Major Challenges in the Assessment of Immunogenicity

EIP Training Course

17 Feb 2020

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Challenges in ADA analysis

- Cut point determination
  - Outlier depletion
  - In-study cut points
- Assay controls (including NC, acceptance criteria)
- Pre-existing antibodies (anti-CCD, anti-PEG)
- Sensitivity
  - Screening assay
  - Confirmatory assay
- Drug Tolerance
- Target Interference
- „Fit for purpose“ ADA assays for non-pivotal clinical trials
- Critical reagents for ADA assays

Cut-Points – Outlier Removal

- FDA:
  - To consider the impact of statistically determined outlier values and true-positive samples when establishing the cut-point
  - To provide justification for the removal of any data points, along with the respective method used to determine their status as outliers
- Outliers can be grouped in analytical and biological outliers
  - Analytical outliers occur if there are one or more aberrant signal among all those from the same individual sample
  - A biological outlier refers to an individual sample. All values of this sample tend to be aberrantly lower or higher than values from other individual samples

Cut-Points – Outlier Removal

- Common approach is to eliminate analytical outliers before biological ones
- Different methods to identify outliers are used
  - Grubbs outlier test for analytical outliers followed by Box Plots on means per subject (biological outliers)
  - Mixed effect ANOVA model
  - Box Plots on subject level residuals (analytical outliers) and means per subject (biological outliers)

Analytical Outlier Removal – Grubbs Test

- Analytical outliers occur if there are one or more aberrant log (S/N) values among all those from the same subject
- Analytical outliers are removed before the exclusion of biological outliers in the following way:
  - The log (S/N) ratios of the same subject from the six assay runs are evaluated
  - An outlier test accommodating the small sample size (n=6) like Grubbs test is used
  - This is conducted through each subject (n=50) and identified analytical outliers are removed

Run	Log (S/N)	Grubbs Z Value	Significant Outlier
1	0.034	0.37435	No
2	0.041	0.08319	No
3	0.029	0.58232	No
4	0.091	1.99654	YES
5	0.027	0.66551	No
6	0.036	0.29116	No

⇐ Analytical outlier: To be removed

Downside: Is an oulier test really valid for only 6 values???

Biological Outlier Removal – Box Plots

- A biological outlier occurs if the mean log(S/N) value of a subject is aberrantly larger or smaller than mean values of other subjects
  - For each Subject the mean log (S/N) is calculated using values from all runs after analytical outliers are removed
  - The boxplot method is applied to all subject mean log (S/N) values
    - Upper criterion: 75th Percentile + 1.5 x (75th Percentile – 25th Percentile)
    - Lower criterion: 25th Percentile – 1.5 x (75th Percentile – 25th Percentile)
- The results from identified biological outliers are removed from all assay runs

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Mean
Individual Sample 1	0.012	0.014	0.013	0.011	0.016	0.01	0.013
Individual Sample 2	0.014	0.012	0.016	0.018	0.015	0.014	0.015
Individual Sample 3	0.011	0.013	0.022	0.018	0.019	0.018	0.017
Individual Sample 4	0.02	0.021	0.019	0.02	0.018	0.019	0.020
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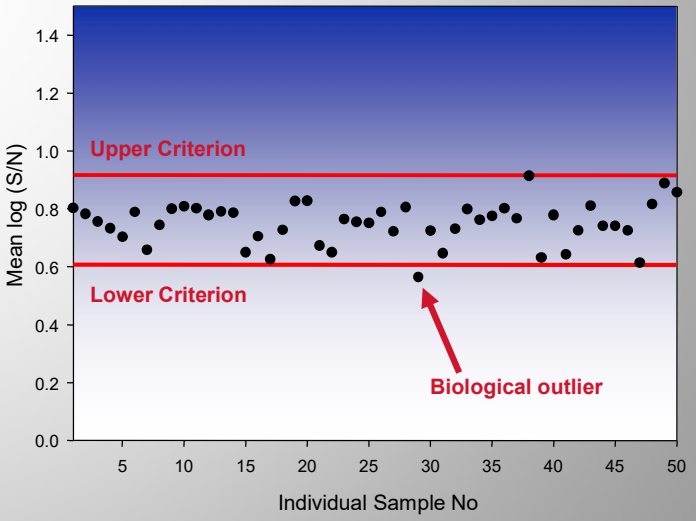
Individual Sample 50	0.011	0.016	0.018	0.011	0.017	0.011	0.014



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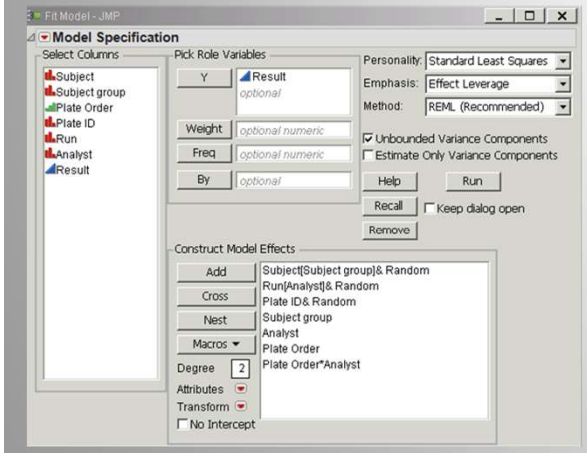
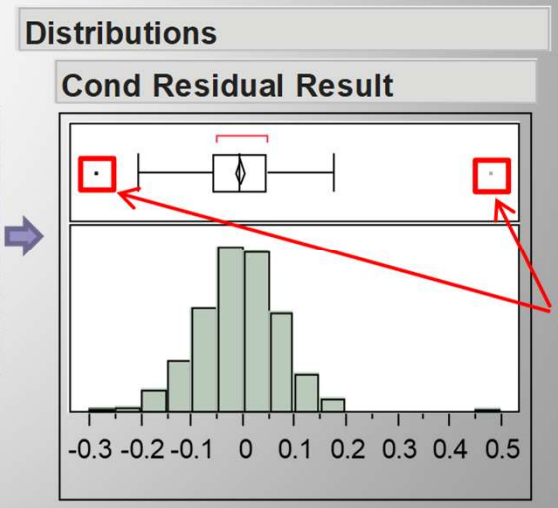
Analytical Outlier Removal – Mixed Effect ANOVA

1. Fit a mixed-effects model on the log (S/N) values
    1. Random effects: Subjects nested within Subject Groups, Run number nested under Analyst, and Plate ID
    2. Fixed effects: Subject Groups, Analyst, Plate testing order, Subject Group x Analyst Interaction
2. Obtain conditional residuals from this model.
3. Use the “outlier box-plot” criteria to identify and remove outliers from the conditional residuals



	Subject	Subject group	Plate Order	Plate ID	Run	Analyst	Result	Cond Residual Result
1	1	1	1	a	1	1	0.8	-0.010932896
2	2	1	1	a	1	1	1.4	0.0897719036
3	3	1	1	a	1	1	0.9	-0.082119822
4	4	1	1	a	1	1	0.9	0.0034736414
5	5	1	1	a	1	1	0.8	-0.096526359
6	6	1	1	a	1	1	0.7	-0.082401741
7	7	1	1	a	1	1	0.9	-0.096385399
8	8	1	1	a	1	1	0.9	0.0177392185
9	9	1	1	a	1	1	0.8	0.0175982587
10	10	1	1	a	1	1	1.4	0.0755063264
11	11	1	1	a	1	1	1.3	0.0610997894
12	12	1	1	a	1	1	0.9	0.0462703729



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Biological Outlier Removal – Mixed Effect ANOVA

1. Refit the ANOVA model without these analytical outliers and obtain Best Linear Unbiased Predictor (BLUP) for each individual sample
2. Use the “outlier box-plot” criteria to identify and remove outliers from BLUPs (biological outliers)

Example_jmp_ANAova_model - Fit Least Squares 2 - JMP

Response Result

Whole Model

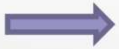
Actual by Predicted Plot

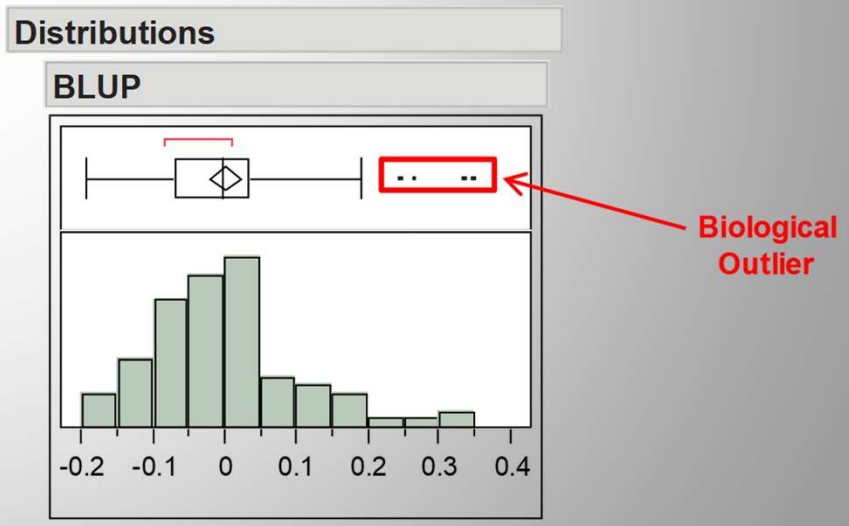
Summary of Fit

Parameter Estimates

Random Effect Predictions

Term	BLUP	Std Error	DFDen	t Ratio	Prob>|t|
Subject group[1]:Subject[1]	-0.099813	0.034601	156.6	-2.88	0.0045*
Subject group[1]:Subject[3]	0.0816794	0.034601	156.6	2.36	0.0195*
Subject group[1]:Subject[4]	0.0277546	0.036339	168.5	0.76	0.4461
Subject group[1]:Subject[5]	-0.009067	0.034601	156.6	-0.26	0.7936
Subject group[1]:Subject[6]	-0.130062	0.034601	156.6	-3.76	0.0002*
Subject group[1]:Subject[7]	0.0968038	0.034601	156.6	2.80	0.0058*
Subject group[1]:Subject[8]	-0.024191	0.034601	156.6	-0.70	0.4855
Subject group[1]:Subject[9]	-0.130062	0.034601	156.6	-3.76	0.0002*
Subject group[1]:Subject[12]	-0.05444	0.034601	156.6	-1.57	0.1177
Subject group[1]:Subject[13]	-0.021815	0.036339	168.5	-0.60	0.5491
Subject group[1]:Subject[14]	0.0816794	0.034601	156.6	2.36	0.0195*
Subject group[1]:Subject[15]	0.2177987	0.034601	156.6	6.29	<.0001*
Subject group[1]:Subject[16]	0.0514307	0.034601	156.6	1.49	0.1392
Subject group[1]:Subject[17]	0.0514307	0.034601	156.6	1.49	0.1392
Subject group[1]:Subject[18]	-0.069564	0.034601	156.6	-2.01	0.0461*
Subject group[1]:Subject[20]	-0.069564	0.034601	156.6	-2.01	0.0461*
Subject group[2]:Subject[21]	-0.135823	0.033565	175.6	-4.05	<.0001*
Subject group[2]:Subject[22]	0.0305454	0.033565	175.6	0.91	0.3641
Subject group[2]:Subject[23]	-0.014828	0.033565	175.6	-0.44	0.6592
Subject group[2]:Subject[24]	-0.135823	0.033565	175.6	-4.05	<.0001*
Subject group[2]:Subject[25]	0.0910428	0.033565	175.6	2.71	0.0073*
Subject group[2]:Subject[26]	0.0795184	0.035366	187.4	2.25	0.0257*
Subject group[2]:Subject[27]	-0.045076	0.033565	175.6	-1.34	0.1810
Subject group[2]:Subject[28]	0.1212916	0.033565	175.6	3.61	0.0004*
Subject group[2]:Subject[29]	-0.060201	0.033565	175.6	-1.79	0.0746
Subject group[2]:Subject[30]	0.1212916	0.033565	175.6	3.61	0.0004*

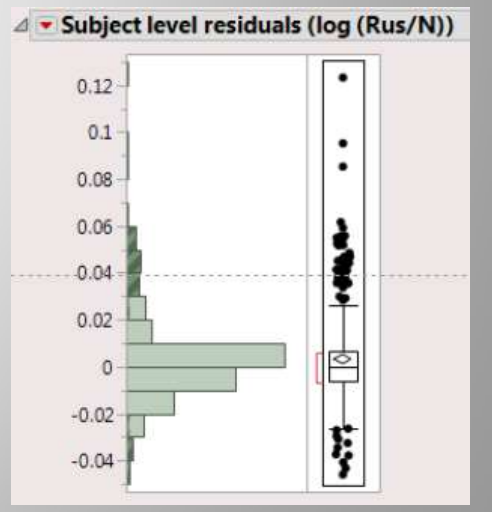




Analytical Outlier Removal – Subject Level Residuals

- Subject level residuals are defined as the absolute value obtained by subtracting each individual (n=6) log(S/N) result from its median log(S/N) result
- Analytical outliers are eliminated from all subject level residuals (n=50 subjects x 6 runs = 300)

Subject	Run	log (S/N)	Median (log (S/N))	Subject level residuals (log (S/N))
1	1	0,0003	-0,0354	0,0357
2	1	-0,0024	-0,0025	0,0001
3	1	0,0184	0,0358	-0,0174
4	1	0,0391	0,0035	0,0356
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
50	6	.	.	.



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In-Study Cut-Point

- FDA:
  - …it is necessary to confirm that the cut-point determined during assay validation is suitable for the population being studied
- It is common practise to establish „in-study cut-points“ if the observed ADA incidence in study pre-treatment samples is outside the 2-11 % window
- However, there is no harmonized approach of how to establish in-study cut-points
  - How many pre-treatment samples tested how many times?
    - 50 samples 6 times as during validation?
  - How to deal with dependent validation parameter (sensitivity; LPC; precision; drug tolerance) if in-study cut-points are significantly diiferent?

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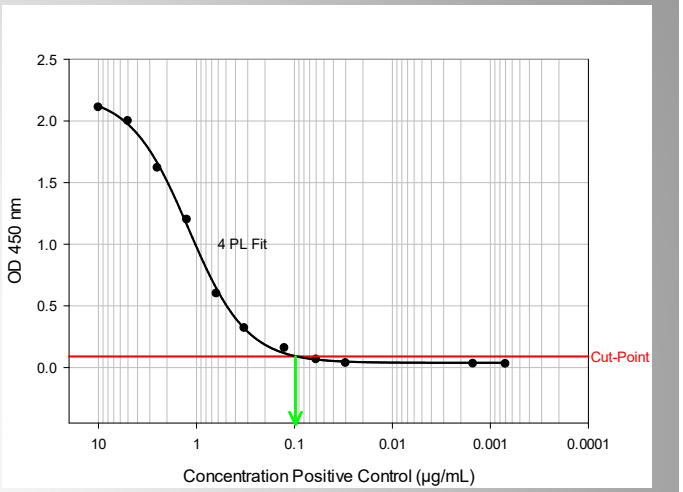
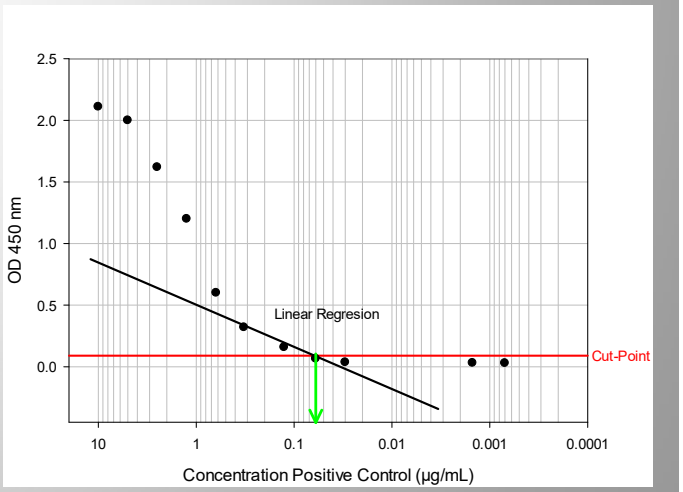
Determination of Assay Sensitivity

- The sensitivity can be calculated by interpolating the linear portion of the dilution curve to the assay cut-point
- The dilution series should be no greater than two- or threefold, and a minimum of five dilutions should be tested
- Interpolation unclear
  - 4PL or 5PL fit
  - Linear regression (how many points)
- To report
  - the mean concentration at the cut-point which will lead to a 50 % failure rate of this concentration
  - Mean + $t_{0.01}$ x SD guarantees a failure rate of 1 %





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Final EMA Immunogencity Guideline, 2017

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

18 May 2017
EMEA/CHMP/BMWP/14327/2006 Rev 1
Committee for Medicinal Products for Human Use (CHMP)

Guideline on Immunogenicity assessment of therapeutic proteins

EMA Immunogenicity Guideline, 2017

The Applicant has to demonstrate that the tolerance of the assay to the therapeutic exceeds the levels of the therapeutic protein in the samples for ADA testing. Due to technical limitations it may not be always possible to develop fully tolerant assays. If this occurs, the best possible assay should be employed and the approach taken should be properly justified.

EMA Immunogenicity Guideline, 2017

10. Summary of the immunogenicity program

The risk-based immunogenicity program

5. Assay strategy

a. Rationale for the choice of assays i. screening, confirmation, and titration

ii. neutralizing

iii. other, e.g. immunoglobulin class, sub-class

b. Specificity and sensitivity of the selected assays in the context of the particular product

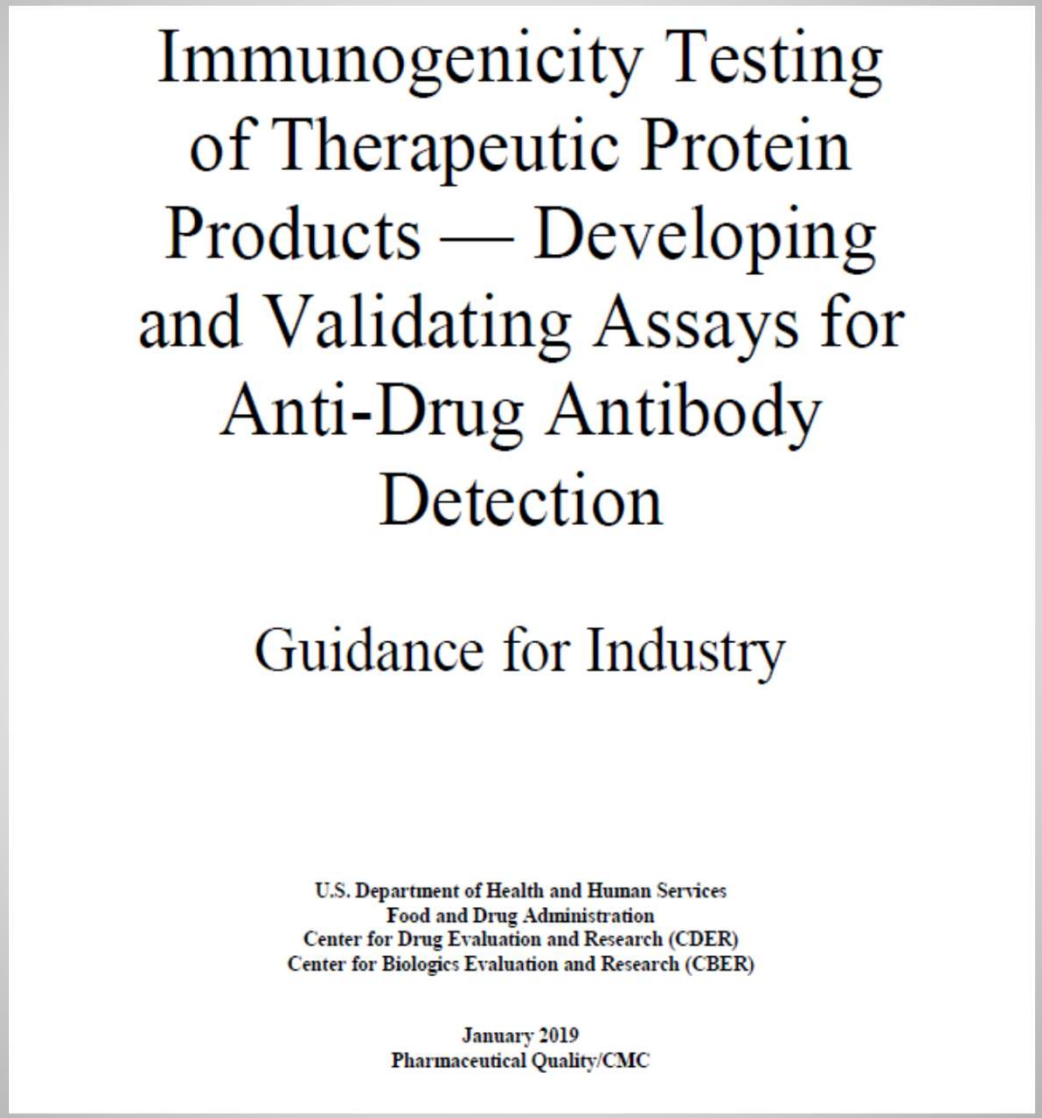
i. selection of the positive control(s)

ii. determination of the threshold for ADA-positivity

c. **Drug and target tolerance** of the assay

d. Matrix interference in different populations

FDA Immunogencity Guidance, 2019



Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

January 2019
Pharmaceutical Quality/CMC

FDA Immunogencity Guidance
- Drug Tolerance -

IV. ASSAY DESIGN ELEMENTS

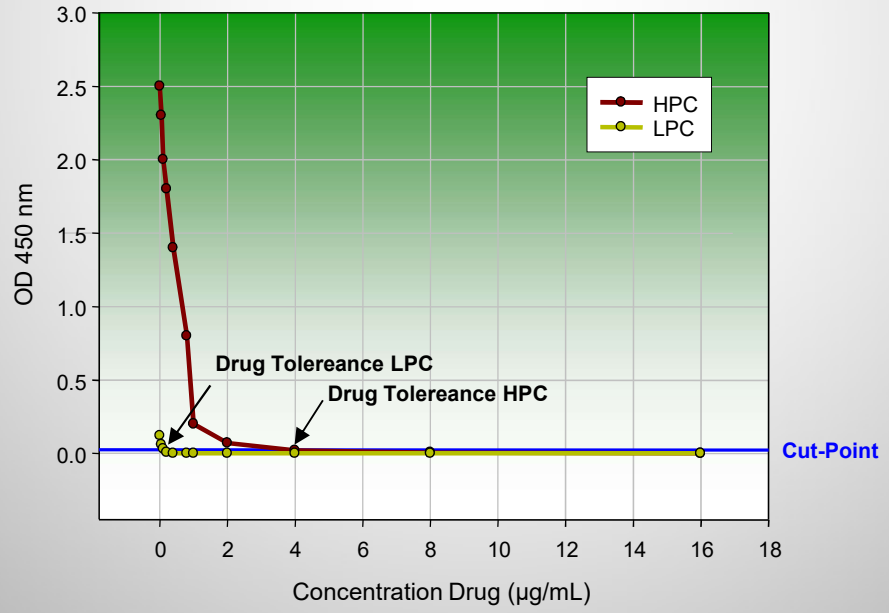
C. Sensitivity

2. Drug Tolerance, Sensitivity, and Assay Suitability

The therapeutic protein product or its endogenous counterpart present in the serum may interfere with the sensitivity of the assay. The assessment of assay sensitivity in the presence of the expected levels of interfering therapeutic protein product, also known as the assay's drug tolerance, is critical to understanding the sensitivity and suitability of the method for detecting ADA in dosed subjects. FDA recommends that sponsors examine assay drug tolerance early in assay development. The sponsor may examine drug tolerance by deliberately adding different known amounts of positive control antibody into ADA-negative control samples in the absence or presence of different quantities of the therapeutic protein product to determine whether the therapeutic protein product interferes with ADA detection. Results obtained in the absence and presence of different quantities of the therapeutic protein product under consideration should be compared. Drug tolerance may be improved using approaches such as acid dissociation that disrupt circulating ADA-drug complexes.

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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FDA Immunogencity Guidance
- Target Interference -

C. Sensitivity

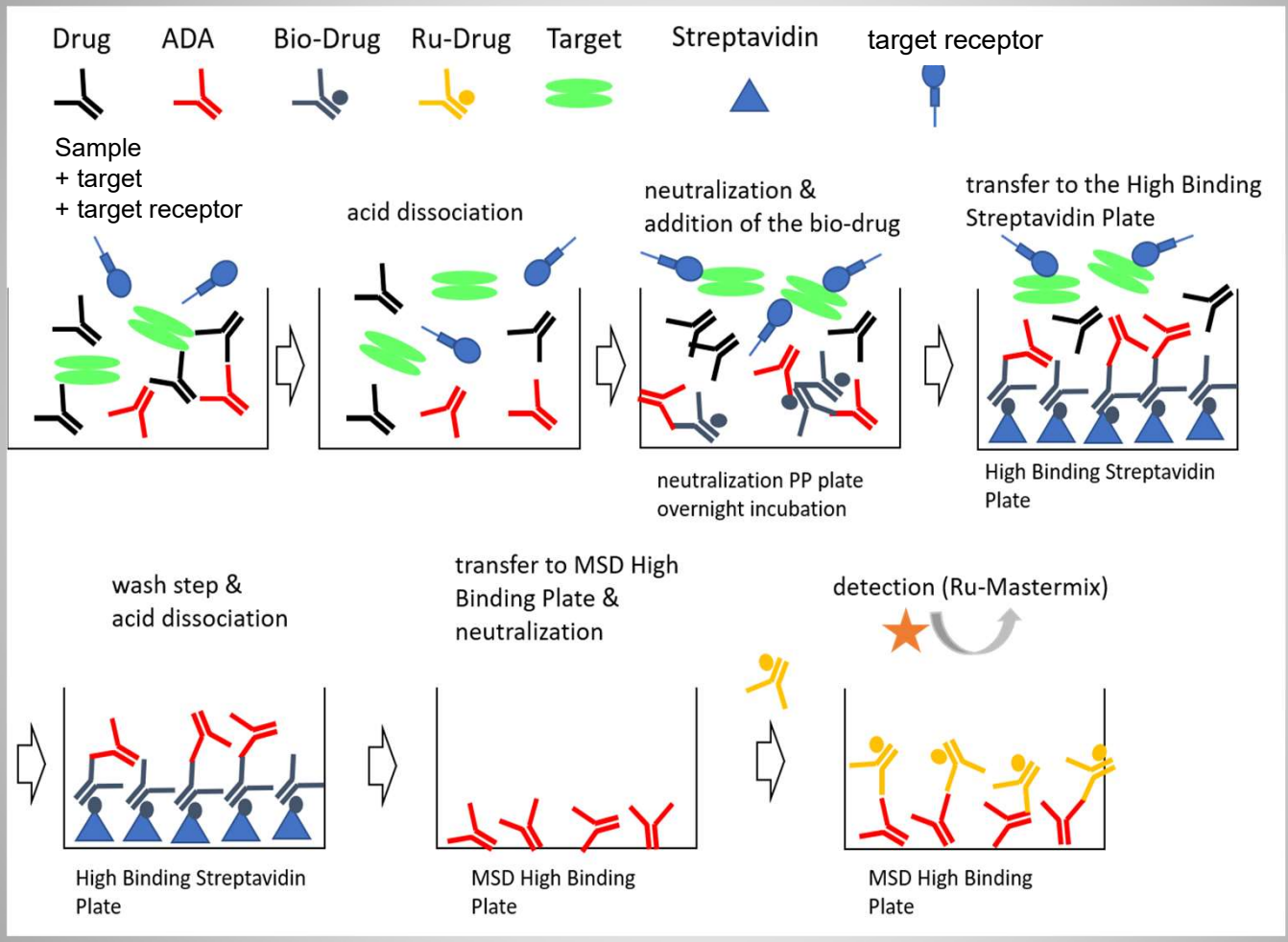
2. Drug Tolerance, Sensitivity, and Assay Suitability

The selectivity of the assay, the nature of the target, and the type of positive control should be taken into consideration when developing the assay because these factors impact the assessment of drug tolerance. For example, acid dissociation may not be appropriate when antibodies are acid labile or the drug target is soluble.

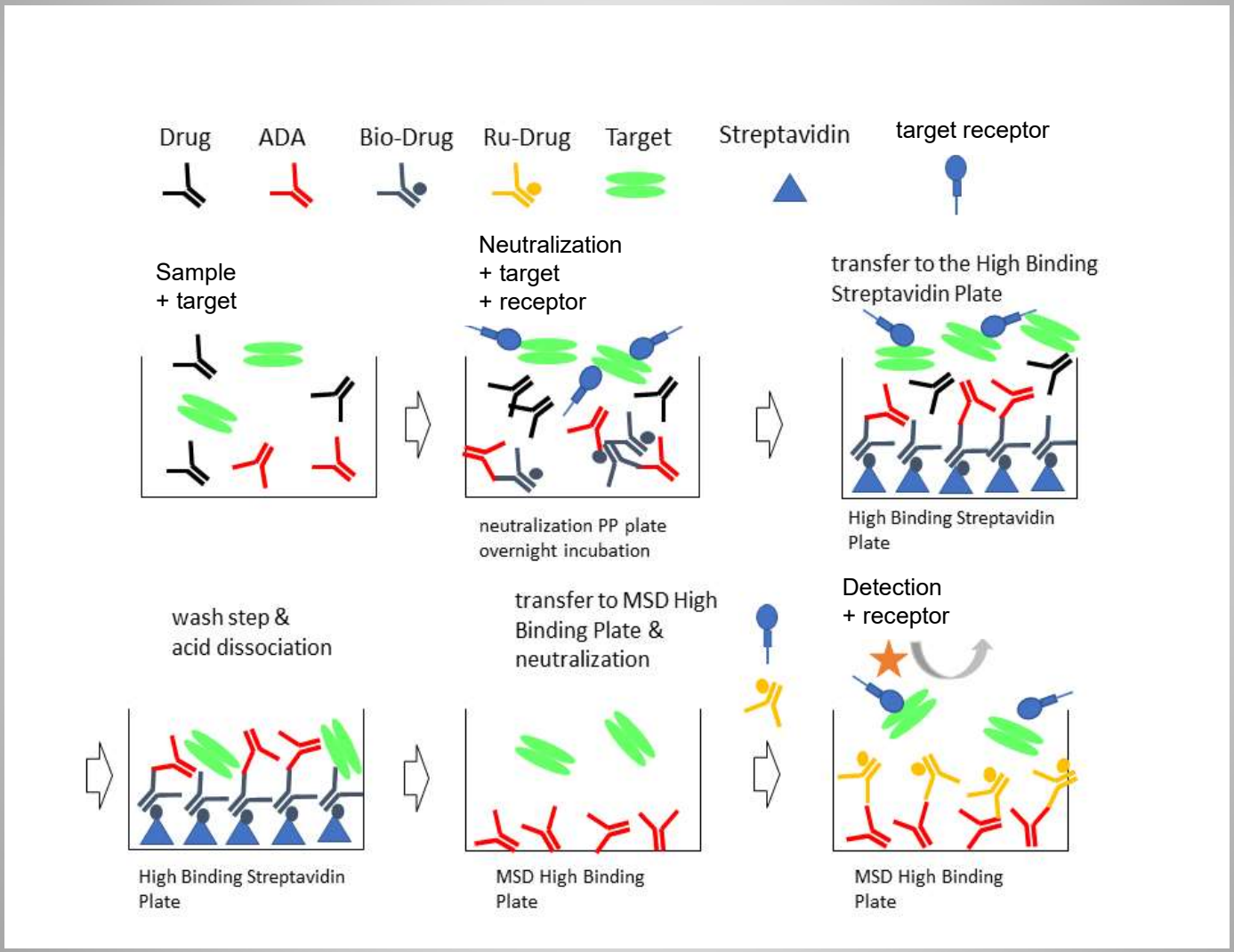
D. Specificity

The assay should specifically detect anti-mAb antibodies but not the mAb product itself, soluble drug target, non-specific endogenous antibodies, or antibody reagents used in the assay

Drug/Target SPEAD



Drug/Target SPEAD



Assessing Drug Tolerance

Control	Drug [µg/ml]	RLU 1	RLU 2	Mean [RLU]	SD [RLU]	% CV
LLPC [4 ng/ml]	1000	47	52	50	4	7,1
	500	48	54	51	4	8,3
	250	48	57	53	6	12,1
	125	52	57	55	4	6,5
	62,5	58	62	60	3	4,7
	0,0	57	63	60	4	7,1
Control	**Drug [µg/ml]**	**RLU 1**	**RLU 2**	**Mean [RLU]**	**SD [RLU]**	**% CV**
LLPC2 [6 ng/ml]	1000	45	50	48	4	7,4
	500	57	49	53	6	10,7
	250	57	53	55	3	5,1
	125	60	54	57	4	7,4
	62,5	59	57	58	1	2,4
	0,0	70	63	67	5	7,4

Assessing Target Interference

no Target Receptor					
	RLU 1	RLU 2	Mean [RLU]	SD [RLU]	% CV
NC+ 2500 ng/ml target	6516	6410	6463	75	1,2
NC+ 1000 ng/ml target	2328	2242	2285	61	2,7
NC+ 250 ng/ml target	549	533	541	11	2,1
NC+ 100 ng/ml target	250	252	251	1	0,6
NC+ 0 ng/ml target	42	45	44	2	4,9

with Target Receptor [5 µg/ml]					
	RLU 1	RLU 2	Mean [RLU]	SD [RLU]	% CV
NC+ 2500 ng/ml target	57	57	57	0	0,0
NC+ 1000 ng/ml target	46	42	44	3	6,4
NC+ 250 ng/ml target	42	40	41	1	3,4
NC+ 100 ng/ml target	54	53	54	1	1,3
NC+ 0 ng/ml target	56	53	55	2	3,9

Assay Summary (extract)

Assay Characteristics	Data
Sensitivity	3.4 ng/ml
Type of Cut Point	FCP
Confirmatory Cut Point (CCP) [%]	28.2
Titration Cut Point (TCP)	1.58
Drug Tolerance at 1,500 ng/ml	>1000 µg/ml
Drug Tolerance at 100 ng/ml	>1000 µg/ml
Drug Tolerance at 6 ng/ml	125 µg/ml
Drug Tolerance at 3 ng/ml	500 µg/ml
Target interference (based on NC)	1000 ng/ml

ADA Assay Transfer

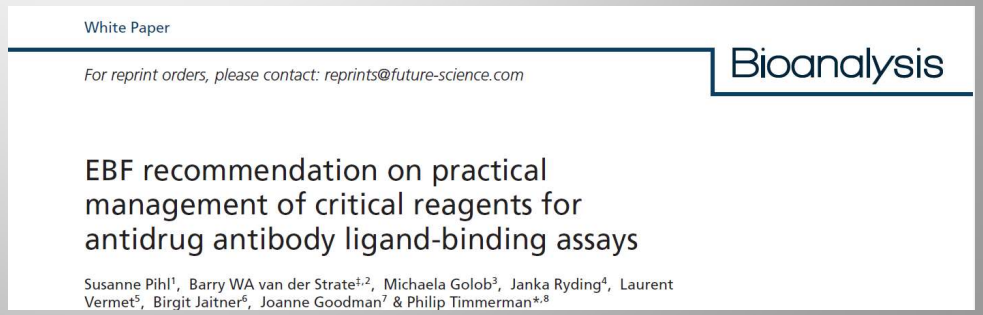
- No harmonized procedure which activities are needed during a transfer of a validated Immunogenicity assay - in contrast to PK assays
- Is a complete revalidation needed - due to interdependencies of validation parameter – see „in-study cut-point“?
- Is it sufficient to show that the controls (NC, LPC, HPC) are still within their acceptance ranges in the transferred assay?

Fit for Purpose ADA Assays

- FDA indicated that validated ADA assays are only needed for pivotal clinical trials (and for high risk therapeutic proteins)
- Assay validation as described in the current guideline is only applicable to these cases
- How would a „fit for purpose“ ADA assay look like for non pivotal trials?
- Proposal
  - Only 3 runs by one analyst for cut-point assessment
  - No dedicated determination of assay sensitivity but test 100 ng/mL during precision runs
  - No acceptance ranges for the controls but all positive controls need to test at or above the assay cut-point (and HPC>LPC)
  - Spiked positive controls need to at least show the % inhibition of the confirmatory cut-point

Critical Reagents for ADA Assays

- As immunogenicity assays are not calibrated, the exchange of reagents might lead to different assay results
  - New lot of (polyclonal) positive control
    - Use of monoclonal positive control(s)
  - Labeled drug (e.g. biotin or SulfoTag)
    - Use of dedicated vendors for labeling (instead of usage of kits)
    - Might be important to define “release criteria” for new batches of labeled drug
  - Pooled matrix serving as negative control
    - Store huge amount of negative control pool
- EBF published recommendations recently
  - Authors are proposing a decision tree for minor and major changes of critical reagent (see next slide)
  - Unfortunately no details on the statistical test and its acceptance criteria for the comparison of old and new reagents are provided


White Paper

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

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

Susanne Pihl[1], Barry WA van der Strate[‡,2], Michaela Golob[3], Janka Ryding[4], Laurent Vermet[5], Birgit Jaitner[6], Joanne Goodman[7] & Philip Timmerman[*,8]

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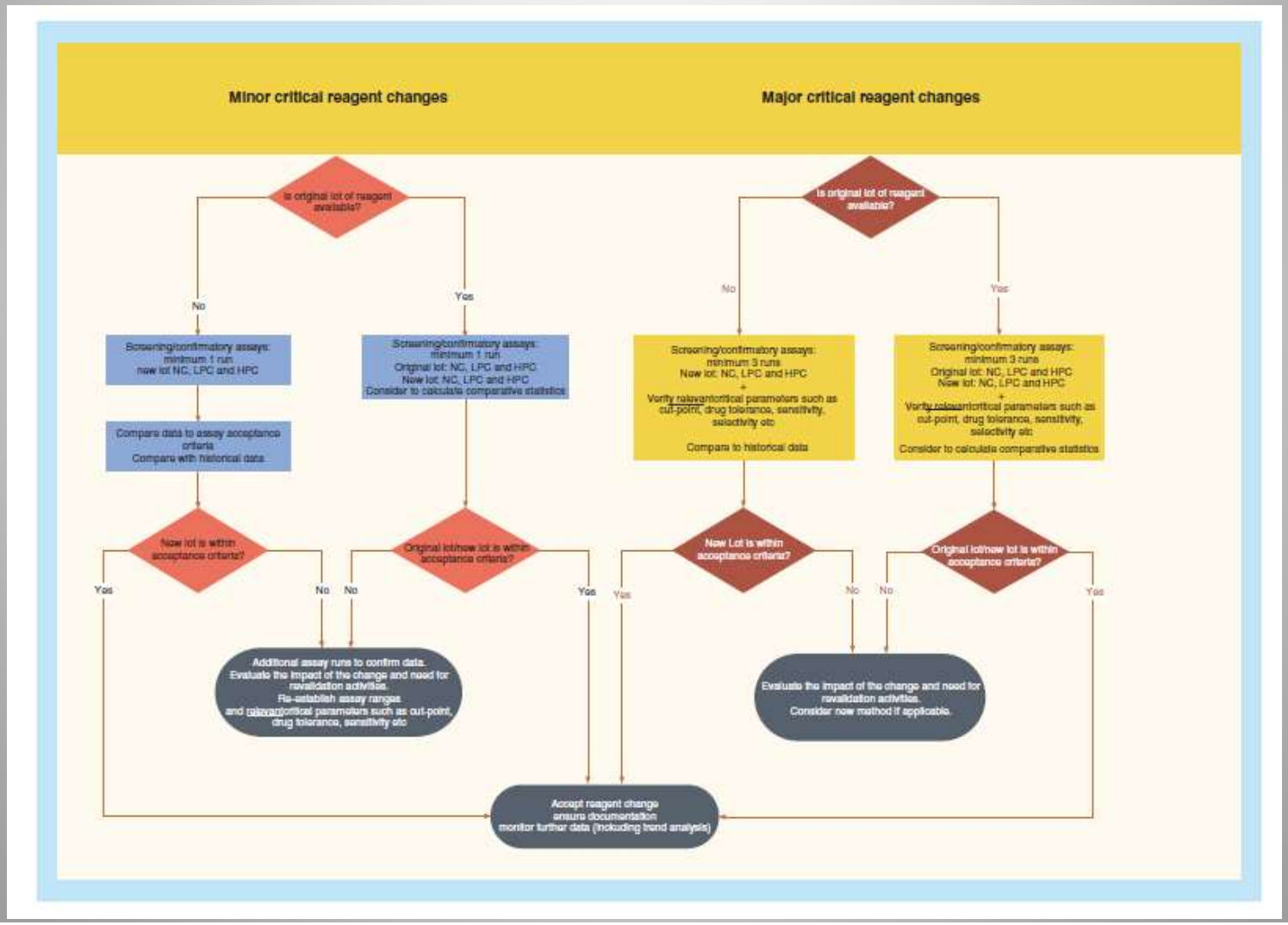
Critical Reagents for ADA Assays



Thank you

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