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

***Presenter: Susanne Pihl (Lundbeck)***
***on behalf of EBF TT-47***

EBF Open Symposium
20 November 2014
Barcelona

# 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

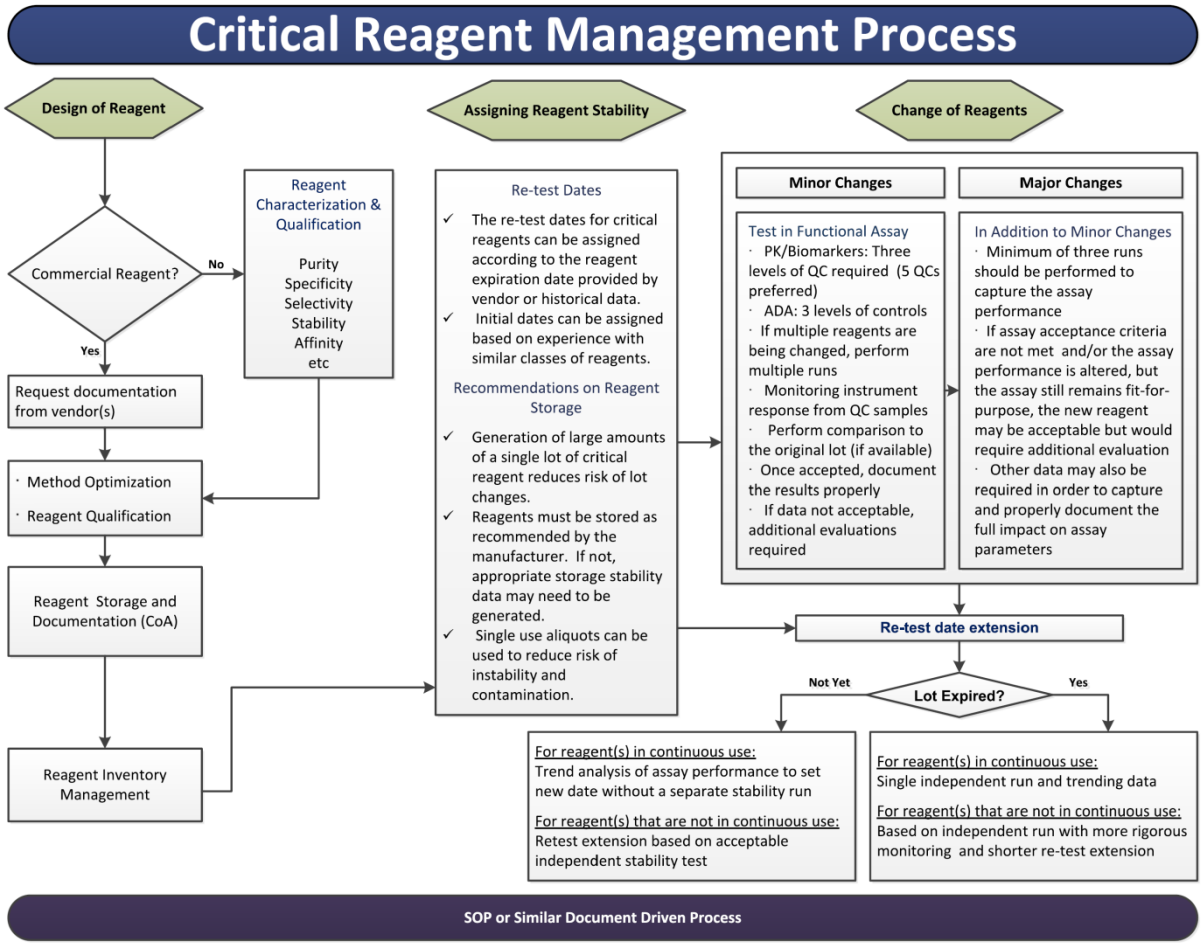



**Fig. 1.** Summary of critical reagent management process

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.

# Ideas for filling the gaps in the current recommendations

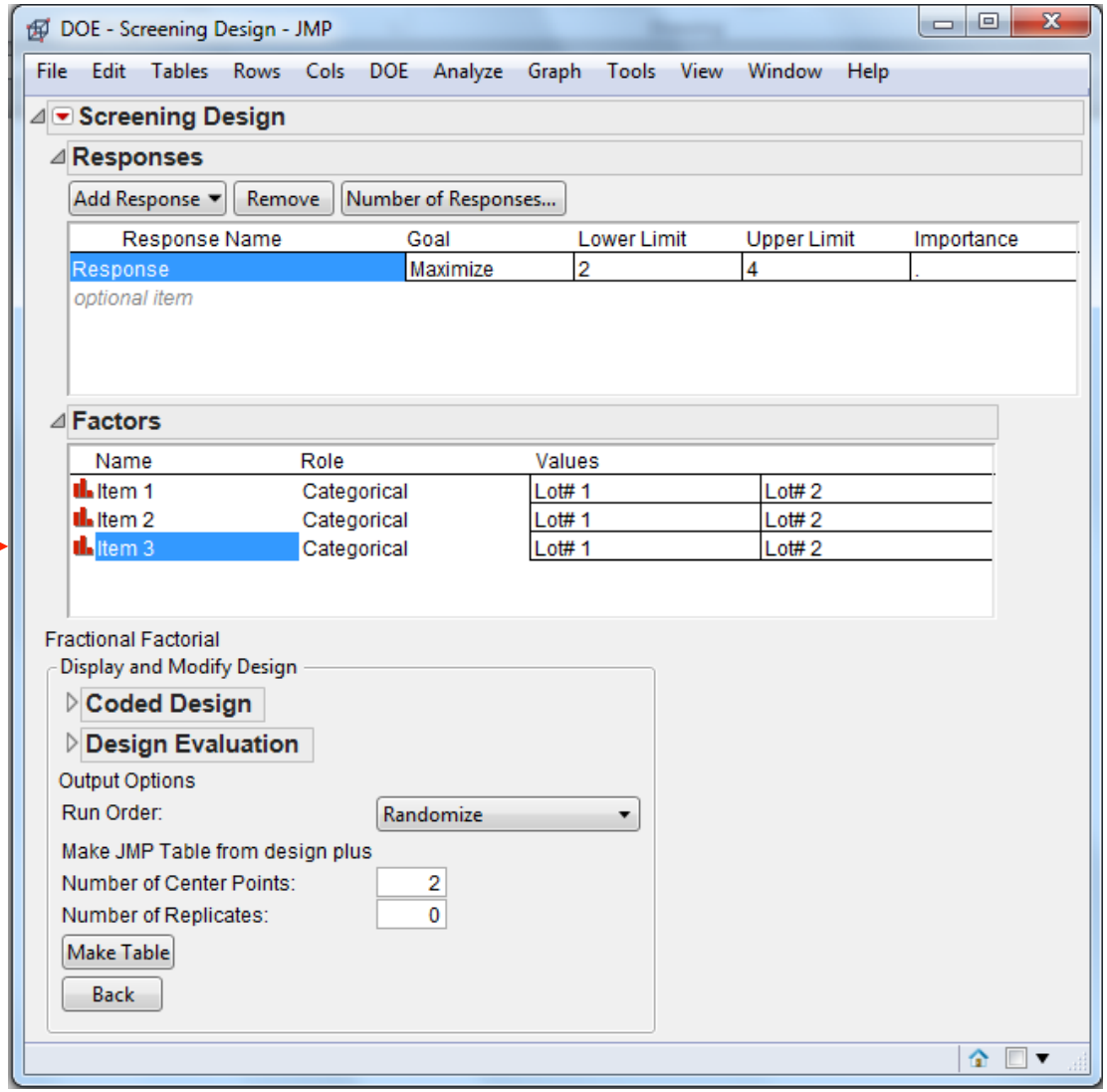
- Practical approach
- Trending – comparison to historical data
- What to do, if the acceptance criteria is not fulfilled
- Fit for purpose – tiered approach
- Identify the differences in acceptance criteria for PK, PD or ADA assays.

# 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
calculated
(can be performe
experiment)

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

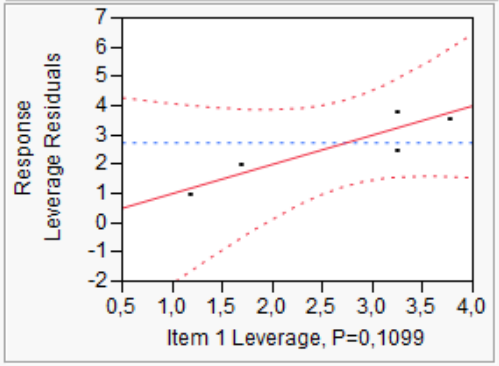
| Response |
|---|
| -1,39 |
| 3,92 |
| 6,99 |
| 5,67 |
| 1,05 |
| 0,15 |

Assay responses are inserted in the table

Effect of different lot# are analyzed

**Item 1**

**Leverage Plot**

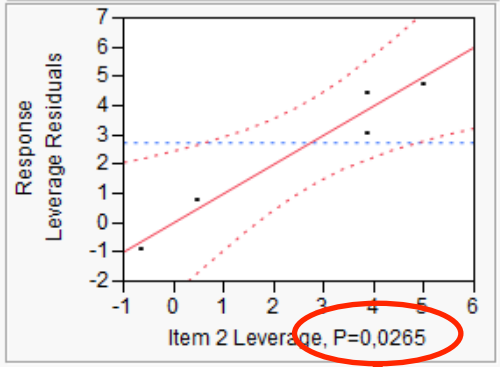



**Least Squares Means Table**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| Lot# 1 | 2,6700000 | 0,45899891 | 3,79750 |
| Lot# 2 | 0,6000000 | 0,59256505 | 0,60000 |

**Item 2**

**Leverage Plot**

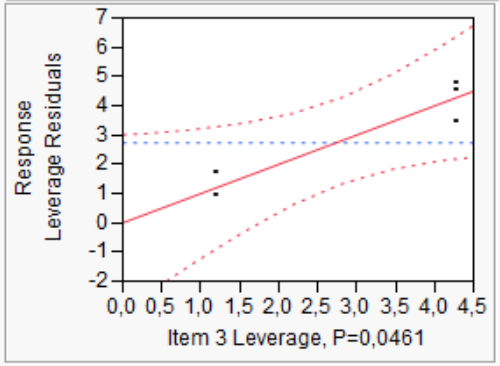



**Least Squares Means Table**

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| Lot# 1 | 3,890000 | 0,45899891 | 4,4075 |
| Lot# 2 | -0,620000 | 0,59256505 | -0,6200 |

**Item 3**

**Leverage Plot**




**Least Squares Means Table**

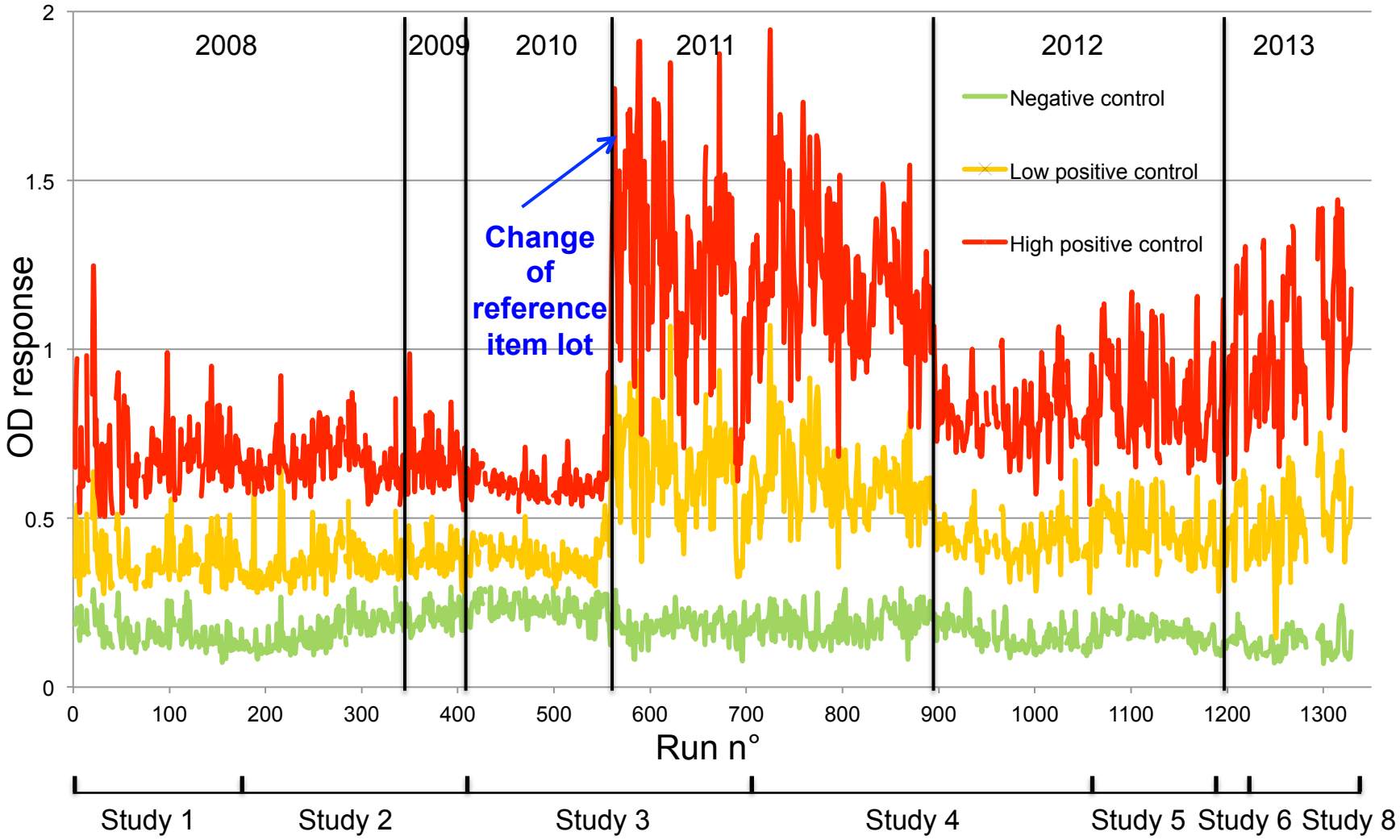
| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| Lot# 1 | 0,0966667 | 0,50744239 | 1,19333 |
| Lot# 2 | 3,1733333 | 0,50744239 | 4,27000 |

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:

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 fulfiled the run acceptance criteria.
- The OD of new and old controls were compared to each other; the defined OD criteria were not fulfilled. Mean $OD_{PCnew}$ deviated ≥ 20% from $OD_{PCold}$
- A new nomalization factor had to be determined

## ➢ Cut point calculation:

In this assay the Cut point is calculated using positive and negative control in this assay.

$$\text{Cut-point} = OD_{NC} + (K \times OD_{PC})$$

## ➢ 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 "$K \times OD_{PC}$" 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.

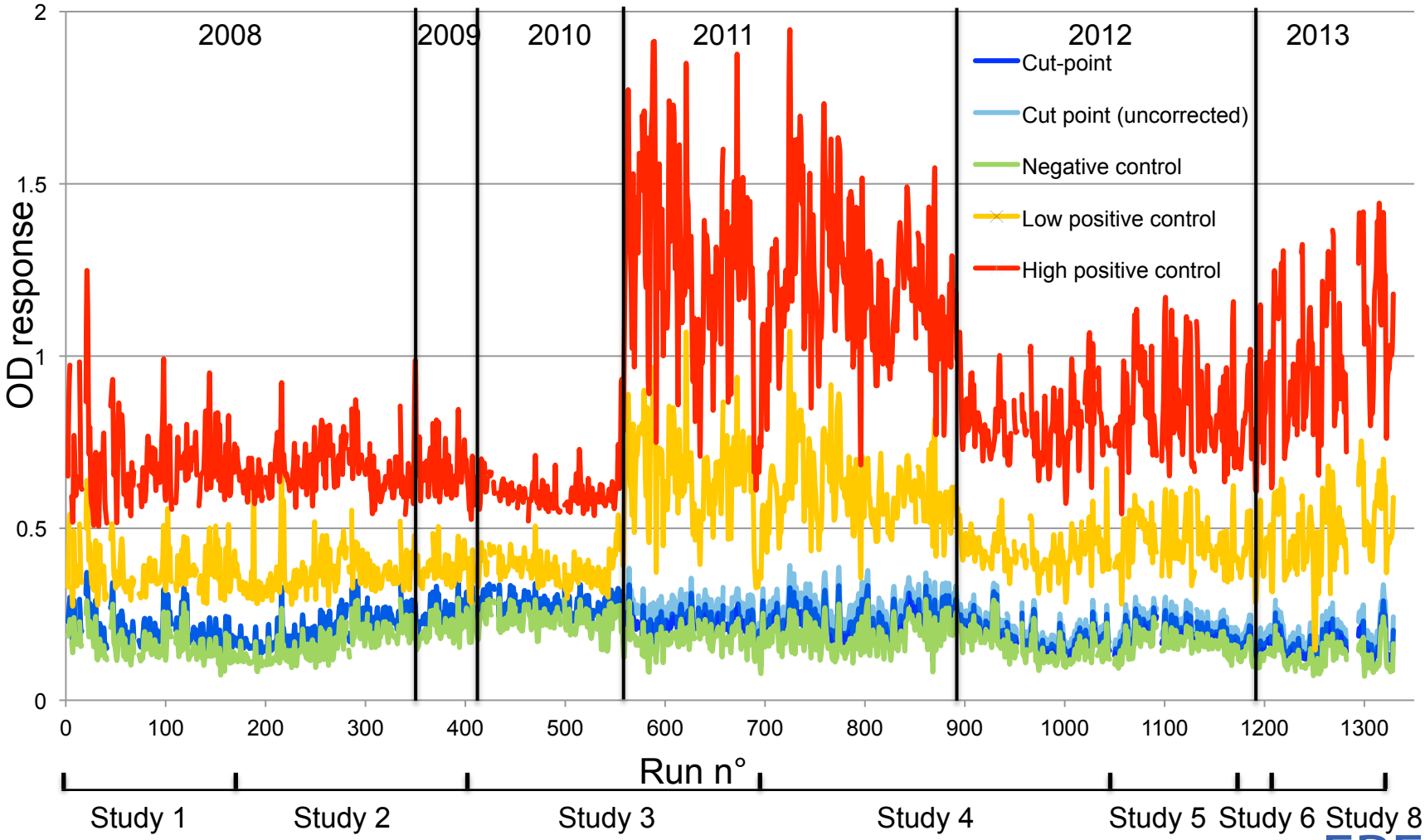
$$K_{(old)} \times OD_{PC(old)} = K_{(new)} \times OD_{PC(new)}$$

$$K_{(new)} = K_{(old)} \times OD_{PC(old)} / OD_{PC(new)}$$

$$K_{(new)} = 0.066 \times 0.601 / 1.225 = 0.033$$

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

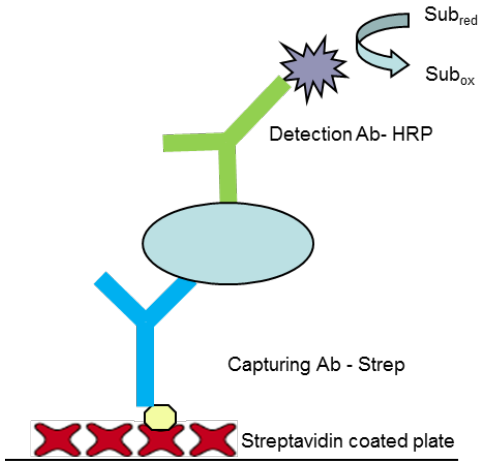
# Trending of Cut-point

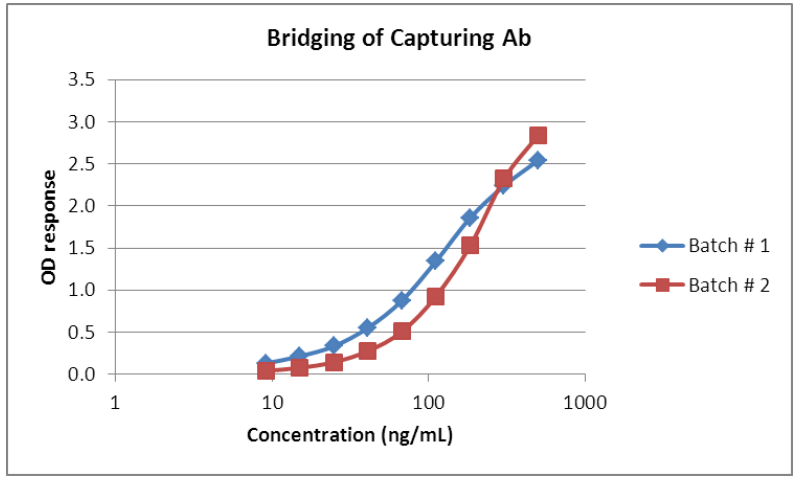


# Case study #3: PK assay

**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.



# 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.

# Acknowledgements


- **Topic team 47**
  - Caroline Laemmli (Celerion)
  - Ian Skitt (Covance)
  - Janka Ryding (Ferring)
  - Laurent Vermet (Sanofi)
  - Lydia Michaut (Novartis)
  - Martin Nemansky (PRA)
  - Ralf Loebbert (Abbvie)
  - Richard Hughes (LGC)

- Sponsor: Arjen Companjen (Crucell)

# 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)
- Geist et al: Characterization of critical reagents in ligand-binding assays: enabling robust bioanalytical methods and lifecycle management (2013)
- O'Hara and Theobald: Life cycle management of critical ligand-binding reagents (2013)
- Xu and Weant: Critical reagent stability for immunogenicity assays (2013)
- O'Hara et al: Ligand binding assays in the 21st century laboratory: Recommendation for characterization and supply of critical reagents (2012)
- 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)

# EBF TT-47