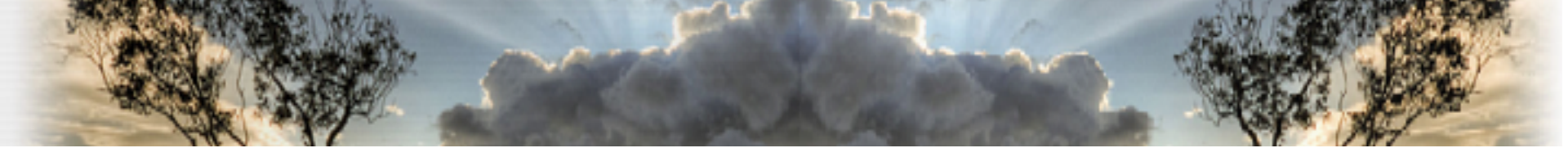


EBF Feedback on ADA critical reagents

Susanne Pihl, on behalf of the EBF

12th EBF Open Symposium
Imagine! A new bioanalytical Earthrise

http://www.e-b-f..eu

Assay characteristics PK vs ADA assays

Table 1. Assay characteristics for pharmacokinetic and antidrug antibody assays.

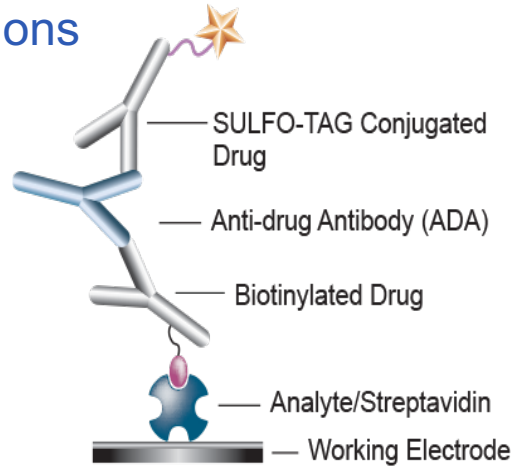
Description	PK assay	ADA assay
Measurement type	Quantitative	Qualitative and semiquantitative (titrations) Tiered approach – multiple assay types
Calibrator	Well characterized reference standard Used in sample testing	Use of a 'surrogate' positive control antibody, usually generated in animals Negative control No standard curve used in sample testing
Control	Quality controls	System suitability controls
Sensitivity	Defined based on mass unit concentration	Defined in relation to the cut-point of the assay. Sensitivity is highly dependent on the surrogate positive control antibody and may not be reflective of sensitivity in clinical samples.
Assay format	Usually a sandwich immunoassay In-house generated or commercially sourced	Depends on the assay format and tier of testing (LBA/CBA/SPR, etc.)

ADA: Antidrug antibody; CBA: Cytometrix bead array; LBA: Ligand-binding assay; PK: Pharmacokinetic; SPR: Surface plasmon resonance.

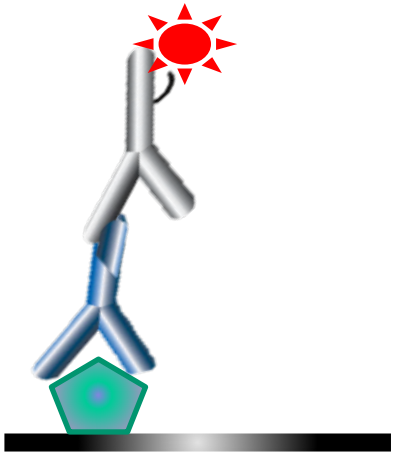
Pihl S, van der Strate BWA, Golob M *et al.* EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays, Bioanalysis, Epub ahead of print, Oct 2019

A Qualitative Assay with Surrogate Controls….

- ADA assay framework is built around a Positive and Negative Control (NC/PC)
- Surrogate:
  - Polyclonal vs Monoclonal
  - Different species
- Coating & Detection concentrations
- MRD
- Sensitivity
- Drug Tolerance



Mesoscale.com



Securing of critical reagent

- (Critical) Reagents:
  - Out of stock
  - Out of production
  - Expire

- Clinical trials:
  - Delayed
  - Extended
  - Additional sampling requested

- Storage capacity is not always unlimited

Overview of critical and non-critical reagents

Table 3. An EBF overview of critical and noncritical reagents for antidrug antibody assays.

Reagent	Considered as critical	Examples
Positive control antibody	Always	Monoclonal/polyclonal antibodies for preparation of LPC and HPC
System suitability controls	Always	Positive controls
Material for confirmatory/characterization assay	Always	For example drug compound that is used for dosing, but also other (multi-domain materials) that can be used for characterization of the immune response (cross-reactivity)
Labeled reagents for bridging assay	Always	New batches of (labeled) coating/detection material. Both in-house or commercially labeled
Labeled reagents for direct assays	Potentially	New batches of coating/detection material. Both in-house or commercially-labeled
Matrix	Potentially	Preparation of assay controls including negative control serum, dilution matrix for titration
Coated surfaces	Potentially	Dependent on platform that is used for analysis
Reagents used in specific processing steps	Potentially	Acid dissociation, SPEAD, PANDA, etc
Standard reagents	Rarely	Blocking reagents, read buffers and substrates

HPC: High positive control; LPC: Low positive control; SPEAD: Solid-phase extraction with acid dissociation; PANDA: Precipitation and acid dissociation.

Pihl S, van der Strate BWA, Golob M *et al.* EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays, Bioanalysis, Epub ahead of print, Oct 2019

Potential impact of lot-to-lot changes

Table 4. Overview of the potential impact of CR lot-to-lot changes on immunogenicity assays.

Reagent change	Potential assay impact
Matrix	May induce drift of negative control responses and impact the assay acceptance criteria and the cut-point
	May induce drift of positive control responses and impact the assay acceptance criteria, the sensitivity and the titration assay result May impact selectivity, especially when changing populations
Positive control antibodies†	May impact the definition of all assay parameters, especially assay sensitivity, drug tolerance and specificity
	May impact the positive control concentration levels
Material for confirmatory/characterization assay	May impact the confirmatory assay reaction (e.g., by change of formulation, process or host cell protein content)
	May impact the functionality in the assay including the cut-point
Labeling of coating/detection reagents	May impact assay responses in general for example, background noise (which further impacts cut-point), positive signal level, response ratios, etc
	May impact assay sensitivity and drug tolerance
	May impact labeling efficiency (applicable for bridging assay formats)
Coated surfaces	May impact assay responses in general for example, background noise (which further impacts cut-point), positive signal level, response ratios, etc
	May impact negative control and positive control functionality
	May impact homogeneity of coated surfaces
Reagents for specific processing steps	May impact the output of the processing step (e.g., acid dissociation, SPEAD, PANDA, etc)

†Additional considerations are if the positive control change is a new purification of anti-sera (from the same bleed), a new bleed or a new immunization.
PANDA: Precipitation and acid dissociation; PC: Positive control; SPEAD: Solid-phase extraction with acid dissociation.

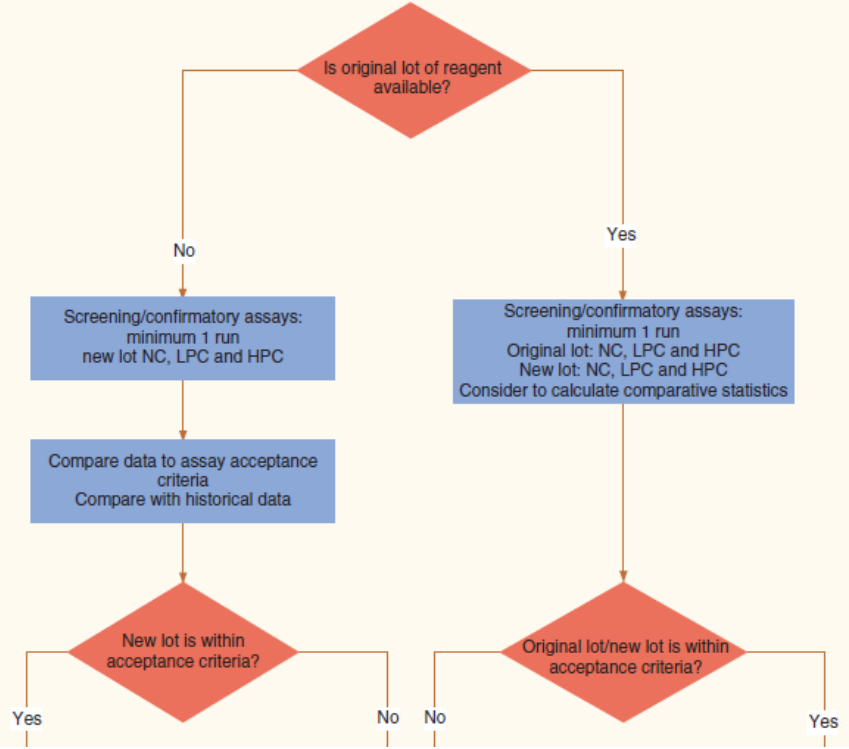
Pihl S, van der Strate BWA, Golob M *et al.* EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays, Bioanalysis, Epub ahead of print, Oct 2019

Minor and major changes

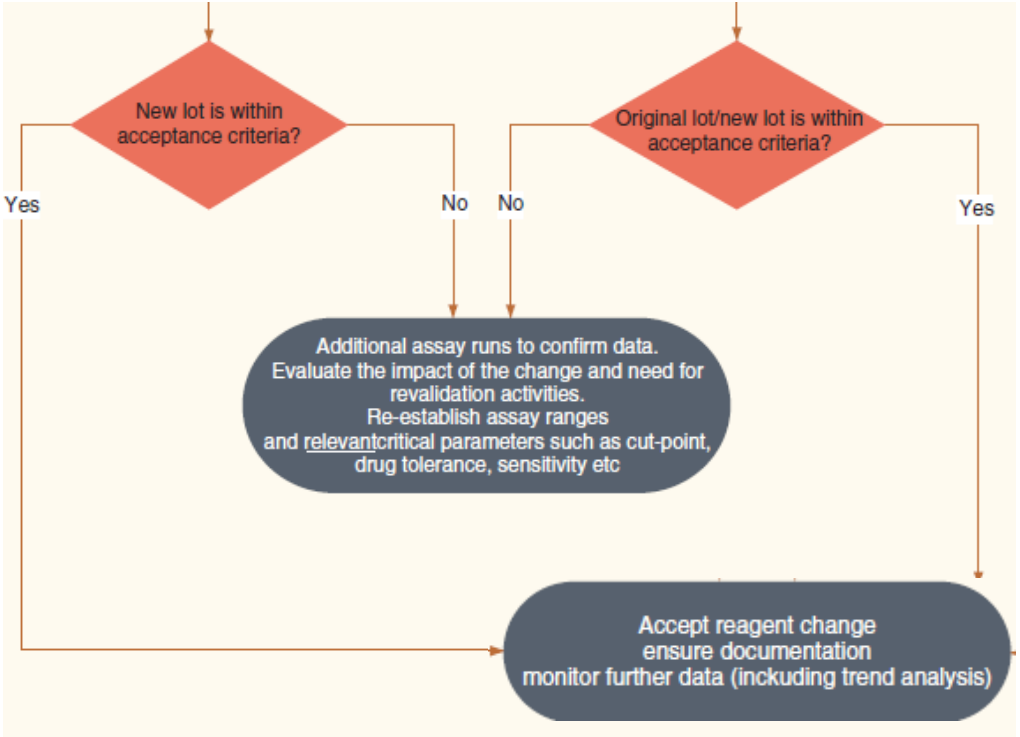
- EBF suggestion in general to follow GBC paper on critical reagents*
  - Definition of minor and major critical reagents
  - ”Minor reagent changes are defined as those that are expected to have minimal effects on assay performance and may therefore be implemented without any deleterious effect on data production”.
  - 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”

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

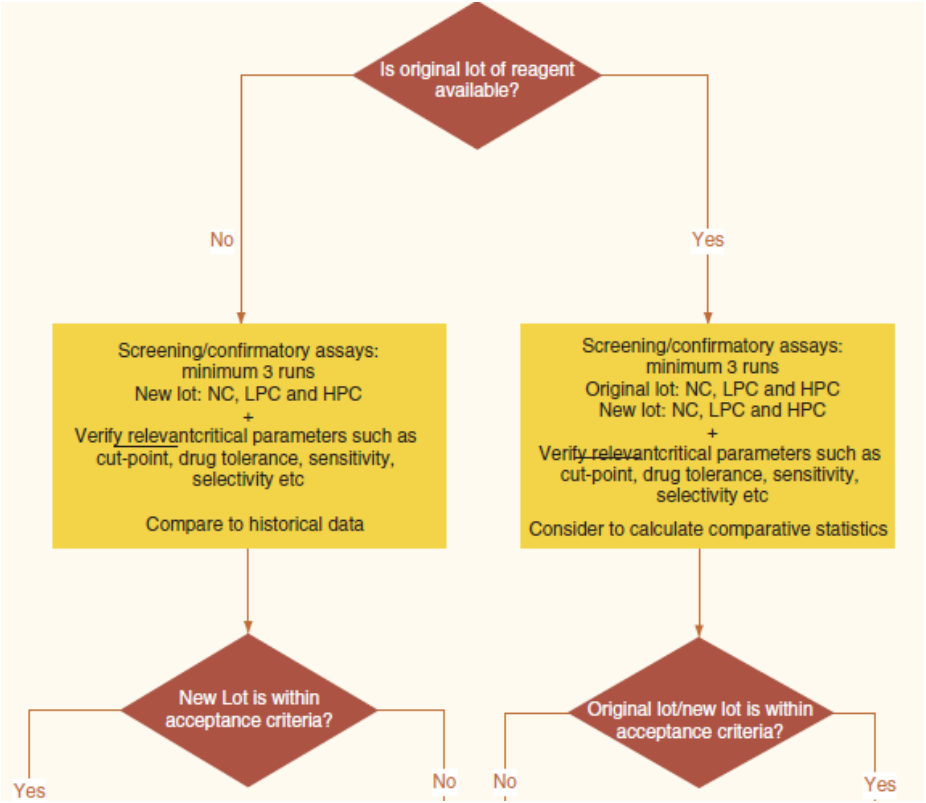
Minor critical reagents changes - testing



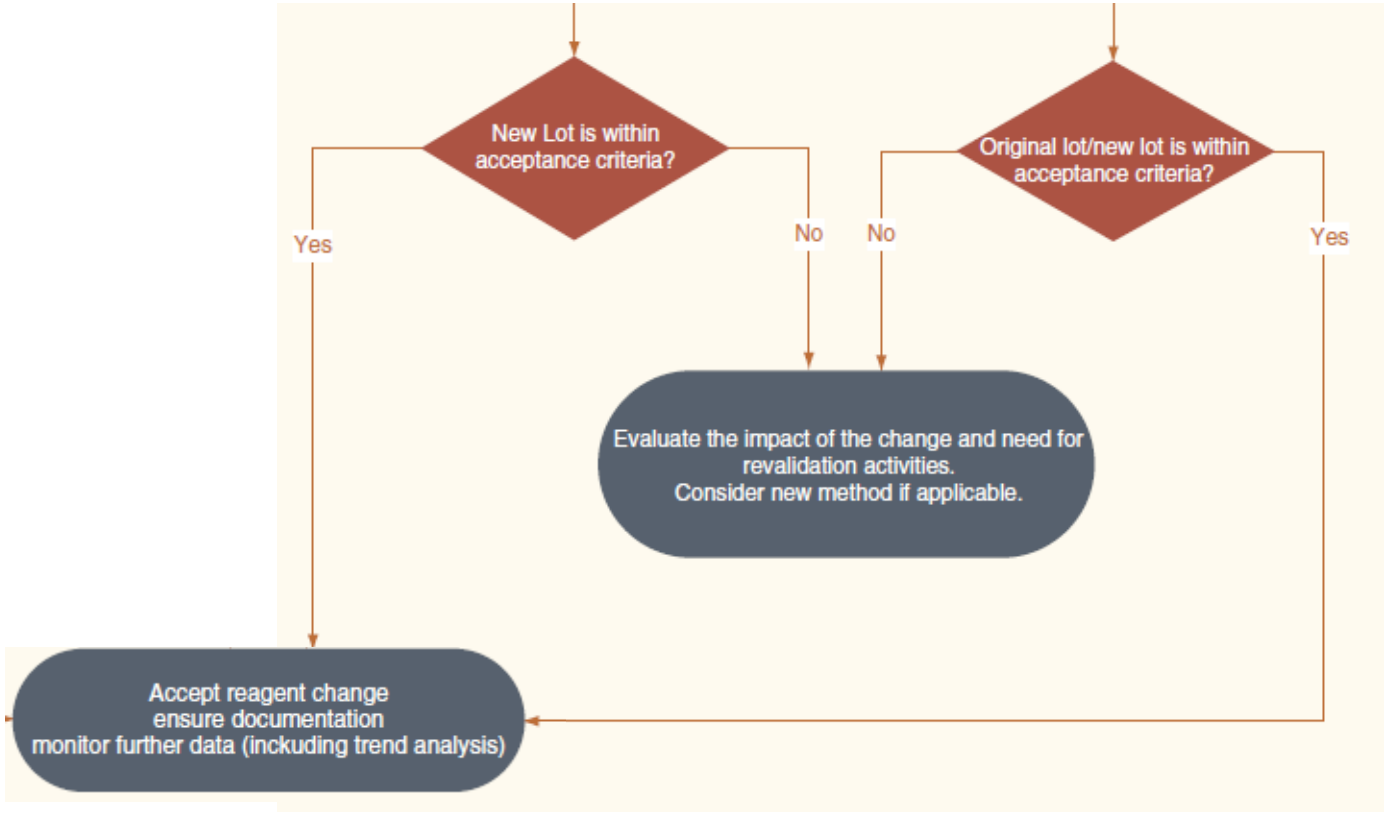
Minor critical reagents changes - acceptance



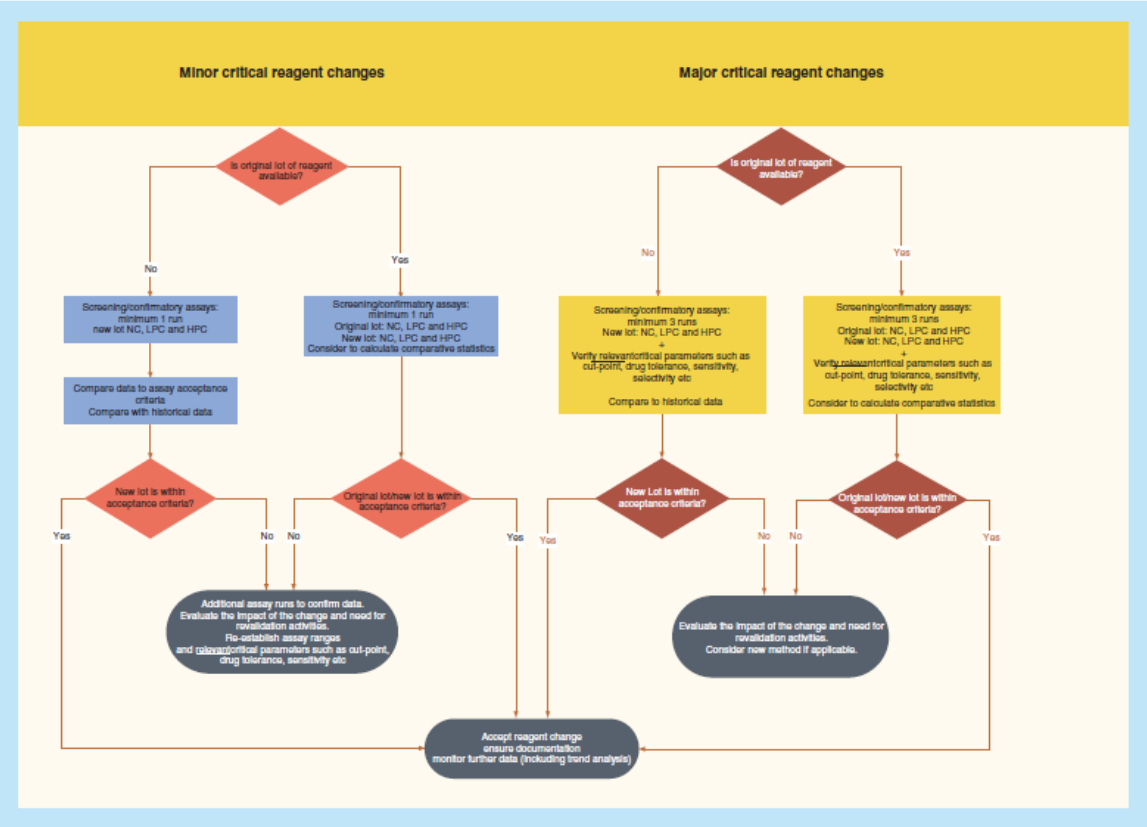
Major critical reagents changes - testing



Major critical reagents changes - acceptance



Minor and major critical reagent changes



Pihl S, van der Strate BWA, Golob M *et al.* EBF recommendation on practical management of critical reagents for antidrug antibody ligand-binding assays, Bioanalysis, Epub ahead of print, Oct 2019

Points to consider

- What changes were implemented to the method?
- What additional assessments were conducted to verify that the new lot is working satisfactorily, or the method has not changed?
- What is the justification to continue with sample analysis after introduction of a new CR lot?
- What consequences were evaluated with regard to previously obtained sample results within a study, that is, is it justifiable to continue with bioanalysis, or has the method been changed to such an extent that (partial) validation is required and sample reanalysis is required?

Summary

- No “Textbook for bridging of the critical reagents”

- Identify the critical reagents
- Secure material as appropriate
- Head-to-head comparison between original and new reagent
- Evaluate the changes and consequences
- Document the changes
- Justify how to move forward

Use your scientific knowledge!

Acknowledgment

- EBF Critical Reagents Team:
  - Susanne Pihl
  - Barry van der Strate
  - Michaela Golob
  - Birgit Jaitner
  - Laurent Vermet
  - Janka Ryding
  - Jo Goodman

- Thank to all European Bioanalysis Forum members for very valuable input

Contact Information

Questions: info@e-b-f.eu

European Bioanalysis Forum vzw

www.e-b-f.eu