Validation of ADA Assays –

Recent experiences and issues

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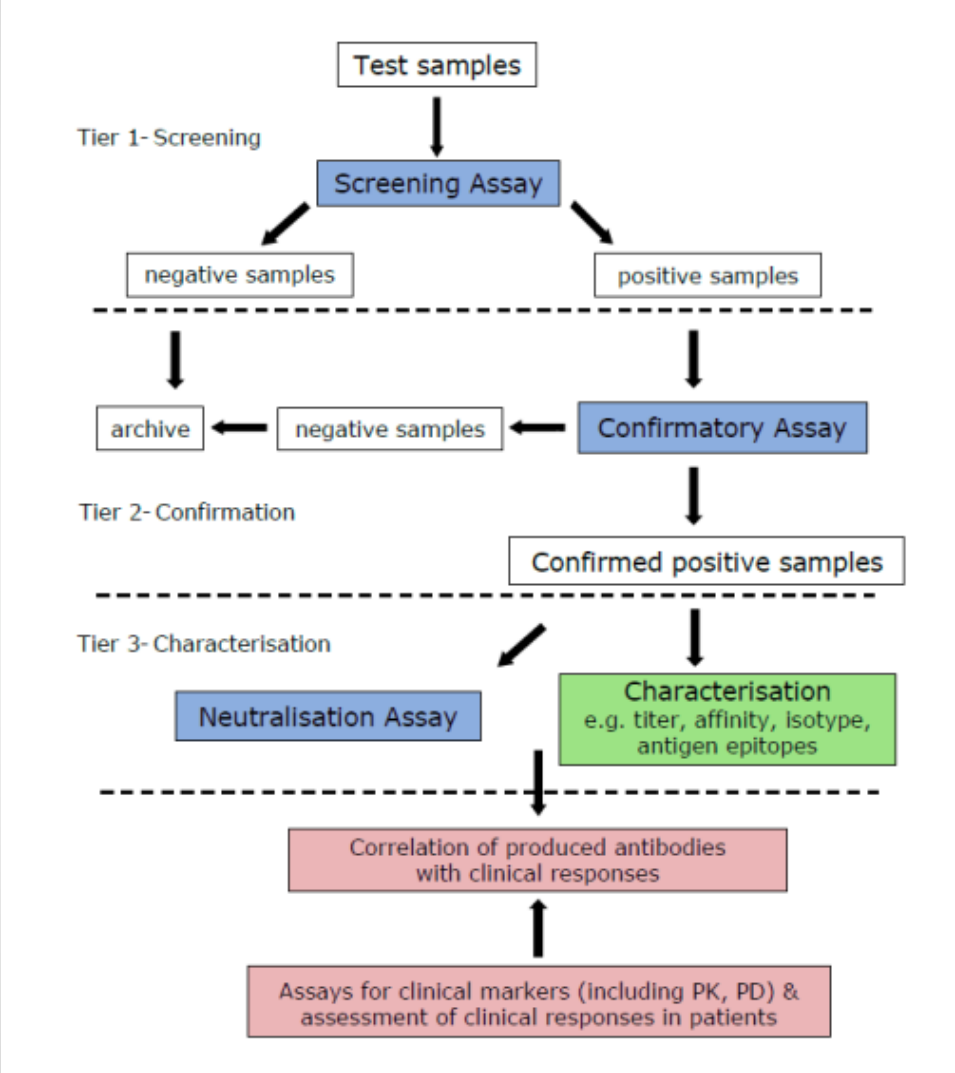
Who is who?

Tiered Immunogenicity Approach



from EMEA/CHMP/BMWP/14327/2006 from 01 Dec 2017

Challenges in ADA analysis

- Cut point determination (Outliers, Pre- versus in-study cut points)
- Assay controls (including NC, acceptance criteria)
- Pre-existing antibodies (anti-CCD, anti-PEG)
- Sensitivity
  - Screening assay
  - Confirmatory assay (spiking concentration)
- Drug Tolerance
- Target Tolerance

Standard Approach for ADA Validation

Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products

Gopi Shankar[a], Viswanath Devanarayan[b], Lakshmi Amaravadi[c], Yu Chen Barrett[d], Ronald Bowsher[e], Deborah Finco-Kent[f], Michele Fiscella[g], Boris Gorovits[h], Susan Kirschner[i,1], Michael Moxness[j], Thomas Parish[k], Valerie Quarmby[l], Holly Smith[m], Wendell Smith[n], Linda A. Zuckerman[o], Eugen Koren[p,*]

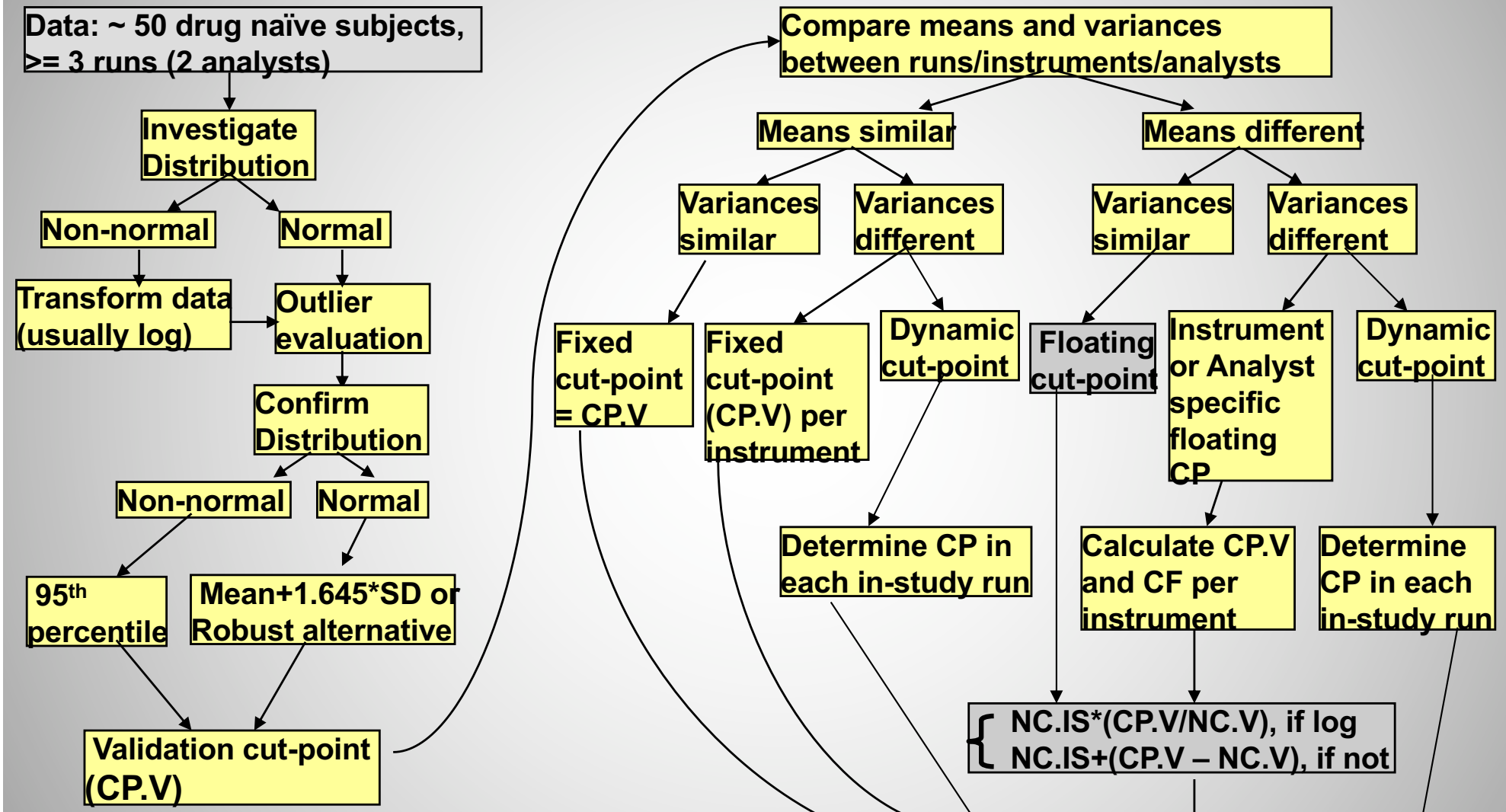
Guideline on Immunogenicity assessment of biotechnology-derived therapeutic proteins

Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products

Guidance for Industry

DRAFT GUIDANCE

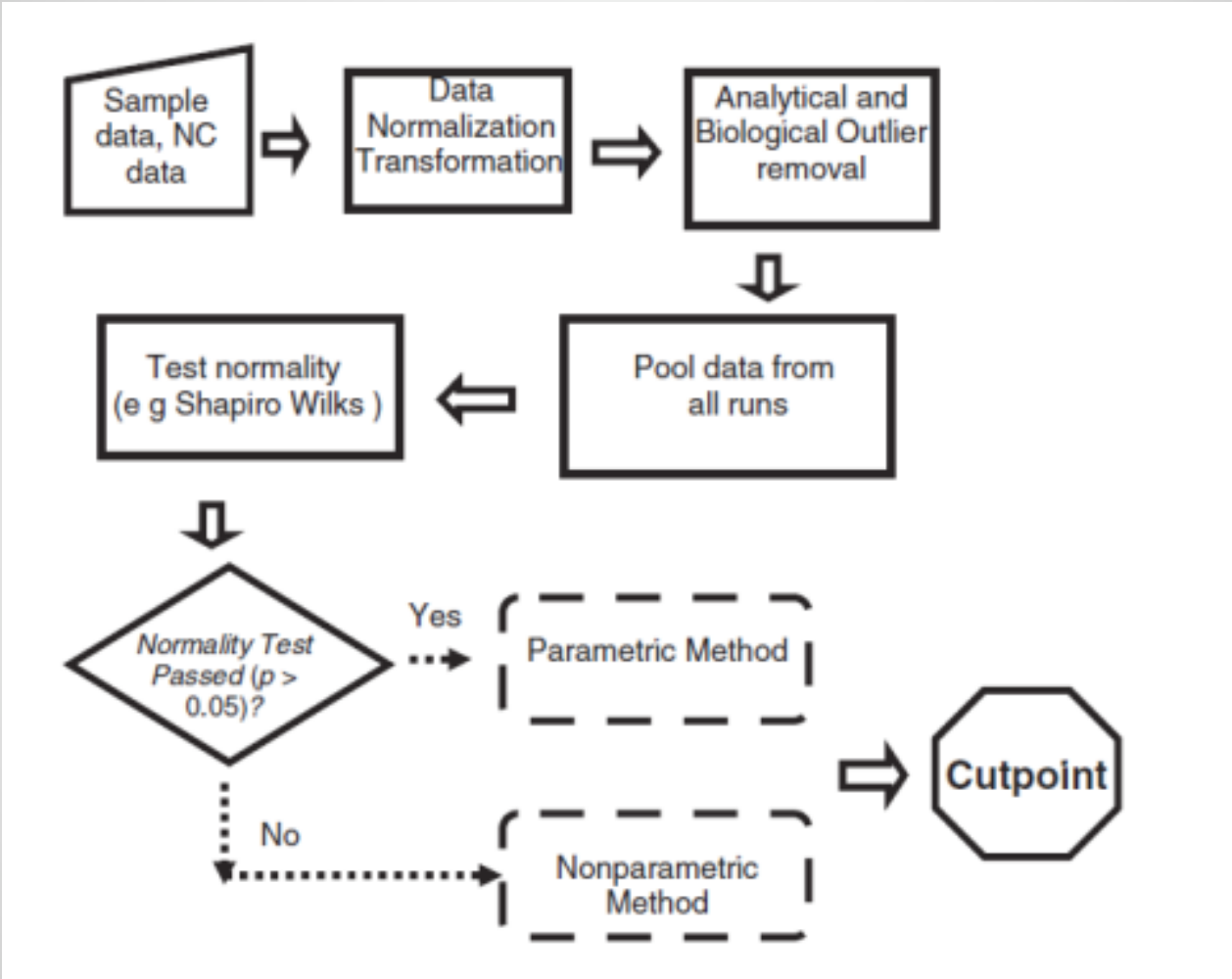
Cut Point Determination (Shankar et al)



NC.V = Neg. Control from Validation runs
NC.IS = Neg. Control from In-Study run

from Shankar et al, 2008

Cut Point Determination (Zhang et al)



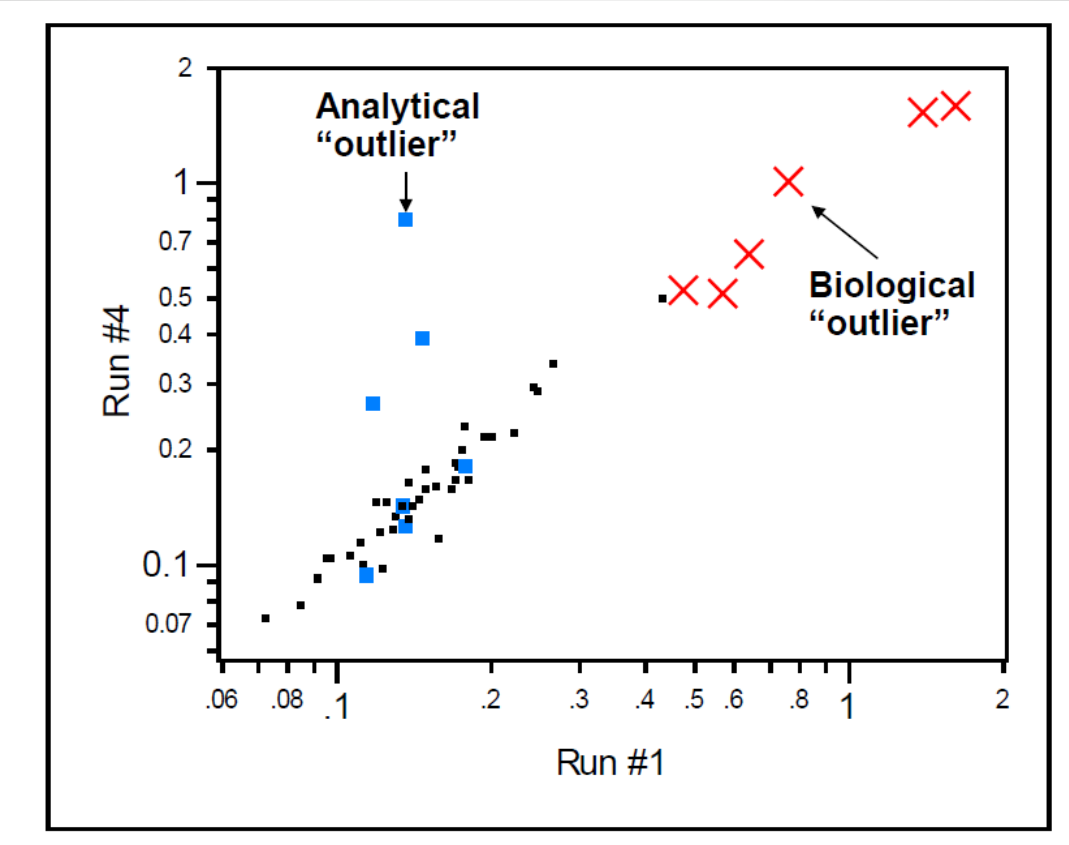
from Zhang et al, JIM, 389 (2013) 79–87

Steps for CP determination

- Sample data normalization by dividing by the average NC response on the same plate
- S/N ratio is obtained for each sample in each run
- Logarithmic transformation applied to all SN values
- Detect and removal of analytical outliers (Grubbs' test)
- Eliminate and delete biological outliers by Boxplot method with 3IQR
- Normality test (Shapiro-Wilks); parametric or nonparametric method
- Anti-log transformation to be applied to get the final cut point.

from Zhang et al, JIM, 389 (2013) 79–87

Analytical or Biological Outlier?



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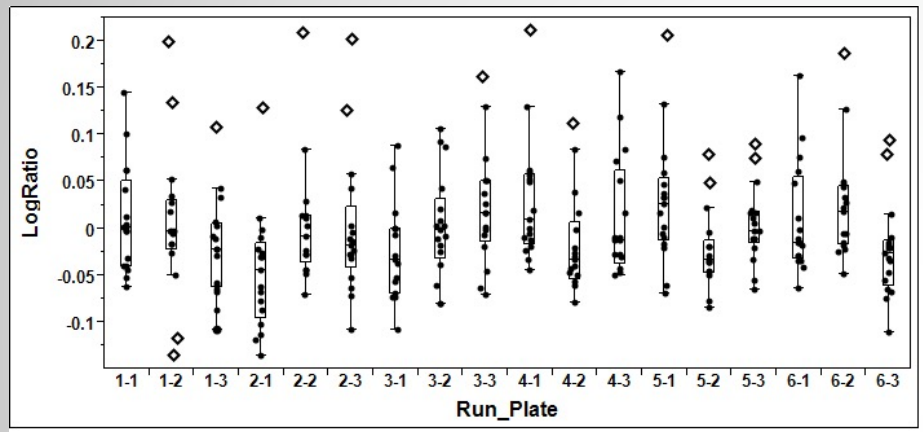
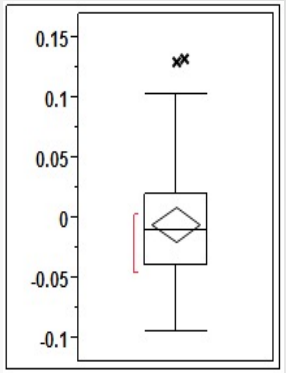
Sequential Outlier Elimination

1. Analytical Outliers



2. Biological Outliers



Subject averaged data

- Identify & exclude analytical outliers (AO) from each assay run/plate separately. Iterate until no more AO
- Then identify & exclude biological outliers (BO) by evaluating the distribution of subject averaged data. Iterate until no more BO.
- Then verify distribution of subject averaged data.

Pre-existing Antibodies

- EMA (2016):

  „***Some*** *individual's/patient's samples may contain pre-existing (pre-treatment) antibodies or possibly other substances which produce significant positive responses in assays, and so screening patients for this is necessary to ensure that post-treatment data can be interpreted correctly in terms of treatment emergent antibodies*."

- FDA (2016):

  Pre-existing antibodies may have clinical effects and may affect the efficacy of the therapeutic protein product being tested. An alternative to the qualitative screening assay approach may be needed to assess the quantity and quality of ADA when pre-existing antibodies are present. For example, testing samples for an increase in ADA using a semi-quantitative assay type such as a titering assay can provide information on the impact of a therapeutic protein product on product immunogenicity that is not provided by a qualitative assay.

Effect of normalized signals

		Analyst	Day	Plate	Residual	Total
ECL values	Variance	179.1	616.3	95.0	155.0	1045.5
	Percent	17%	59%	9%	15%	100%
Normalized ECL	Variance	0	0	0	0.0020	0.0010
	Percent	0	0	0	100%	100%

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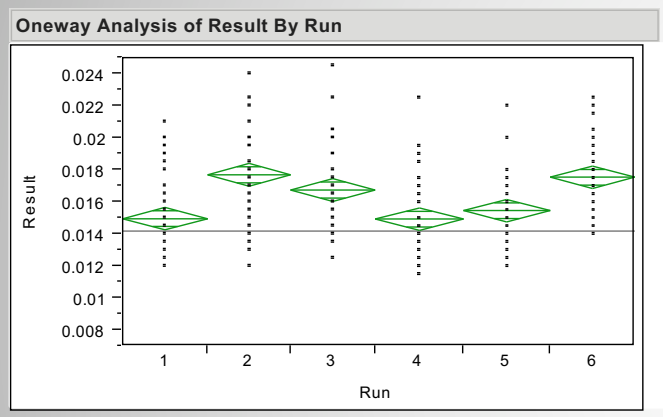
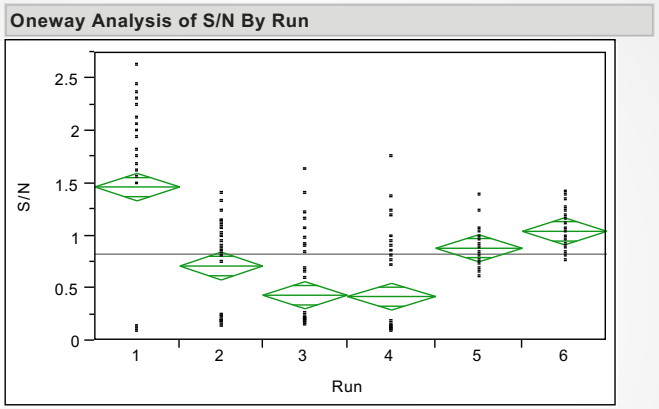
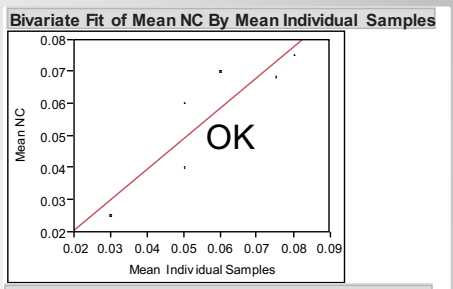
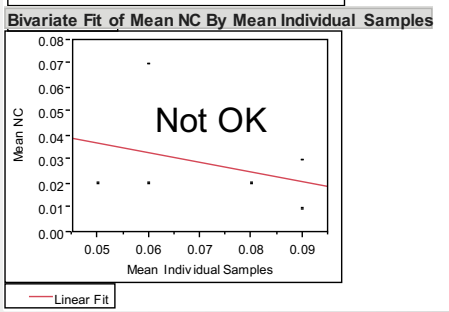
Suitability of the Negative Control

- Often a floating Cut-Point is used (e.g. log(S/N))
- However, normalization is not necessarily beneficial









- Check suitability of the negative control by plotting the mean response of the individual samples per run against the mean of the negative control of the same run
  - Normalization is not beneficial if the relationship has zero or negative slope value

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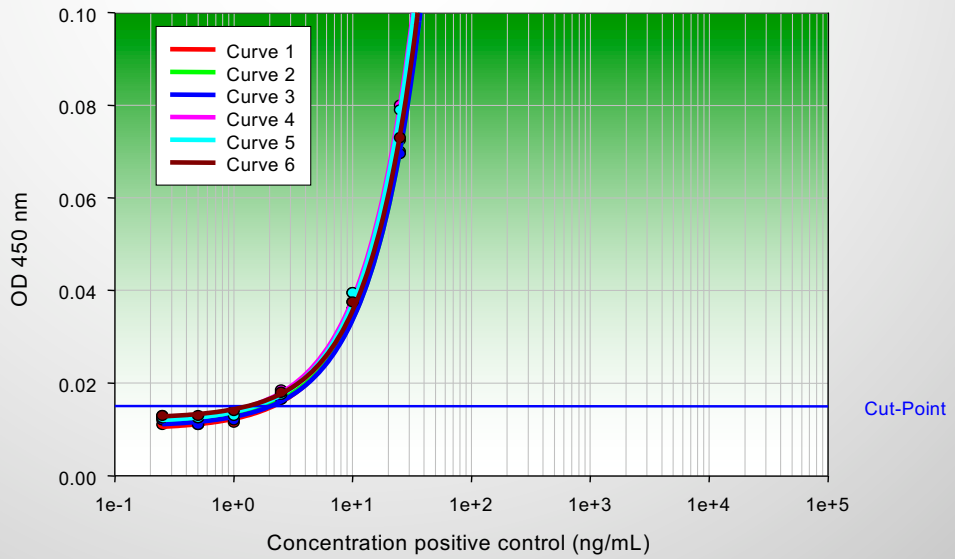
Acceptance Ranges for Assay Controls

- Shankar et al: Acceptance ranges for the controls are defined based on the variability (SD) of the assay
  - For the positive controls both upper and lower limits are defined as:
    mean response ± $t_{0.005}$,df ×SD
  - For the negative control an upper limit is defined as:
    mean response + $t_{0.01}$,df ×SD
- As the validation is performed in a limited timeframe the SD might not be representative for the variability throughout sample testing
  - This might lead to a significantly higher failure rate than the anticipated 1%
- Potential solutions.
  - Use of ratios (e.g. to NC) as acceptance ranges
  - Re-determine acceptance ranges once significant assay failures are experienced

Sensitivity

- Sensitivity *per se* is highly dependent on the positive control reagent(s)
- Assay sensitivity represents the lowest concentration at which the antibody preparation consistently produces a positive result.
- Shankar: Mean of the interpolated positive control at least 6 assay runs plus $t_{0.05,df}$×SD
- FDA: Sensitivity should be at least 100 ng/ml as such antibody concentrations may be associated with clinical events



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Sensitivity – Issues (I)

- Calculated LPC may not test positive during precision runs
  - Potential solution: Test calculated LPC + at least one higher concentration during precision runs
    - Sensitivity = lowest concentration that tests positive in all precision runs (or all runs during validation)

LPC (25 ng/ml)					Intrabatch statistics	
Operator	Batch-no.	Mean OD (n=2)			Mean OD (n=3)	CV (%)
1	1	0.021	0.02	0.019	0.02	5.00
1	2	0.005	0.003	0.004	0.004	25.0
1	3	0.036	0.034	0.038	0.036	5.56
1	4	0.003	0.003	0.002	0.003	21.7
1	5	0.011	0.009	0.012	0.011	14.3
1	6	0.04	0.039	0.041	0.040	2.50
2	7	0.041	0.036	0.042	0.040	8.10
2	8	0.036	0.034	0.038	0.036	5.56
2	9	0.021	0.020	0.019	0.020	5.00
2	10	0.001	0.001	0.003	0.002	69.3
2	11	0.033	0.036	0.038	0.036	7.06
2	12	0.003	0.003	0.004	0.003	17.3
Overall statistics					Interbatch statistics	
Mean (n=54)		0.021			0.021	
CV (%)		74.7			76.6	
Cut-point		0.015				

Below screening cut-point

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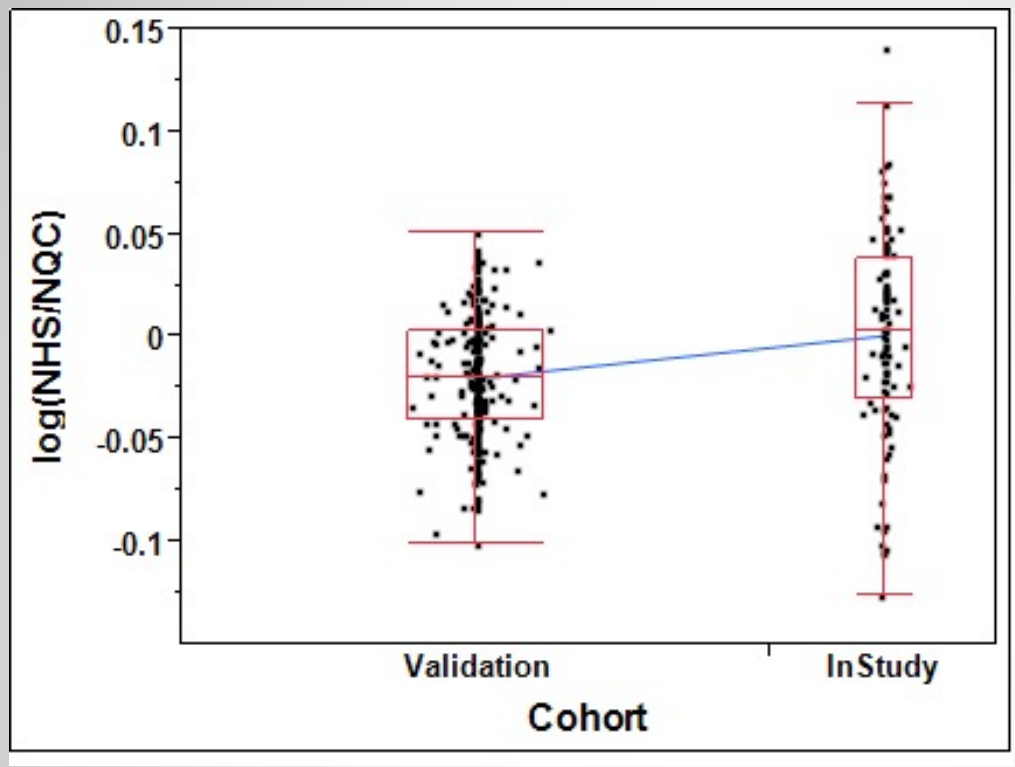
Sensitivity of confirmatory assay

- Sensitivity is usually determined on the screening assay level
- However, the sensitivity of the confirmatory assay might be lower (different) than the sensitivity of the screening assay

Sensitivity Screening Assay	Sensitivity Confirmatory Assay
25 ng/mL	33 ng/mL

- As only samples testing positive in both screening and confirmatory assay will be defined as “positive” it might be misleading to define assay sensitivity only based on the screening assay
- Proposal: report both sensitivities (screening & confirmatory assay)
- FDA also recommends that the sensitivity of the confirmatory assay be confirmed using a low concentration of the positive control antibody

Pre-study versus in-study cut point



- SCP factor from validation data ~ 1.1
- Variability of in-study data significantly higher, $p<0.0001$
- Results in > 30% false positive rate of clinical baseline samples

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Pre-study versus in-study cut point

- New screening cut-point based on pre-treatment samples require re-assessment of several validation parameter:
  - Confirmatory cut-point
  - Sensitivity
  - LPC

Specificity

- FDA: Specificity refers to the ability of a method to detect ADA that bind the therapeutic protein product but not assay components such as surfaces or reagents.

- PK guidances: Specificity is related to the concept of cross-reactivity with structurally related compounds
  - endogenous compounds, isoforms, variants forms of the analyte, or physico-chemically similar compounds)
  - concomitant medication.

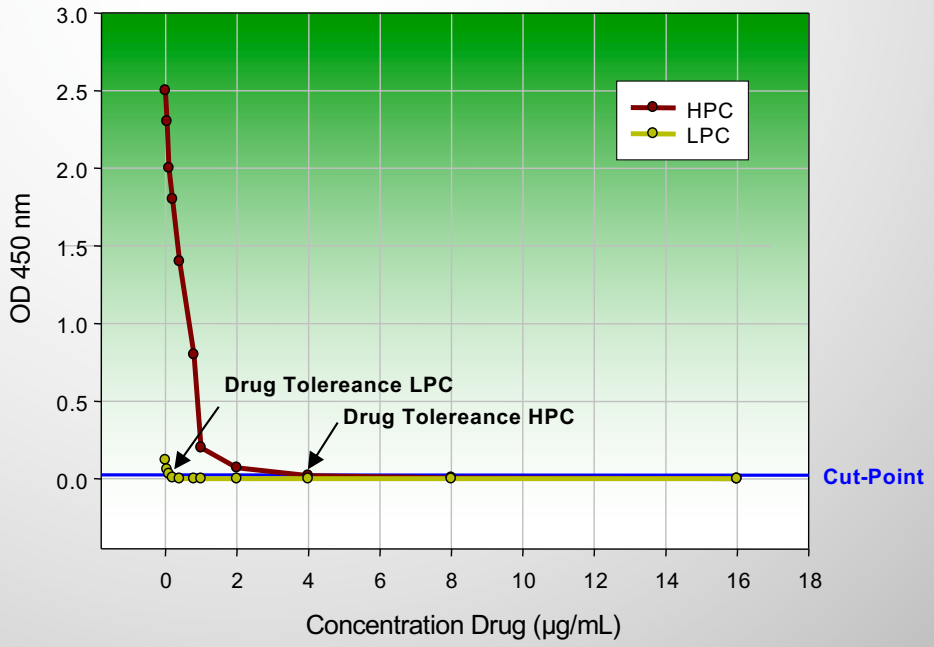
Selectivity

- FDA: The selectivity of an ADA assay is its ability to identify therapeutic protein product-specific ADA in a matrix that may contain potential interfering substances.

- PK guidances: Selectivity is the ability to measure the analyte of interest in the presence of unrelated compounds, e.g.
  - degrading enzymes
  - heterophilic antibodies or rheumatoid factor
  - Lipemic and haemolysed samples

Drug Tolerance

- Potential for interference by the drug present in the serum
- Effect of various concentrations of study drug on the HPC, MPC and LPC should be tested.
- More challenging with ADA sensitivities as low as 10 ng/ml.



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Thank you

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