Critical reagents in LBA

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Focus Workshop

(*In collaboration with the AAPS and JBF*)

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Why are we discussing critical reagents in LBA?

1. There is no clear definition
2. No specific guidance from regulators including no recommendation on how to deal with a change of lot
3. No unified approach can be used due the variation of types of critical reagents and platforms
4. High variability in the approaches described in the current literature, leaning towards being very extensive
5. Challenge with lack of communication or warming from commercial suppliers, when reagents are changed

Content

- Current guidelines
- Recommendation for ICH M10
  - Definition of critical reagents
  - Lot changes
  - Long-term stability
  - Documentation
- Topics not to be included in the critical reagent section for ICH10

Keep in mind

Critical reagents are not reference standard

ICH M10: Only PK assay

EMA (2011): 7.1.1.12. Reagents

- **Critical reagents, including binding reagents** (e.g. binding proteins, aptamers, antibodies or conjugated antibodies) **and those containing enzymatic moieties have direct impact** on the results of the assay and therefore **their quality must be assured**. Accordingly, when **changing reagent batches** during validation or sample analysis the analytical **performance of the method must be verified** to ensure that it is not altered compared with the original or previous batch.

- **Conditions guaranteeing the maintenance of the stability** of both non critical reagents (e.g. buffers, diluents or acidification reagents) and more importantly of the critical reagents should be documented in order to ensure that the performance of the method is not affected over time.

FDA (draft 2013): Key Reagents

- **Key reagents**, such as **reference standards**, antibodies, tracers, and matrices **should be characterized appropriately and stored under defined conditions**. **Assay reoptimization** or validation may be important when there are **changes in key reagents**. For example:
- Labeled analytes (tracers)
  - Binding should be reoptimized
  - Performance should be verified with standard curve and QCs.
- Antibodies
  - Key cross-reactivities should be checked.
  - Tracer experiments above should be repeated.
- Matrices
  - Tracer experiments above should be repeated.

Japan (LBA, 2013): 6.5. Critical reagents

- **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**.

Topics to include in ICH M10 guideline

- Definition of critical reagents
- Critical reagents throughout the lifecycle of the assay
  - Lot-change
  - Long-term stability
- Documentation

Critical reagents throughout the lifecycle of the assay

- To ensure the analyte concentrations in study samples are comparable during the assay's lifespan

  - Validation
  - Change of critical reagents
    - Run partial validation, if required
  - Monitoring of the assay over time
    - Including long-term stability
  - Ongoing study sample analysis

Definition of critical reagents - 1

- Critical reagents are defined in the guidelines, white papers and other publications

- Recommendation:
  - Include a definition of critical reagents in the guideline

Definition of critical reagents - 2

- Outcome of an internal EBF workshop

- EBF definition from March 2016

  For LBA, critical reagents are (often biological) reagents/molecules that are involved in binding and staining reactions that can alter the outcome of the assay even without being noticed.

  Consequently, these reagents influence the validity of an LBA assay.

  As a result, lot-switching of critical reagents requires additional bridging/qualification experiments before reagents are used for sample analysis.

Lot-change of critical reagents - 1

- Minor / major changes as defined by the GBC group*:
- ”Minor reagent changes are defined as those that are expected to have minimal effects on assay performance and may therefore be implemented without any deleterious effect on data production”.
- Examples:
  - new reagent lot derived from a previously qualified stock
    - such as a new purification of polyclonal sera from the same animals
    - a new conjugation using the same protein lot when the conjugation process has been demonstrated to be well controlled

* King et al: Ligand binding assay critical reagents and their stability: Recommendations and best practices from the global bioanalysis consortium harmonization team, The AAPS Journal Vol. 16, No. 3, May 2014.

Lot-change of critical reagents - 2

- Major changes as defined by the GBC group*:
- Major changes: ”This is the most extensive reagent qualification level and is directed primarily towards the replacement of critical reagent where the original source of a reagent is no longer available”
- Examples:
  - antibody lots obtained from new animals,
  - new clones for monoclonal antibody production, or
  - new cell lines for the generation of recombinant material

* King et al: Ligand binding assay critical reagents and their stability: Recommendations and best practices from the global bioanalysis consortium harmonization team, The AAPS Journal Vol. 16, No. 3, May 2014.

Lot-change of critical reagents - 3

- Testing of change of reagents should be performed in the assay.

- CoA (or technical datasheet) for the new reagent should at a minimum include:
  - Name of reagent
  - Lot no
  - Catalogue no (for commercial reagents)
  - Concentration, if applicable
  - Retest date (expiry date)
  - Manufactory date
  - Storage recommendation

Lot-change of critical reagents - 4

- Recommendation to include in ICH M10 guideline:
  - Pragmatic and flexible baseline approach for testing of lot-changing and stability
  - Keeping in mind the wide range of types of critical reagents and platforms might not all be covered

Lot-changing of critical reagents – Minor

- EBF suggestion for minor changes:

Minor change:

- New purification of the same lot
- Relabelling
- Change of standard reagents

<u>Change of standard reagents:</u>

One qualification run with standards and 3 QC levels

If normal run acceptance criteria are fullfilled, then the new batch is accepted – min requirements

To be documented in relevant paperwork for the method

<u>New purification or relabelling of the same lot:</u>

One qualification run with standards and 3 QC levels including the original lot if possible

If normal run acceptance criteria are fullfilled within the same lot, then the new batch is accepted – min requirements

To be documented in relevant paperwork for the method

Lot-changing of critical reagents - Major

➢ EBF suggestion for major changes:

Major change:

- Change to production method of antibodies
- New bleed of polyclonal antibodies (possible change in epitope pattern changes)
- New supplier

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Same Clone: Looking into the possibilities of performing partial validation

Different clone: Different antibody and thereby a different method – new validation

New bleed: comparing to the previous bleed - Looking into the possibilities of partial validation

Partial validation:

(3 accuracy and precision runs with 5 QC levels, selectivity, specificity)

Long-term stability monitoring and testing - 1

- Suggest to add a general guidance*

Table II. General Guidance for Reagent Storage Conditions

Reagent type	Storage condition	Retesting[a]	Extension[b]
Purified proteins/antibody[c]	≤−60°C	8 years from preparation date	2 years
	2°C to 8°C	6 months from thaw date	6 months
Labeled proteins/antibody[c]	≤−60°	4 years from preparation date	1 year
	2°C to 8°C	6 months from thaw date	NA
Lyophilized reagents	≤−60°C	8 years from receipt date without reconstitution	2 years
	2°C to 8°C	4 years from receipt date without reconstitution	NA[b]
Commercial biological matrix	≤−60°C	3 years from receipt date	1 year
	2°C to 8°C	1 week from thaw or receipt date	NA

NA not applicable
[a] For specific reagents, scientific judgment may need to be applied based on the nature of the individual components; when appropriate, document the basis for the retest date in the analytical or reagent preparation procedure
[b] Retest date can be further extended upon additional supporting data
[c] For reagents in non-lyophilized form

* King et al: Ligand binding assay critical reagents and their stability: Recommendations and best practices from the global bioanalysis consortium harmonization team, The AAPS Journal Vol. 16, No. 3, May 2014.

Long-term stability monitoring and testing - 2

- Or as a minimum allow extention of critical reagents longer than the expiry date or retest data as long it is monitored
  - As an example by control chart
- After a long gap in analysis, suggest:
  - Perform some type of verification that the method still perform as previous – could be part of assay set-up or possible testing of new preparation of standard and QC samples
  - After successful evaluation, samples can be analysed
- Recommend to use re-test data instead of expiry data as this is ongoing testing

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Documentation

- Requirement in the guideline
  - Requirements for documentation of source and origin of critical reagent should be part of CoA or technical datasheet
  - Lot-changes and stability testing should be documented in relevant paperwork for the method

- Documentation as part as method development
  - Documentation of characterization and selection of critical reagents should be part of development

Topics not to be included in the critical reagent section for ICH M10

- Reference standard
  - Not classed in scope as critical reagent for PK assay
- Selection of critical reagents
  - Part of assay development, which is not part of the guideline
- Level of characterization of critical reagents
  - Risk based approach
  - Different possibilities for commercial available and customized reagents

Recommendation for ICH M10 guideline - 1

1. Definition:
   - For LBA, critical reagents are (often biological) reagents/molecules that are involved in binding and staining reactions that can alter the outcome of the assay even without being noticed.
   - Consequently, these reagents influence the validity of an LBA assay.
   - As a result, lot-switching of critical reagents requires additional bridging/qualification experiments before reagents are used for sample analysis.
   - Critical reagents should be identified and documented in the bioanalytical method

2. Anticipation of need and monitoring throughout the lifecycle of the assay
   - Limit the guideline to only include recommendation from assay validation and onwards.
   - Limit the minimum requirements to include lot-change and reagent stability testing in the bioanalytical assay

Recommendation for ICH M10 guideline - 2

3. Pragmatic and flexible baseline approach for testing for lot-change and stability
   - Include a definition of minor and major lot-changes as defined in the GBC paper and include a flexible baseline approach with different requirements for minor and major lot-changes
   - Stability testing should be based on performance in the assay, rather than expiry dates from supplier, and retest dates can be set from experience and it is suggested to add a generic table for recommendation of suggested re-test periods

4. Documentation
   - Requirements for documentation of source and origin of critical reagent by CoA or technical datasheet
   - Characterization and selection to be part of development
   - Lot-changes and reagent stability testing should be documented in relevant paperwork for the method

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