reference materials, blank matrices, and critical reagents (receipt/release, use, and storage), record of samples (receipt/release, preparation, and storage), record of analyses, record of instrument (calibration and settings), record of deviations, record of communications, and raw data such as analytical data.

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline, more detailed requirement, recommendation for summary table.
The FDA expects the sponsor to maintain data at the analytical site to support summary data submitted in Validation and Analytical Study Reports.

Underline: key words in the item

Validation report

MHLW LBA Guideline (2014)
Summary of the validation
Information on the reference standards
Information on the blank matrices
Information on the critical reagents
Analytical method (including description related to the MRD)
Validated parameters and the acceptance criteria
Validation results and discussion
Rejected runs together with the reason for rejection
Information on reanalysis
Deviations from the protocol and/or SOP, along with the impact on study results
Information on reference study, protocol, and literature

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline, more detailed requirement, recommendation for summary table.

Underline: key words in the item

Study sample analysis report

MHLW LBA Guideline (2014)
Summary of the study sample analysis
Information on the reference standards
Information on the blank matrices
Information on receipt and storage of study samples
Information on the critical reagents
Analytical method <u>(including description related to the MRD)</u>
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

EMA Guideline (2011)
Similar to Japanese Guideline.

FDA Guidance (2018)
Similar to Japanese Guideline, more detailed requirement, recommendation for summary table.

<u>Underline</u>: key words in the item

Issues not stated in MHLW Guideline (1 of 4)

FDA Guidance (2018)

Endogenous Compounds

For analytes that are also endogenous compounds, the accuracy of the measurement of the analytes poses a challenge when the assay cannot distinguish between the therapeutic agent and the endogenous counterpart. In such situations, the following approaches are recommended to validate and monitor assay performance. Other approaches, if justified by scientific principles, can also be considered.

- The biological matrix used to prepare calibration standards should be the same as the study samples and free of the endogenous analyte. To address the suitability of using an analyte-free biological matrix, the matrix should be demonstrated to have: (1) no measurable endogenous analyte; and (2) no matrix effect or interference when compared to the biological matrix. The use of alternate matrices (e.g., buffers, dialyzed serum) for the preparation of calibration standards should be justified. The QCs should be prepared by spiking known quantities of the analyte in the same biological matrix as the study samples. The endogenous concentrations of the analyte in the biological matrix should be evaluated before QC preparation (e.g., by replicate analysis). The concentrations for the QCs should account for the endogenous concentrations in the biological matrix (i.e., additive) and be representative of the expected study concentrations.

- Parallelism should be evaluated for assays for endogenous compounds.

Underline: key words in the item

Issues not stated in MHLW Guideline (2 of 4)

FDA Guidance (2018)

Biomarkers
The recommendations in this guidance only pertain to the validation of assays to measure in vivo biomarker concentrations in biological matrices such as blood or urine. Considerable effort also goes into defining the biological function of biomarkers, and confusion may arise regarding terminology (e.g. biomarker method validation vs biomarker qualification).

Biomarkers are increasingly used to assess the effects of new drugs and therapeutic biological products in patient populations. Because of the important roles biomarkers can play in evaluating the safety, activity, or effectiveness of a new medical product, it is critical to ensure the integrity of the data generated by assays used to measure them. Biomarkers can be used for a wide variety of purposes during drug development; therefore, a FFP approach should be used when determining the appropriate extent of method validation. When biomarker data will be used to support a regulatory decision making, such as the pivotal determination of safety and/or effectiveness or to support dosing instructions in product labeling, the assay should be fully validated.

For assays intended to support early drug development (e.g., candidate selection, go-no-go decisions, proof-of-concept), the sponsor should incorporate the extent of method validation they deem appropriate.

Method validation for biomarker assays should address the same questions as method validation for drug assays. The accuracy, precision, sensitivity, selectivity, parallelism, range, reproducibility, and stability of a biomarker assay are important characteristics that define the method. The approach used for drug assays should be the starting point for validation of biomarker assays, although the FDA realizes that some characteristics may not apply or that different considerations may need to be addressed.

Underline: key words in the item

Issues not stated in MHLW Guideline (3 of 4)

FDA Guidance (2018)

Diagnostic Kits

Diagnostic kits are sometimes co-developed with new drug or therapeutic biological products as analytical methods that are used during the development of new drugs and therapeutic biologics. The recommendations in this section of the guidance do not apply to commercial diagnostic kits intended for point-of-care patient diagnosis (e.g., companion diagnostic kits), which are addressed in the following CDRH guidance documents:

- Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product
- In Vitro Companion Diagnostic Devices

However, when commercial diagnostic kits are repurposed as analytical methods to measure the concentrations of drugs, therapeutic biologics, or biomarkers in development, the FDA has the following recommendations:

- LBA kits with various detection platforms are sometimes used to determine analyte concentrations in pharmacokinetic or pharmacodynamic studies when the reported results must exhibit sufficient precision and accuracy. Because such kits are generally developed for use as clinical diagnostic tools, their suitability for use in such studies should be demonstrated.
- Diagnostic kit validation data provided by the manufacturer may not ensure that the kit method is reliable for drug development purposes. In such situations, the performance of diagnostic kits should be assessed in the facility conducting the sample analysis. Validation considerations for kit assays include, but are not limited to, the following examples:
  - Site-specific validation should be performed. The specificity, accuracy, precision, and stability of the assay should be demonstrated under actual conditions of use. Modifications from kit processing instructions should be completely validated.
  - Kits that use sparse calibration standards (e.g., one- or two-point calibration curves) should include in-house validation experiments to establish the calibration curve with a sufficient number of standards across the calibration range.
  - Actual QC concentrations should be known. Concentrations of QCs expressed as ranges are not sufficient for quantitative applications. In such cases, QCs with known concentrations should be prepared and used, independent of the kit-supplied QCs.
  - Standards and QCs should be prepared in the same matrix as the subject samples. Kits with standards and QCs prepared in a matrix different from the subject samples should be justified, and appropriate cross-validation experiments should be performed.
  - If the analyte source (i.e., reference standard) in the kit differs from that of the subject samples (e.g., the sample is a protein isoform of the reference standard), testing should evaluate differences in assay performance of the kit reagents.
  - If multiple kit lots are used within a study, lot-to-lot variability and comparability should be addressed for any critical reagents.
  - Individual batches using multiple assay plates (e.g., 96-well ELISA plates) should include sufficient replicate QCs on each plate to monitor the accuracy of the assay. Acceptance criteria should be established for the individual plates and the overall analytical run.

Underline: key words in the item

Issues not stated in MHLW Guideline (4 of 4)

FDA Guidance (2018)

Bridging Data From Multiple Bioanalytical Technologies

The FDA encourages the development and use of new bioanalytical technologies. However, the use of two different bioanalytical technologies for the development of a drug may generate data for the same product that could be difficult to interpret. This outcome can occur when one platform generates drug concentrations that differ from another platform. Therefore, when a new platform is used in the development of a drug, the data it produces should be bridged to that of the other method. This is best accomplished by assessing the output of both methods with a set of incurred samples (a minimum of 20 samples). In cases where one method produces data with a constant bias relative to the other, concentrations can be mathematically transformed by that factor to allow for appropriate study interpretation. Sponsors are encouraged to seek feedback from the appropriate FDA review division early in drug development. The use of two methods for BE studies in ANDAs is discouraged.

Dried Blood Spots

Dried blood spot (DBS) technology has been under development for several years. The benefits of DBS include reduced blood sample volumes collected for drug analysis as well as ease of collection, storage, and transportation. Additional validation of this sampling approach is essential before using DBS in regulatory studies. This validation should address, at a minimum, the effects of the following issues: storage and handling temperatures, homogeneity of sample spotting, hematocrit, stability, carryover, and reproducibility, including ISR. Correlative studies with traditional sampling should be conducted during drug development. Sponsors are encouraged to seek feedback from the appropriate FDA review division early in drug development.

Underline: key words in the item