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

Susanne Pihl¹, Barry WA van der Strate², Michaela Golob³, Laurent Vermet⁴, Birgit Jaitner⁵, Joanne Goodman⁶, Marianne Scheel Fjording⁷ & Philip Timmerman^{*,8}

¹Susanne Pihl, Ascendis Pharma A/S, Copenhagen, Denmark

²PRA Health Sciences, Assen, The Netherlands

³Nuvisan GmbH, Grafting, Germany

⁴Sanofi Research & Development, Montpellier, France

⁵Novartis Pharma AG, Basel, Switzerland

⁶MedImmune, Cambridge, UK

⁷Novo Nordisk A/S, Måløv, Denmark

⁸European Bioanalysis Forum, Havenlaan 86c b204, 1000 Brussels, Belgium

*Author for correspondence: chair@e-b-f.eu

Critical reagents play a crucial role in ligand-binding assays; the robustness and reliability of an assay is defined by the quality and long-term availability of these reagents. However, neither regulatory guidelines nor relevant scientific papers provide clear directions for set-up, life cycle management and, more importantly, the acceptance criteria required for the testing of the critical reagents for pharmacokinetic, biomarker and immunogenicity assays. The ambiguity from current guidelines can be a challenge for the bioanalytical community. Members of the European Bioanalysis Forum community undertook a more pragmatic approach on how to assess the impact of critical reagents. In this paper, a review and corresponding gap analysis of the current guidelines and relevant papers will be provided as well as decision trees proposed for lot-to-lot changes of critical reagents for pharmacokinetic assays.

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Introduction & scope

Critical reagents play an important, but often overlooked role, in ligand-binding assays (LBAs). Unlike chromatographic methods, immunoassays rely on binding properties of the reagents to quantify the analyte of interest. The reagents used for LBAs are often produced using biological systems and may be prone to production variability. The reagents can be proteins, antibodies and conjugated antibodies. The unique binding characteristics define the capability of the assay to reproducibly and consistently quantify the analyte. Therefore, it is important to understand whether any change in the reagents significantly impacts the performance of the assay (functional assay) and as a result the reagent is deemed as critical.

Current the European Medicines Agency (EMA), US FDA and the Japanese Ministry of Health, Labour and Welfare (MHLW) guidelines [1–3] do not provide clear strategies for assessing critical reagents for LBAs. The guidelines simply state that “critical reagents have direct impact on the results of the assay and therefore their quality must be assured” [1], “The sponsor should appropriately characterize and document (i.e., determine the identity, purity and stability) all reference standards and the critical reagents” [2] and “the quality of critical reagent should be appropriately maintained throughout the period of use in analytical method validation and study sample analysis” [3]. As the process behind these statements is not defined in regulatory guidance, it remains a challenge for the bioanalytical community to agree on the best practices [4–11] for defining, qualifying and monitoring critical reagents.

The Global Bioanalysis Consortium (GBC) released a recommendation on the life cycle management of critical reagents [10]. To further expand on this topic, the European Bioanalysis Forum (EBF) herein provides a gap analysis of current publications and in addition provides a practical recommendation on the assessment of critical reagents in LBAs used in support of pharmacokinetic (PK) studies. However, some scientific assessments proposed in the manuscript may be project specific and should not be required for all projects. Immunogenicity assay reagents (LBA or cell based), and biomarker assay reagents are out of scope for this recommendation.

Review of the current recommendations & definitions

There is agreement in the literature for the need to verify assay performance when critical reagents are changed and that the efforts to qualify reagents need to be documented. This documentation includes, but is not limited to, information regarding reagent identity, concentration, storage conditions and stability testing. A comprehensive list with recommendations regarding documentation is provided by the aforementioned GBC paper [10].

Current guidelines and scientific papers listed in Table 1 offer a valuable insight into the bioanalytical community perspective on critical reagents, but there are clear gaps that would benefit from more in-depth discussion. The major gaps are as follows:

- No formal definition of a critical reagent is provided. Most published papers offer high level definitions.
- No practical recommendations on reagent monitoring processes. Many papers list reagent monitoring and trend analysis as an effective life cycle management tool, however the actual process and execution is not presented in detail.
- Limited recommendations on life cycle management of reagent stability and expiry/re-testing, particularly in relation to commercial expiry dates versus in-house established dates. Although this area is partially addressed in a few publications [5,10], expiry and re-test date management is not clearly outlined.
- No practical recommendation on process control or execution for reagent lot-to-lot changes. This is a critical aspect of reagent handling and companies have different approaches. Specifically details on the design and evaluation of change control experiments would be of value.

Identifying critical & noncritical reagents

LBA involves a choice of several platforms, detection systems, single or multiplex and/or plate or beads formats. Each of these approaches use reagents that require careful monitoring.

Assay reagents are considered critical if their quality, nature, structure or specificity has a direct impact on the assay performance. These reagents define the assay readout and are most often, but not exclusively, capture/detection antibodies or peptides/proteins. In contrast, noncritical reagents can easily be replaced in an LBA without the need for lot-to-lot assessment. Some examples of critical and non-critical reagents are given in Table 2. In summary, the EBF defines critical reagents as reagents that require specific testing of newly introduced lots, where a loss of performance can lead to suboptimal results in routine bioanalysis and assay validation. In contrast, noncritical reagents changes can be monitored within study, and therefore no special testing runs are required.

In principle there are two approaches for obtaining critical reagents. The first is to obtain a large batch of the critical reagent to avoid any changes during a drug development program or a study that requires analysis over a long period of time. This approach will require the determination of a re-test date or monitoring of long-term stability of the critical reagents. The second approach is to have smaller batches of critical reagents, avoiding the requirement of long-term stability. However, this approach may inevitably lead to (multiple) lot-to-lot changes to confirm performance of the new lot. The approach chosen depends on the strategy and capability of the company. The careful identification of critical reagents must be performed in balance with scientific considerations taking the availability of the reagents into consideration.

It should be acknowledged that not all reagents can be tested for criticality during method development and validation. This can also be performed during sample analysis or transfer of the method to another facility. Reagents which are non-critical in some (if not most) LBAs, can indeed prove to be critical in other cases. This is often identified when the assay is transferred between laboratories and different providers are used to supply what are previously considered as noncritical reagents. In cases where the recipient laboratory employs reagents from other sources than the originator laboratory, method transfer between laboratories offers the opportunity to assess the impact that individual reagents may have on assay performance. If an impact on assay performance is discovered, alternative reagent lot(s) or preferred suppliers used in the recipient laboratory should be tested in at least one

Table 1. Overview of current guidelines and white papers.

Study (year)	Definition of critical reagents	Process recommendation for lot-to-lot changes	Recommendation for reagent stability	Ref.
EMA (2012)	Binding proteins Conjugates Antibodies Aptamers	Verification of method performance	Storage conditions and maintenance should be documented	[1]
FDA (2018)	Reference standards Antibodies Labeled analytes Matrices	Evaluate binding and re-optimize assays Verify performance with a standard curve and QCs Evaluate cross-reactivities	Storage conditions should be defined	[2]
MHLW (2014)	Conjugates Antibodies Components with performance impact	Perform partial validation when minor changes are made to an analytical method that has already been fully validated	Storage conditions should be used that ensure consistent quality	[3]
Nowatzke and Woolf (2007)	Conjugates Antibodies Enzymatic moieties Matrix	Verification of method performance Similarity assessment using a combination of calibrators and QCs	Not discussed	[4]
Rup and O'Hara (2007)	Conjugates Antibodies Cell lines	Verification of method performance Method re-optimization Test old and new lots in parallel Trend analysis	Default expiry dates for commercial reagents Trend analysis may extend reagent expiration date	[5]
Viswanathan <i>et al.</i> (2007)	Conjugates Antibodies	Not discussed	Storage conditions and maintenance should be documented Default expiry dates for commercial reagents Stability data required for newly established storage conditions	[6]
Staack <i>et al.</i> (2011)	Conjugates Antibodies Peptides Receptors or ligands (including fragments)	Not discussed	Monitor aggregation of reagents Characterize with functional and biophysical methods	[7]
O'Hara <i>et al.</i> (2012)	Components with performance impact Conjugates Antibodies Complex drugs/biologics Solid supports Matrix	Depends on extent of change and expected impact Verification of method performance Method acceptance criteria established <i>a priori</i>	Storage conditions and maintenance should be documented Trend analysis may extend reagent expiry date	[8]
O'Hara and Theobald (2013)	Components with performance impact	Verification of method performance Method acceptance criteria established <i>a priori</i>	Expiry dates should be based on formulation, storage conditions and available biophysical and functional data	[9]
King <i>et al.</i> (2014)	Drugs/biologics Conjugates Antibodies Proteins Peptides Matrix	Verification of method performance Similarity assessment i.e., test old and new lots in parallel (if available) Minor lot changes require 1 run with minimum 3 control levels (biomarker, PK, immunogenicity) Major lot changes require 3 runs with minimum 3 control levels (biomarker, PK, immunogenicity)	Default expiry dates for commercial reagents. If such information does not exist, dates can be assigned based on experience with similar classes of reagents Stability data required for newly established storage conditions Trend analysis may extend reagent expiry date	[10]
Bradford (2015)	Reagents that are specific to the analyte Drugs/biologics Conjugates Antibodies Matrix Receptors or ligands	Verification of method performance Similarity assessment, i.e., test old and new lots in parallel (if available)	Investigate conjugate stability Trend analysis	[11]

bridging run, head-to-head with the original assay reagents, to ensure the new lot(s) meet *a priori* acceptance criteria.

The most practical strategy for identifying critical reagents is to run direct comparisons of alternative lots of reagent against an original lot (head-to-head comparison). This is ideally done at the end of method development when the majority of performance characteristics have been defined and the assay format is fixed. Nevertheless, even when assays are developed from scratch, this may not be feasible due to limited reagent availability, or it is not possible to secure large quantities for a longer period. In this case, it is recommended that sufficient amounts of the original reagent are retained for bridging experiments between different reagent lots.

Table 2. A European Bioanalysis Forum overview of critical and noncritical reagents of ligand-binding assays.

Reagent	Considered as critical	Examples
Antibodies (capture/detection reagents)	Always	Monoclonal or polyclonal antibody used as capture reagent in a PK assay
Commercial or in-house conjugated reagents, including in-house conjugated beads	Always	In-house labeled reagents, e.g., Biotin/Sulfo-TAG labeled capture and detection antibodies
Commercial kits	Always in case of change of critical reagent lot	Change of capture reagents in the kit lot
Solid phase	Potentially	Plates (ELISA, MSD, Aushon etc.), paramagnetic beads, Bioaffy CDs (Gyrolab) etc.
Biological matrix	Potentially	If endogenous counterpart is present
Standard reagents	Rarely	Blocking reagents (BSA, Superblock, etc.), enzymatic substrates for detection (TMB, etc.), buffer components (PBS, Tween-20 etc.)

Solid-phase reagents such as plates or beads from vendors with a good history of supply, and lot-to-lot reproducibility, may be considered noncritical. Evaluation of multiple lot numbers during method development (assay prevalidation) may be advisable. Further assessment during validation may be required if the solid phase is considered variable, before proceeding to sample analysis. Alternatively, if variability is found during method development, it is advisable to source a single lot number of solid phase reagents for completion of the validation and sample analysis. However, this approach is not always possible and poses some restrictions on future analysis by the same method. Low binding and uncoated plates, such as those used for transfer to assay plates, electrochemiluminescence (MSD) or colorimetric assays (like ELISA) can be considered noncritical. Coated plates, such as streptavidin-coated MSD or ELISA plates, Ni²⁺ NTA, and Gyrolab CDs may be considered critical reagents.

For commercial kits particular consideration should be paid when using these for the purpose of a pharmacokinetics (PK) assay. If possible, evaluate the supplier history by considering their reliability to supply the kit for the duration of the study, and the frequency of kit changes made historically. It is recommended to secure sufficient commercial kits of the same lot number for the duration of the validation and for at least the sample analysis for the first study. If this is not possible, multiple lots should ideally be evaluated during method development, although this may not always be possible since kits are often produced in ‘on-demand’ batches. Therefore, the impact of lot changes should be closely monitored.

Most biological matrices may be considered noncritical for PK. However, in some applications, when an endogenous counterpart of the drug is present in the matrix, it may be necessary to prescreen lots or individual sources of matrix especially where endogenous components may cause assay problems such as high background or baseline levels.

Lot-to-lot changes of critical reagent: recommendations & considerations: a pragmatic approach

The above section has described a practical strategy for defining which reagents can be considered critical and noncritical during assay development and validation. However, challenges also exist when changing a critical reagent lot mid-study or when re-establishing an assay after a considerable period of time. The purpose of lot-to-lot changes testing is to verify that assay performance is not altered by switching to the new reagent lot, compared with the original reagent. A new critical reagent lot needs to be tested prior to its use in a study. The practices of accepting (or not accepting) a new critical reagent lot varies within the bioanalytical community.

It is the recommendation of the EBF that all critical reagents are tested in the PK assay using the assay run acceptance criteria and compared with expected assay performance, thus keeping unexpected results to a minimum. This EBF recommendation is also in line with previous recommendations provided by King (2014) [10], O’Hara (2012) [8]. The EBF suggests following the GBC recommendation on critical reagents [10] regarding the definition of minor and major critical reagents: “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.” Conversely a major change requires “the most extensive reagent qualification level and is directed primarily towards the replacement of a critical reagent where the original source of a reagent is no longer available.” The GBC advocates three qualification runs for major changes and one run for minor changes [10]. Furthermore, this paper recommends acceptable re-test dates based on the reagent type and the storage conditions.

In addition to the GBC recommendation, the EBF recommendations for both major and minor changes are to review the Certificate of Analysis (CoA)/technical datasheet and assess whether there is a change in lot, clone or

Figure 1. European Bioanalysis Forum recommendation for minor changes.

Figure 2. European Bioanalysis Forum recommendation for a new production of monoclonal antibody using the same clone.

concentration. In such cases it is recommended to perform a head-to-head comparison between the new and old lot in the PK assay. The evaluation may result in no further testing is required to an optimization or re-development of the assay. However, it is important to understand what impact the change in critical reagent may have on the assay and to evaluate which tests should be included in order to implement the new lot.

For evaluation of possible scenarios of critical reagent changes, EBF recommends the following decision trees (Figures 1–4), however it is important to keep in mind that these are the minimum requirements and it may be required to include additional experiments, based on scientific rationale or company policy.

Figure 3. European Bioanalysis Forum recommendation for a new production of monoclonal antibody using a different clone

Figure 4. European Bioanalysis Forum recommendation for new production of a polyclonal antibody (new bleed from same or new animal).

Preferably, qualification and evaluation of a reagent change are performed well in advance of the introduction of the new reagent in bioanalysis. There are several options when replacing a critical reagent depending on the availability and stability:

- 1) Both the old and new lots of the critical reagent are available and are within re-test date;
- 2) Both the old and new lots of the critical reagent are available but the old lot is outside the re-test date;
- 3) Only the new lot of critical reagent is available (old lot is depleted).

Scenario 1 is always preferable as it offers a way to bridge the reagents. Hence it is recommended to retain some of the old material to allow a head-to-head experiment prior to a critical reagent change. Having the old lot as comparator in the bridging run helps to assess the critical impact of the change and serves as an internal assay control even if the old lot is used outside the re-test date as mentioned in scenario 2. However, this should be taken into account during the evaluation of results from the head-to-head comparison in the PK assay. Scenario 3 is not optimal but nevertheless quite common if reagent consumption is not effectively monitored or if the reagent is not

available at a certain time. For this reason, bioanalytical laboratories should consider having active reagent logging systems plus effective planning and communication between responsible parties to mitigate the risk of running out of a comparator reagent prior to qualification of new material.

The timing of the qualification of a new lot is also dependent on the reliability of the reagent supplier and communication with these vendors; suppliers might not always inform customers regarding a change in the production of the critical reagent and, as such, production alterations might have a significant impact on the assay performance. This should be kept in mind even when ordering the same catalog number but different lots of the critical reagent.

In summary, the bioanalyst should decide upfront, if the introduction of a new lot of (critical) reagent is a minor or a major change and what impact the possible changes may have on the assay functionality. Thereafter, performing a functional test in the PK assay by comparing the new reagent against the old reagent and then evaluating the results is needed. Based on this evaluation, a justification to either perform additional qualification/validation experiments, or to proceed with bioanalysis is required. The outcome of these experiments, in other words, the results, the evaluation and conclusions/justifications should be documented.

Stability

Reagent stability and expiry/re-test date management can be a challenge. Reagent stability, characterization and life cycle management is discussed in Rup (2007), O'Hara (2012) and King (2014) [5,8,10]. Many companies use commercial reagents in their assays. These usually come with vendor-specified expiry dates. Rarely can the vendor supply details on stability assessments and not uncommonly the expiry date is specified as, for example, 12 months from delivery date. There is an overall consensus in the industry that assigning a re-test date over an expiry date is more practical. EBF recommends that stability testing should be based on functional performance in the PK assay by confirming that the *a priori* assay acceptance criteria are still met. Re-test dates should be set from experience or procedures and generic stability tables and used for recommendation of suggested re-test periods, rather than strictly applying the expiry dates provided by the supplier.

It is recommended to monitor relevant assay data such as quality control (QC) level data or instrument responses, which yields valuable supportive information to monitor stability of the critical reagent, but is also useful to evaluate lot-to-lot testing of critical reagents during the life cycle of an assay. Monitoring of data throughout assay use/during bioanalysis offer a way to early identify issues (e.g., assay drift) and thereby provide an opportunity to take corrective action.

Documentation

Documentation is an important, but often neglected, part of reagent handling. Survey results from the GBC identified large inconsistencies in reagent-related documentation practices between companies [10]. A likely reason for this is that current EMA, FDA and MHLW guidelines do not provide a clear direction. An outline of best practices for documenting reagents can be found in King (2014) [10].

It is recommended to have a formal procedure for reagent handling, qualification and documentation practices. Such information will help support life cycle management and lot-change decisions. The testing and evaluation of lot-to-lot changes and reagent stability testing should be documented in the relevant method documentation.

Critical reagent information can be captured in CoA, a technical datasheet or in other relevant documentation such as a reagent book.

The EBF recommendation for the minimum information for a critical reagent is as follows:

- Name of reagent;
- Lot number;
- Source (e.g., cell line, method of expression), if applicable;
- Origin source for modified reagents (e.g., labeled reagents);
- Catalog number (for commercial reagents);
- Concentration, if applicable;
- Re-test date/expiry date;
- Manufacture date;
- Storage condition recommendation.

Conclusion & recommendation

Definition, qualification and monitoring of critical reagents play an important, but often overlooked, role in LBA. For LBA, critical reagents are (often biological) reagents/molecules that are involved in binding reactions that can alter the outcome of the assay. Consequently, these reagents influence the validity of an LBA assay and of the resulting data. However, to date there is no clear definition for critical reagents in regulatory guidelines or how to manage changes.

Whilst some scientific assessments proposed in this manuscript may be project specific and should not be required for all projects, the EBF recommendations for critical reagents in PK assays are:

- Identify the critical reagent per PK assay and clearly document in the method documentation (Section 3);
- Identify the noncritical reagents and monitor during routine sample analysis (Section 3);
- Ideally, ensure enough material is available to support an entire study and/or drug development program (Section 3);
- Use GBC definitions to evaluate what constitutes a minor or major change (Section 4);
- Where one single lot cannot be sourced for the entire period of use, retain enough material of the old lot for head-to-head comparisons of new lots (Section 4);
- Evaluate lot-to-lot changes of critical reagents using the CoA and test in the PK assay using *a priori* criteria (Section 4);
- Application of re-test dates instead of expiry dates to monitor and evaluate reagent stability (Section 5);
- Monitor QC and assay data during the assay life cycle (Section 5);
- Document reagent identity, lot-to-lot changes and evaluation/extension of re-test dates in the relevant paperwork for the method (Section 6);
- For commercial kits that are used for PK purposes, secure sufficient kits of the same lot number. If this is not possible then test new batches when available (Section 4).

Decision trees have been provided for evaluation of lot-to-lot changes depending on the degree of change (minor and major) and type of reagent (standard reagents/relabeling, monoclonal antibodies produced from the same or different clone and polyclonal antibodies from a new animal bleed). However, it is important to keep in mind that this is a recommendation for the minimum requirements and that additional testing might be required based on the scientific knowledge of the impact of a critical reagent on an assay.

The EBF recommends performing a functional evaluation of lot-to-lot change and stability testing in the PK assay, but also acknowledges that further characterization of the reagents may be implemented to minimize the chance of surprises during testing of new reagent lots. Whether additional reagent characterization is performed is a business decision and needs to be balanced against the additional time and cost of the characterization. This is especially important in early phases of drug development when attrition is high. It should also be noted that assay data robustness is independent of the level of reagent characterization applied and a highly characterized assay reagent does not automatically generate more robust assays.

Future perspective

It is important that the industry and regulatory authorities agree on the appropriate level of testing for critical reagents, and ensure that the difference between critical reagents (which are a component in the assay) and a reference standard, which is used directly for concentration determination, is recognized. Going forward, the bioanalytical community would also welcome a more open communication with the commercial suppliers to ensure more consistency across different reagent lots and potentially longer stability/re-test dates. It would be beneficial for the industry if commercial vendors provide more information regarding reagent characteristics including a fully comprehensive CoA or technical datasheet. Furthermore, commercial vendors should ensure timely communication to the end users when changes or a cease in production occurs to allow for timely assessment of the impact in the assay and provide adequate time to take any required mitigation steps. The EBF is also discussing the requirements for critical reagents for immunogenicity assays and biomarkers and expect to provide recommendations for immunogenicity assays in these areas as the next step.

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