EBF recommendation on practical management of critical reagents for ligand-binding assays

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Introduction

- In scope: Critical reagents within validated ligand-binding assays for PK, ADA and pivotal biomarkers (PD)

- Out of scope: Cell-based assays, drug reference standard and biomarker calibration/reference standard will be discussed separately
Why a team on critical reagents?

- Critical reagents are crucial to the assay performance due to their unique characteristics.
- They can be binding reagents such as binding proteins, antibodies and conjugated antibodies as well as positive and negative control.
- Typically produced via biological processes and prone to lot to lot variability.
- Difficult to know the real extent of the change (for both “home-made” or commercially available reagents)
- Fill the gaps in the current guidelines / recommendations on critical reagents.
Regulatory guidelines

EMA and FDA general states:

- Changing of reagent batches should be verified (EMA).
- Conditions guaranteeing the maintenance of the stability of critical reagents should be documented (EMA).
- Critical reagents should be characterized appropriately (FDA).
- No real definition of critical reagents are given.
Current specific publications

Several white papers and articles regarding critical reagents have been published over the past years (list in backup slide).

- Main statement: ligand-binding assays are not better than the critical reagents selected.

- Main focus: supply/preparation, selection and characterization.

- Main scope: what to do (and not as much, how to do it).
Current GBC recommendation

Critical Reagent Management Process

Design of Reagent

- Commercial Reagent?
  - Yes
    - Request documentation from vendor(s)
      - Method Optimization
      - Reagent Qualification
    - Reagent Storage and Documentation (CoA)
    - Reagent Inventory Management
  - No
    - Reagent Characterization & Qualification
      - Purity Specificity Selectivity Stability Affinity etc

Assigning Reagent Stability

- Re-test Dates
  - The re-test dates for critical reagents can be assigned according to the reagent expiration date provided by vendor or historical data.
  - Initial dates can be assigned based on experience with similar classes of reagents.

- Recommendations on Reagent Storage
  - Generation of large amounts of a single lot of critical reagent reduces risk of lot changes.
  - Reagents must be stored as recommended by the manufacturer. If not, appropriate storage stability data may need to be generated.
  - Single use aliquots can be used to reduce risk of instability and contamination.

Change of Reagents

- Minor Changes
  - Test in Functional Assay
    - PK/Biomarkers: Three levels of QC required (5 QC's preferred)
    - ADA: 3 levels of controls
      - If multiple reagents are being changed, perform multiple runs
      - Monitoring instrument response from QC samples
      - Perform comparison to the original lot (if available)
      - Once accepted, document the results properly
      - If data not acceptable, additional evaluations required

- Major Changes
  - In Addition to Minor Changes
    - Minimum of three runs should be performed to capture the assay performance
    - If assay acceptance criteria are not met and/or the assay performance is altered, but the assay still remains fit-for-purpose, the new reagent may be acceptable but would require additional evaluation
    - Other data may also be required in order to capture and properly document the full impact on assay parameters

SOP or Similar Document Driven Process

- For reagent(s) in continuous use:
  - Trend analysis of assay performance to set new date without a separate stability run
  - For reagent(s) that are not in continuous use:
    - Retest extension based on acceptable independent stability test

- For reagent(s) in continuous use:
  - Single independent run and trending data
  - For reagent(s) that are not in continuous use:
    - Based on independent run with more rigorous monitoring and shorter re-test extension

Fig. 1. Summary of critical reagent management process


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Ideas for filling the gaps in the current recommendations

- Practical approach
- Trending – comparison to historical data
- What to do, if the acceptance criteria is not fulfilled
- Fit for purpose – tiered approach
- Identify the differences in acceptance criteria for PK, PD or ADA assays.

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Case Study # 1: One way to define a critical reagent

The design of experiment approach

- Difference in assay response upon changing a reagent lot determines the criticality of the reagent
- Critical reagents can be identified using a “design of experiment” (DoE) approach by comparing different lot#
- Assessment of assay response change by DoE upon changing a lot can be done using software like JMP
- Next follows an example of determination of the effect of changing a reagent lot of a presumed critical reagent
We compare 2 different lot# of 3 reagents:
Item 1
Item 2
Item 3

A screening design DoE analysis is used
A total of 6 assay runs are calculated (can be performed in 1 experiment).

Changing lots of item 2 appears to have a significant impact and therefore item 2 should be considered critical.

Assay responses are inserted in the table.

Effect of different lot# are analyzed.

Significant difference.
Case study #2: ADA assay:

➢ Trend analysis for the change of reference item (positive control)
ADA assay: Trending of controls

Change of reference item lot

Run n°

Study 1 Study 2 Study 3 Study 4 Study 5 Study 6 Study 8

OD response

2008 2009 2010 2011 2012 2013

2008 2009 2010 2011 2012 2013

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Change of reference item:
Within a running study the reference item (rabbit polyclonal antibody, affinity purified) was depleted and a new reference item (from a new rabbit) was produced.

Approach:
Controls prepared with old and new reference item in the same plasma pool were analysed together on the same plate (analysis in 4 wells, 2 independent runs).

- The new controls fulfilled the run acceptance criteria.
- The OD of new and old controls were compared to each other; the defined OD criteria were not fulfilled. Mean OD_{PC_{new}} deviated ≥ 20% from OD_{PC_{old}}
- A new normalization factor had to be determined
Cut point calculation:
In this assay the Cut point is calculated using positive and negative control in this assay.

\[
\text{Cut-point} = \text{OD}_{NC} + (K \times \text{OD}_{PC})
\]

Practical approach:
To keep the sensitivity, the false positive rate and the titer determination as constant as possible over the course of the study, a new normalization factor was calculated mathematically.

The relevant term “\(K \times \text{OD}_{PC}\)” had to be kept constant.
**Calculation of K:**

Within the Study 3 data over 157 runs (old reference item) and 9 runs (new reference item) were used.

\[
K_{(\text{old})} \times \text{OD}_{PC(\text{old})} = K_{(\text{new})} \times \text{OD}_{PC(\text{new})}
\]

\[
K_{(\text{new})} = K_{(\text{old})} \times \frac{\text{OD}_{PC(\text{old})}}{\text{OD}_{PC(\text{new})}}
\]

\[
K_{(\text{new})} = 0.066 \times 0.601 \div 1.225 = 0.033
\]

This new K factor was then used for cut-point calculation of future runs including controls prepared with the new reference item.
Trending of Cut-point

- Cut-point
- Cut point (uncorrected)
- Negative control
- Low positive control
- High positive control

Run n°

Study 1  Study 2  Study 3  Study 4  Study 5  Study 6  Study 8

OD response

2008  2009  2010  2011  2012  2013

Study 1  Study 2  Study 3  Study 4  Study 5  Study 6  Study 8

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Case study #3: PK assay

PK method with a new lot of capturing antibody:

- The bridging run was **accepted**: all QCs (concentrations) passed.
- However; the OD curves were **not parallel**:
  - LLOQ was 3.3x lower while ULOQ was 1.1x higher.
- As per Investigation SOP evaluation is triggered when OD values deviate more than 50% → "Is this the same material?"
- Due to concern regarding validated LLOQ, **partial re-validation** was performed: precision, accuracy and selectivity.

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Outlook of the topic team

This topic team is aiming for:

- Providing a practical approach for testing of critical reagent.
- Designing a stepwise (tiered) approach.
- Looking into different approaches for ADA, PK and pivotal biomarker (PD) assays using examples (fit-for-purpose qualification).
- Collect further experience outside the team.
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Backup slides
Summary of current specific publications

- King et al: Ligand binding assay critical reagents and their stability: Recommendations and best practices from the global bioanalysis consortium harmonization team (2014)
- O'Hara and Theobald: Life cycle management of critical ligand-binding reagents (2013)
- Xu and Weant: Critical reagent stability for immunogenicity assays (2013)
- Staack et al: Quality requirements for critical assay reagents used in bioanalysis of therapeutic proteins: what bioanalyst should know about their reagents (2011)
- Nowatzke and Woolf: Best practices during bioanalytical method validation for the characterization of assay reagents and the evaluation of analyte stability in assay standards, quality controls, and study samples (2007)
- Rup and O’Hara: Critical ligand binding reagent preparation/selection: when specificity depends on reagents (2007)