

New FDA Immunogenicity Guideline

EIP Training Course

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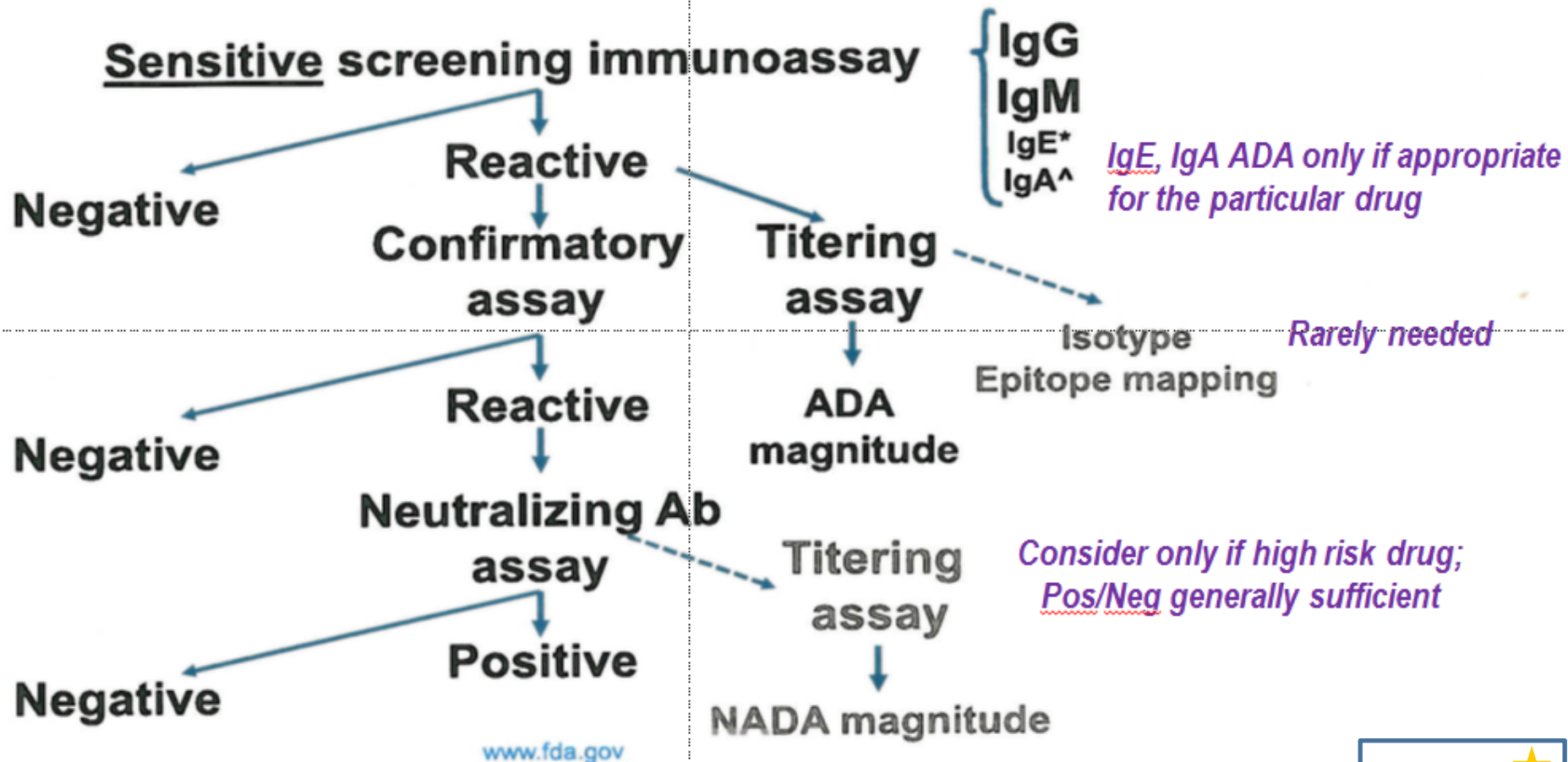
Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection

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

Multi-Tiered Immunogenicity Approach

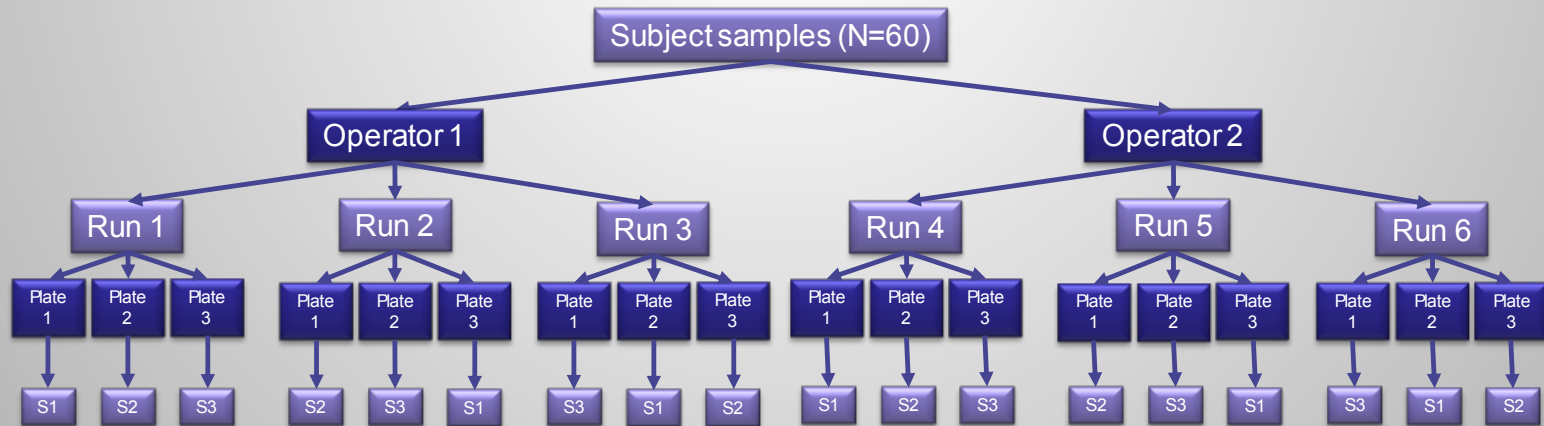


Immunogenicity Assay Critical Parameters

- Parameter
 - Cut points (screening; confirmatory)
 - Sensitivity
 - Drug tolerance 
 - Specificity and selectivity
 - Minimum required dilution 
 - Precision,
 - Reproducibility
 - Robustness
- Some parameter are preliminarily assessed during assay development and verified during validation
 - Cut-point, sensitivity, drug tolerance
- Other parameter are only assessed during assay development but results should be presented in the validation report
 - MRD
 - Therapeutic protein product concentration for NAb assay

Screening Cut-Point (I)


- The cut-point should be determined statistically with an appropriate number of treatment-naïve samples, generally around 50, from the subject population
 - Each sample should be tested by at least two analysts on at least three different days for a total of at least six individual measurements
 - The sponsor should consider the impact of statistically determined outlier values and true-positive samples when establishing the cut-point
 - Balanced study designs should be used for cut-point determination.



S1: Subject samples 1-20
S2: Subject samples 21-40
S3: Subject samples 41-60


Screening Cut-Point (II)

- Usual calculations (e.g. mean + 1.645 x SD) are set to yield 5% false positive rate on average (50 % of the time)
 - Using this calculation the false positive rate can range from 2-11%

 Current thinking of FDA is to apply a 90 % one-sided lower confidence interval for the 95th percentile

- This would assure **at least** a 5 % false positive rate (90 % of the time)
- However, this formula will yield ~10% false positives on average

Confirmatory Cut-Point

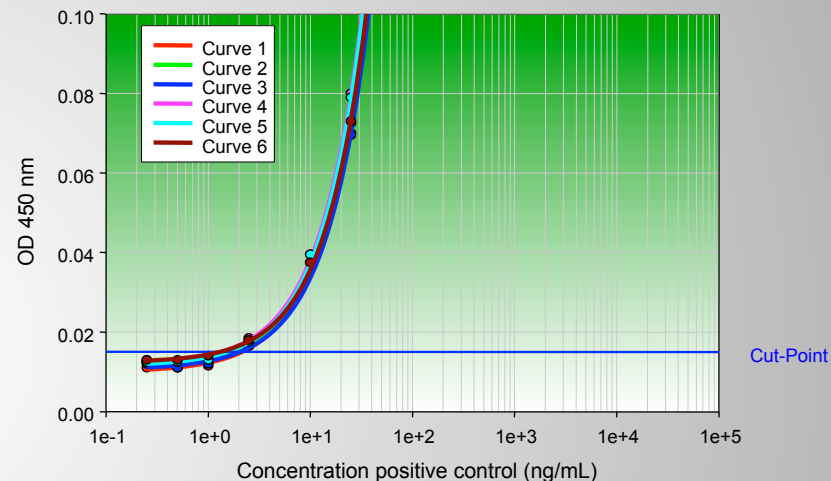
- The Confirmatory cut-point is most commonly established by evaluating the mean and the variance of drug naive samples in presence and absence of drug
- FDA recommends a 1 % false positive rate
 - The use of tighter false-positive rates such as 0.1% is not recommended
- FDA recommends that the sensitivity of the confirmatory assay be demonstrated using a low concentration of the positive control antibody
 -  FDA expects that the selected confirmatory assay will have similar sensitivity to the screening assay but higher specificity
- The confirmatory assay needs to be fully validated

Assay Sensitivity (I)

- Draft: *“Although traditionally FDA has recommended sensitivity of at least 250–500 ng/mL, recent data suggest that concentrations as low as 100 ng/mL may be associated with clinical events.”*
- EIP comments:
 - Recommend highlighting that assay sensitivity is highly dependent on the positive control used in the evaluation
 - Also, drug tolerance is not taken into account in this section. Sensitivity of the assay and drug interference are related factors (the higher the sensitivity, the higher the drug interference at the level of sensitivity measured)
- Final: *“Assay sensitivity is assessed using positive control antibody preparations that may not represent the ADA response in a specific subject...Because the measurement of assay sensitivity can be affected by onboard drug, it is also important to determine assay sensitivity in the presence of the expected concentration of onboard drug”*

Assay Sensitivity (II)

- Procedure
 - 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
 - Positive control can be affinity purified polyclonal or monoclonal antibodies
 - During routine performance of the assay, a low positive system suitability control should be used to ensure that the sensitivity of the assay is acceptable across assay runs
 - The low positive control should be consistently demonstrated as positive in both screening and confirmatory tiers



Drug Tolerance

- Evaluate positive control ADA detection in the presence of different amounts of drug
 - Vary both the concentration of the positive control and the amount of drug „checker board“

Positive control (ng/ml)	Drug (amount in serum)	Drug (amount in serum)	Drug (amount in serum)
100	Lower than expected	Expected	Higher than expected
250	LTE	E	HTE
500	LTE	E	HTE
750	LTE	E	HTE

Specificity & Selectivity

- Specificity
 - Specificity refers to the ability of a method to exclusively detect the target analyte (ADAs)
 - Incubation of positive and negative control antibody samples with the purified therapeutic
 - Inhibition of signal of positive controls in the presence of the relevant therapeutic protein indicates that the response is specific
 - No effect on negative control expected
- Selectivity
 - Selectivity the ability of the assay to identify ADAs specific to the therapeutic protein product in the presence of other components in the sample
 - Spiking the positive control(s) in the presence or absence of matrix
 - Comparing the **recovery** of ADA in buffer alone with that in the matrix can provide input on the degree of interference from matrix components

MRD

- Determination of MRD usually involves serially diluting treatment-naïve ADA-negative samples (at least 10), as well as testing known amounts of purified antibody at high, medium, and low concentrations in serially diluted matrix in comparison to the same amount of positive control antibody in diluent

- Determine the mean signal (S) and standard deviation at each dilution. Determine the mean signal (B) and standard deviation of the assay blank
- Calculate the Z-factor according to

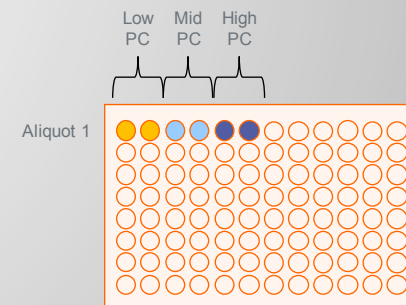
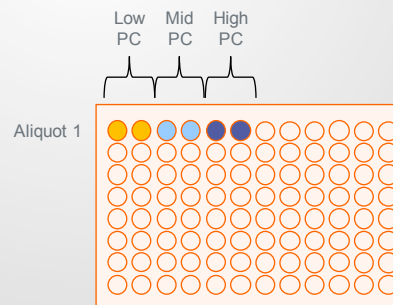
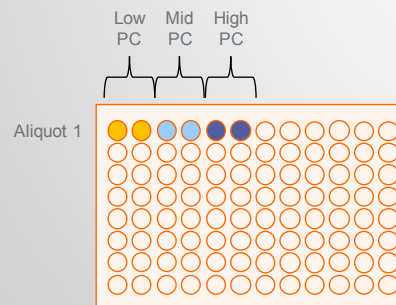
$$Z = \frac{[(\text{mean}(S) - 3SD(S)) - [\text{mean}(B) + 3SD(B)]]}{\text{mean}(S) - \text{mean}(B)}$$

- Aim for the highest Z-factor (excellent assays show a Z-factor between 0.5 and 1)
- FDA recommends that MRD not exceed 1:100
 - All sample dilutions, such as the MRD and acid dissociations, should be factored into the calculations of titers and sensitivity

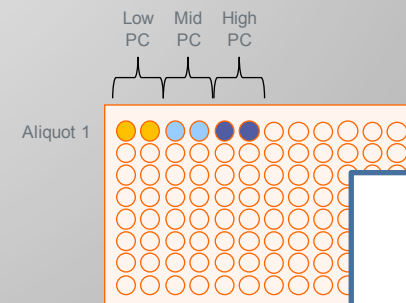
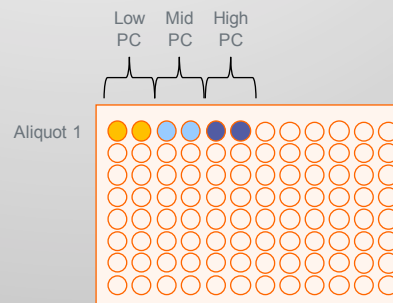
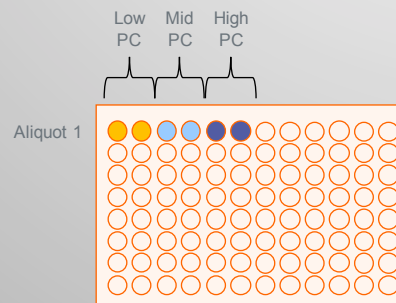
Inter-Assay Precision

- FDA recommends that inter-assay precision be evaluated on different days and by different analysts using the same instrument platform and model, although different instruments should be used to include all sources of variability. This design results in at least six **independent determinations** for each sample
- Samples should include negative controls and positive samples whose testing yields low, intermediate, and high values of the assay dynamic range

Analyst 1
Day 1
Instrument A

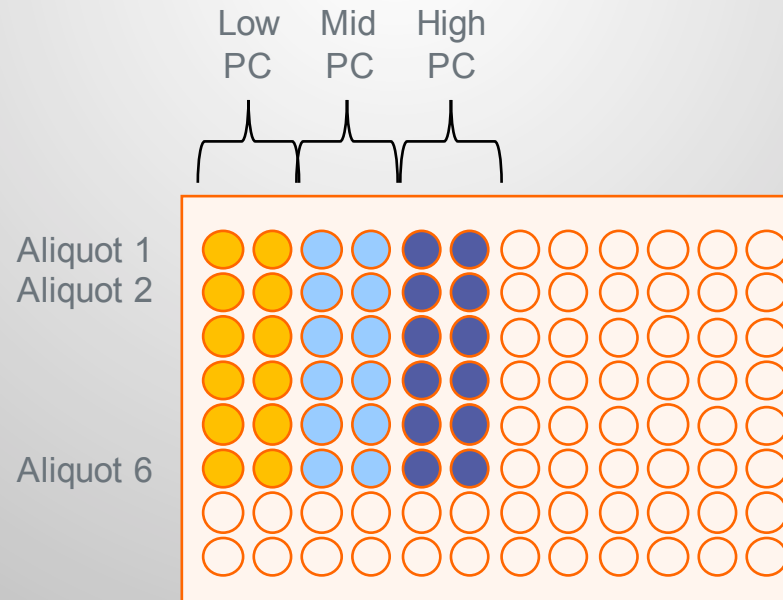


Analyst 2
Day 2
Instrument B



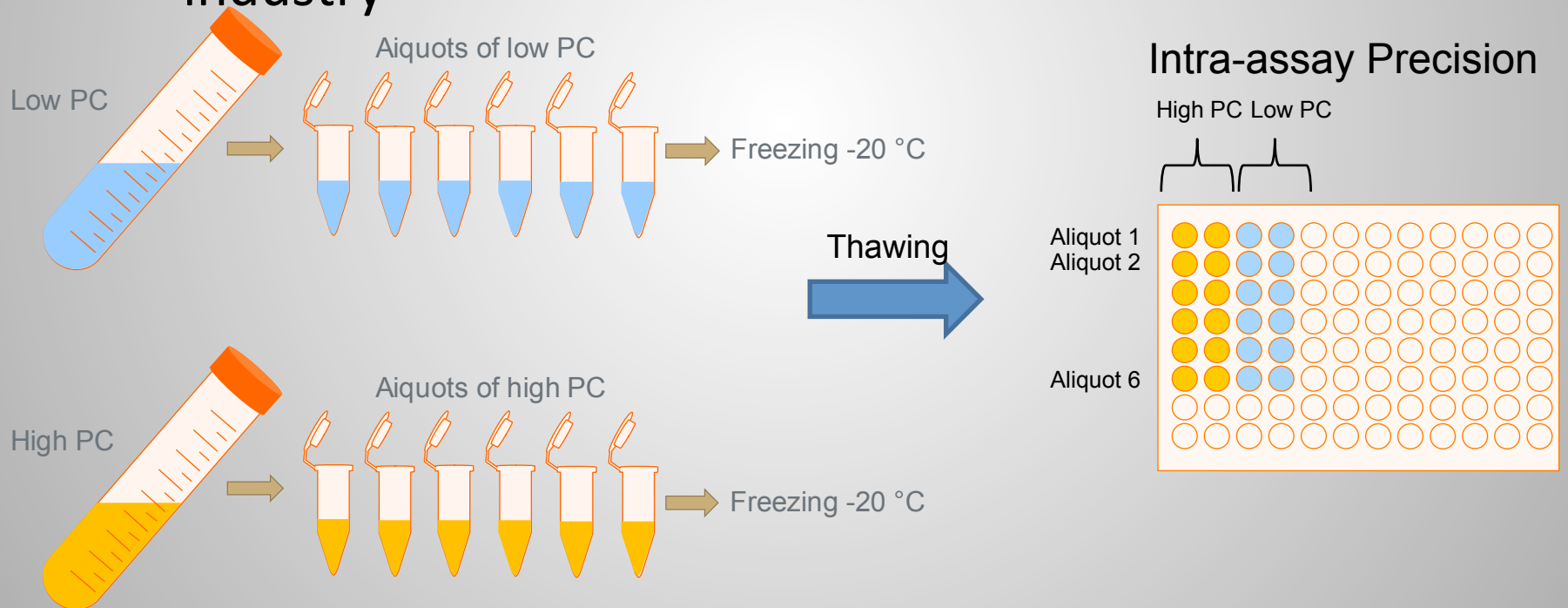
Intra-Assay Precision

- Intra-assay precision should be evaluated with a minimum of six **independent preparations** of the same sample per plate **independently prepared by the same analyst**
- Samples should include negative controls and positive samples whose testing yields low, intermediate, and high values of the assay dynamic range



Intra-Assay Precision

- What are “Independent Aliquots”?
 - Different interpretations in biopharmaceutical industry



Are these really independent preparations?

Stability

- Draft: *“However, studies evaluating long-term stability of positive control antibodies may be useful”*
- EIP comment:
 - FDA should acknowledge that the stability of antibodies frozen in matrix is known (and should be independent of the CDRs). Therefore dedicated long term stability studies using the positive control are not adding value
- Final: *“However, studies evaluating short-term stability, including, as relevant, freeze-thaw cycle and refrigerator- and room-temperature stability of positive control antibodies, may be useful”*

Integrated Summary of Immunogenicity

- The “Integrated Summary of Immunogenicity” should be included in eCTD section “5.3.5.3 Reports of Analysis of Data from More than One Study”
- It should include:
 - 1. Immunogenicity risk assessment**
 - Discussion of risk factors (product-, process-, clinical-, and patient-related factors) and how these may impact the immunogenic potential (likelihood & clinical sequelae of ADAs)
 - 2. Tiered strategy and bioanalytical assays with stage- appropriate information**
 - Description of the immunogenicity testing strategy (3-tiered approach)
 - Characterization of the various methods that were developed & used throughout the program
 - 3. Clinical study design and sampling strategy**
 - Discuss how selected immunogenicity sampling time points help to
 - Reveal the incidence, persistence, and clinical significance of ADAs and NAbS
 - Minimize drug interference (report drug concentration at ADA sampling time points)
 - 4. Clinical immunogenicity data analysis**
 - Summary results of ADAs and NAbS for all clinical studies (incidence, titers, kinetics)
 - Impact of ADAs on PK/PD, efficacy and safety
 - 5. Conclusions and risk mitigation**
 - Discuss impact of immunogenicity on the benefit/risk of drug to the patient
 - Discuss how immunogenicity will be monitored post-marketing (if warranted)