Drug Tolerance and Target Interference

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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
- Drug Tolerance
- Target Interference

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

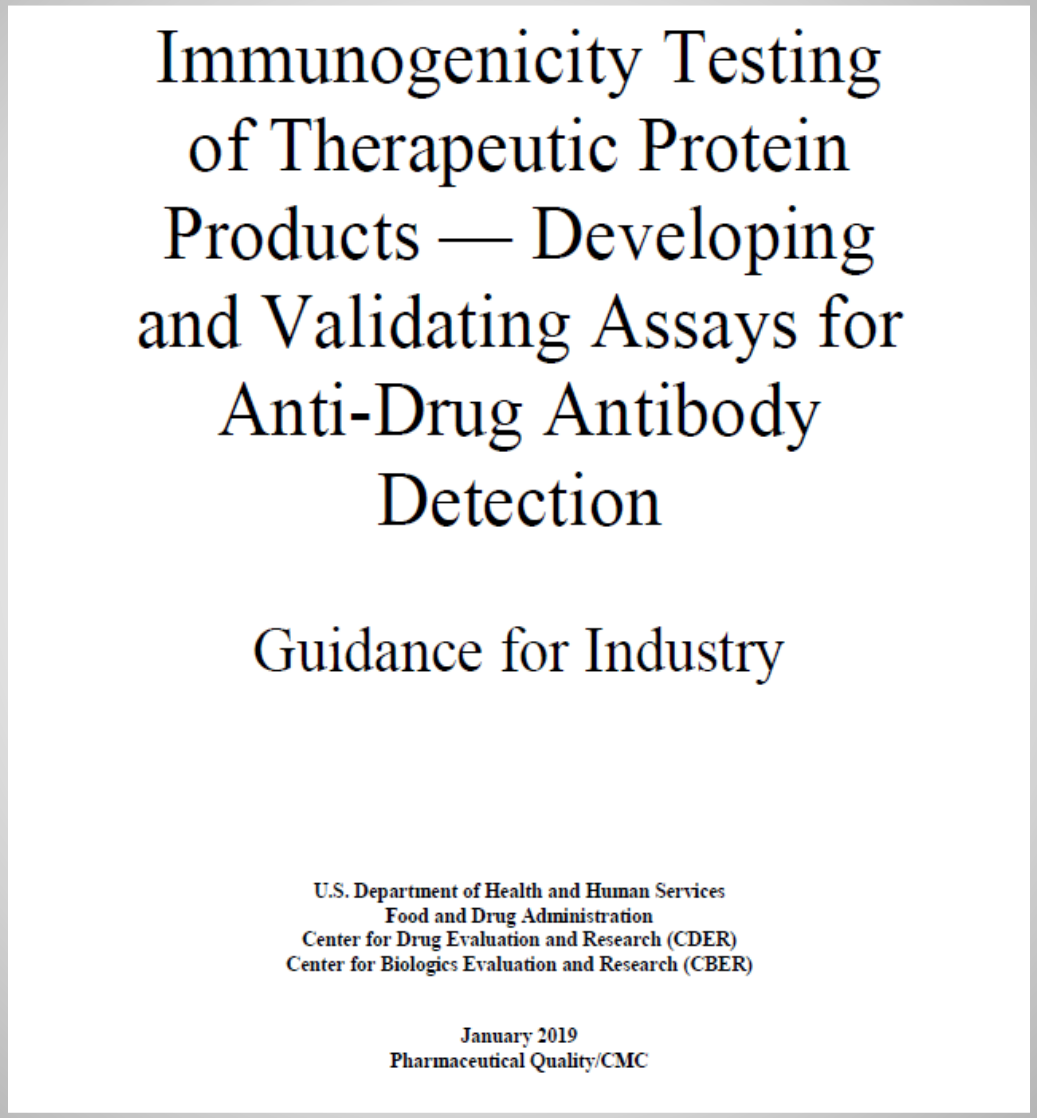
i. selection of the positive control(s)

ii. determination of the threshold for ADA-positivity

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

d. Matrix interference in different populations

Final 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

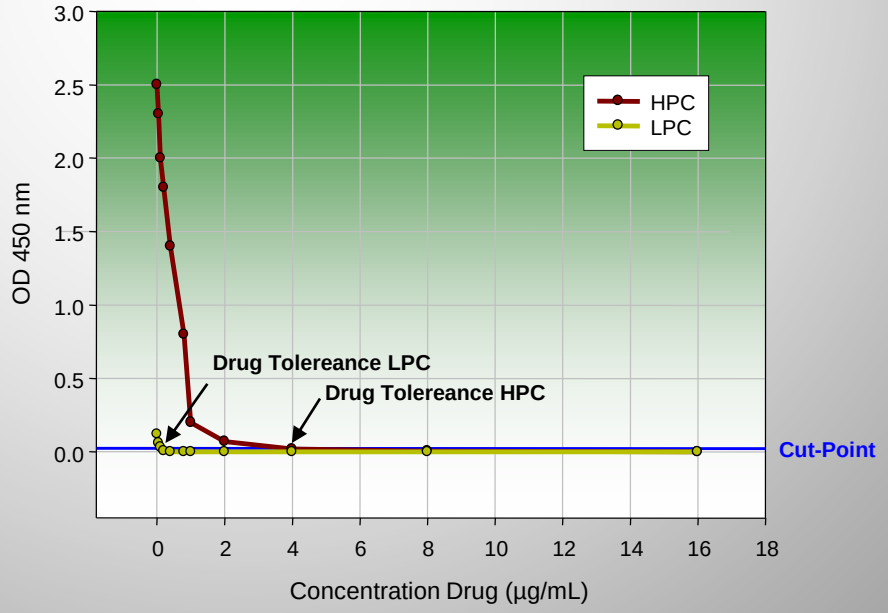
C. Sensitivity

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.



EIP European Immunogenicity Platform

FDA Immunogencity Guidance
- Target Interferece -

C. Sensitivity

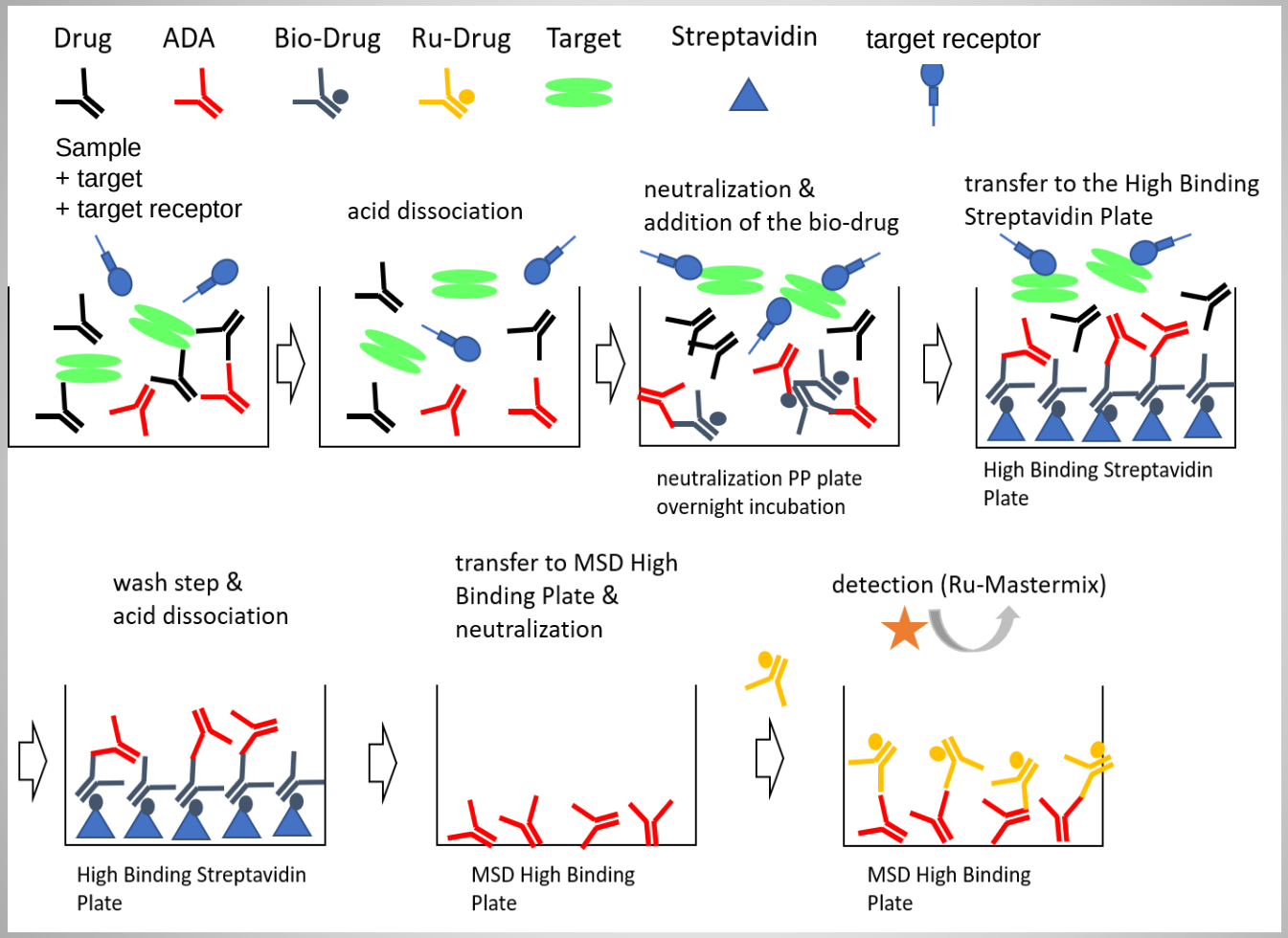
2. Drug Tolerance, Sensitivity, and Assay Suitability

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.

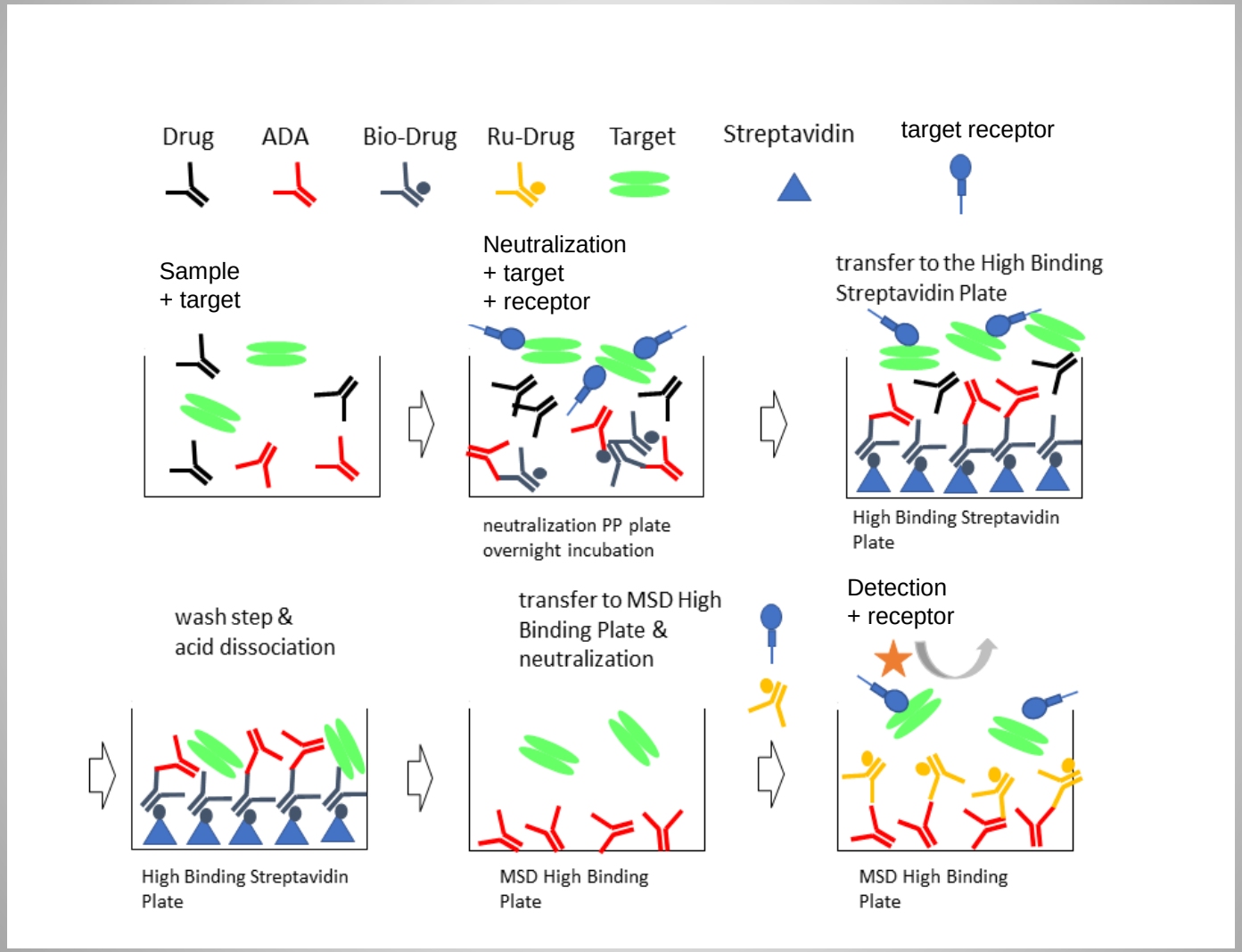
D. Specificity

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

Thank you

EIP
European Immunogenicity Platform