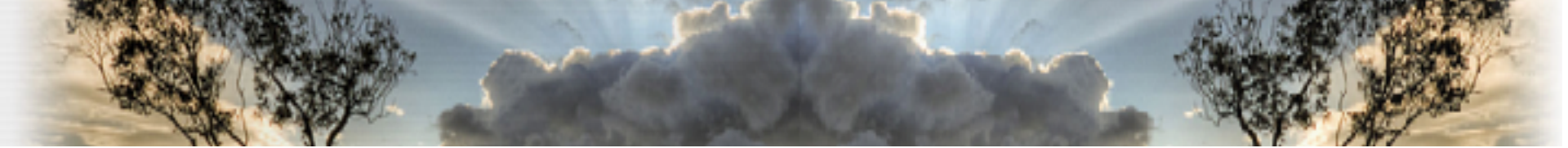


EBF Feedback for critical reagents in LBA biomarker assays

Susanne Pihl, on behalf of the EBF

Biomarkers in Pharma R&D
A roadmap from *Context of Use* to *Using the data*

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

Definition of a critical reagent

- Reagents that have the ability to significantly impact the performance of the assay (functional assay) are deemed as critical.
- In scope are all critical reagents except the reference standard*
- For biomarker assays commercial kits are often used. The kit may be considered
  - a) as one reagent with a retest/expiry date applicable for the entire kit and all components
  - b) as multiple reagents where the kit components have individual retest/expiry dates

* Kunz, Goodman, Loevgren et al. Addressing the challenges of biomarker calibration standards in ligand-binding assays: a European Bioanalysis Forum perspective. Bioanalysis: (2017), 9 (19), 1493-1508

Overview of critical and non-critical reagents

Reagent	Considered as Critical	Examples
Commercial kits	Always	Sometimes it is not known, which reagent of the kit has changed Change of capture reagents in the kit lot
Un-labelled ligand binding reagent (antibodies) (capture/detection reagents)	Always	Monoclonal or polyclonal antibody
Commercial or in-house conjugated reagent, including in-house conjugated beads	Always	In-house labeled reagents, e.g. Biotin/Sulfo-TAG labeled capture and detection antibodies
Biological matrix (used for dilution buffer)	Potentially	Endogenous counterpart is present
Solid phase	Potentially	Plates (ELISA, MSD etc.), paramagnetic beads, Bioaffy CDs (Gyrolab) etc.
Standard reagents	Rarely	Blocking reagents (BSA, Superblock, etc.), enzymatic substrates for detection (TMB, etc.), buffer components (PBS, Tween-20 etc.)

Challenge of securing of critical reagents

- 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. This 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. This will require testing of lot-to-lot changes
- (Critical) Reagents:
  - Out of stock, out of production or expire
- Clinical trials:
  - Delayed, extended and/or additional sampling requested
- Storage capacity is not always unlimited

Context of use

- The context of use should be taken into account as many of the biomarkers are exploratory and only a limited number of biomarkers are safety, efficacy or diagnostic markers

- Different approaches should be used for the different type of assays and will also depend of the business risk, study set-up and timing in the development
  - used for defining low, medium and high risk for critical reagent

- The highest standard should be implemented for safety, efficacy and diagnostic markers

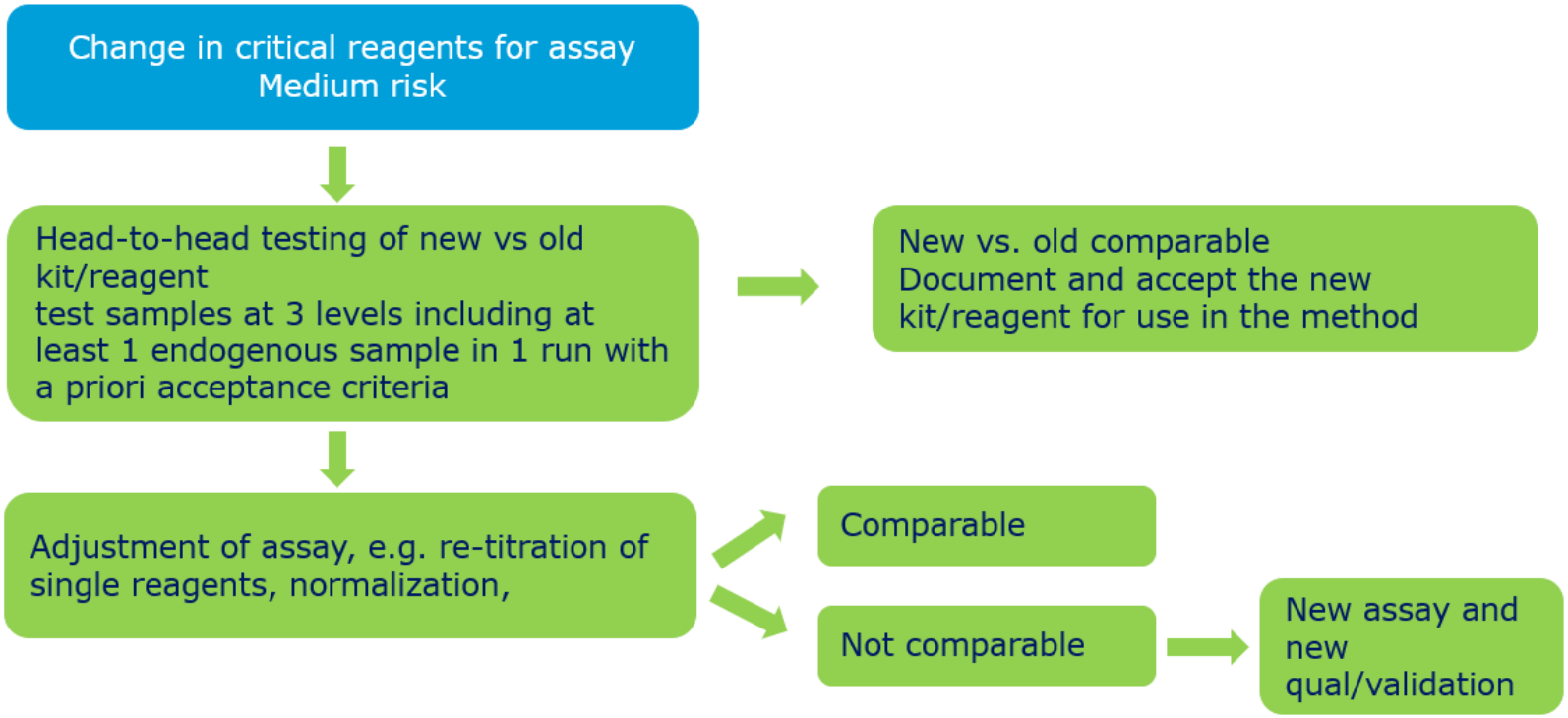
Identifying low, medium and high risk

Risk	Risk of impact
Low	Exploratory biomarker – research level No comparison of data between studies is needed No risk of wrong decision for subjects or internal decision
Medium	Exploratory or probably validated biomarker Able to compare data between studies Trust for internal decision
High	Safety, efficacy and diagnostic markers Trust for internal decision, subjects and regulatory authorities

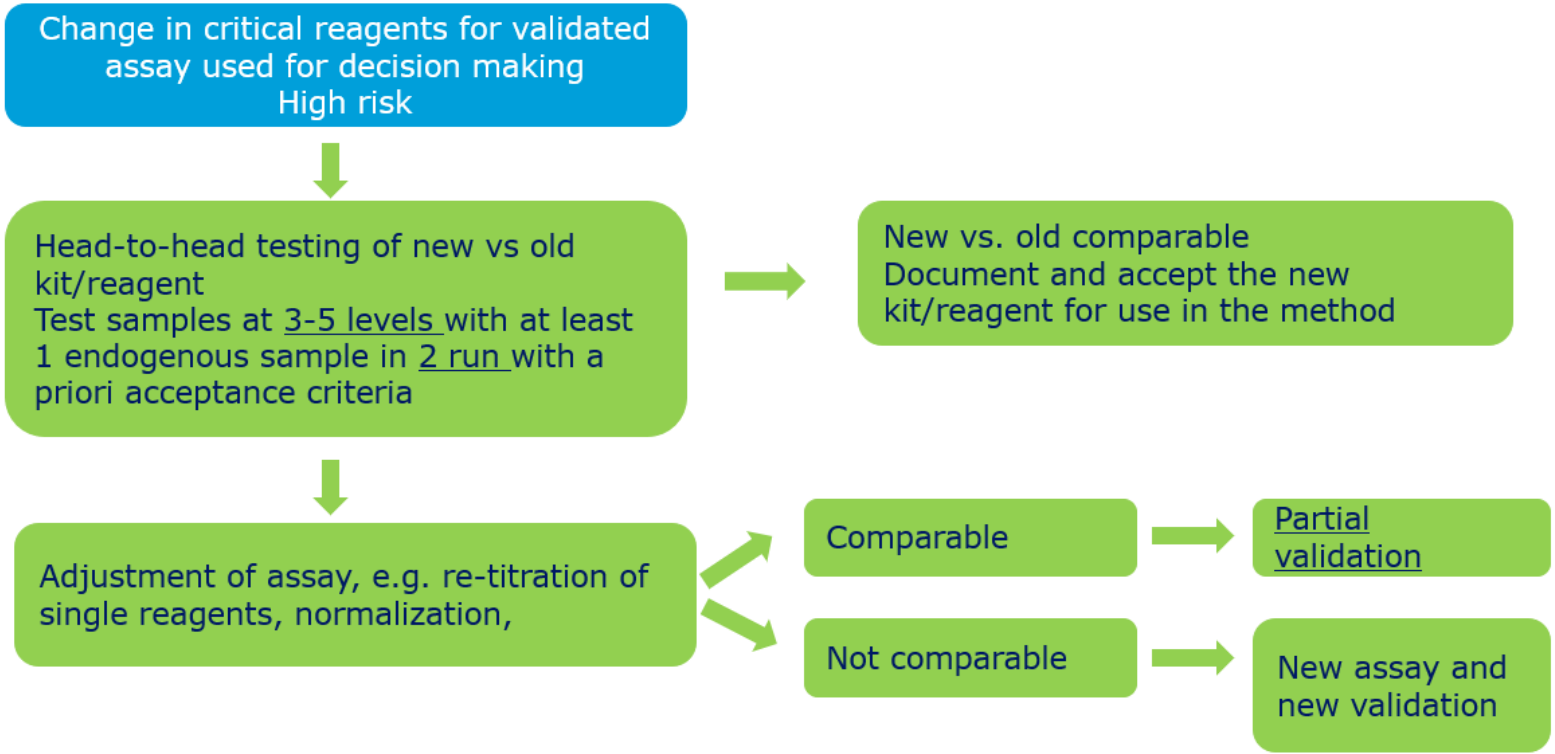
Lot-to-lot changes of critical reagents – low risk

- It might often be acceptable to use the new kit lot without any further testing
- Trust the vendor!

Lot-to-Lot changes of critical reagents – medium risk



Lot-to-Lot changes of critical reagents – high risk



Stability testing of critical reagents

- One of the challenges are that the reference material used for the original testing has been through the same stability period
- For biomarkers, the endogenous matrix samples can be used for the stability testing and can therefore be started early by using isochronic design. Isochronic design can distinct between stability of the test samples and stability of the critical reagents
- Isochronic design*
  - Samples are prepared in one pool and one aliquot will be stored at the reference temperature (below -130ºC) for the entire period and stability samples will be moved to the reference temperature after the defined stability timepoints
  - Analysis of samples at one time point after storage for different periods of time at a testing temperature

Pihl, Andersen, Bruzelius et al: An alternative design for long-term stability testing of large molecules: a scientific discussion paper from an EBF Topic Team. Bioanalysis (2015) 7(24), 3073–3083

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 that the method has not changed?
- What is the justification to continue with sample analysis after introduction of a new critical reagent 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 lot-to-lot changes of the critical reagents”

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

Use your scientific knowledge!

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