



FDA Draft Guidance on Immunogenicity Testing

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Guidance for Industry Assay Development for Immunogenicity Testing of Therapeutic Proteins

DRAFT GUIDANCE

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I. Introduction

- “FDA guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.”

I. Introduction

- Recommendations to facilitate development of immune assays for assessment of the immunogenicity of therapeutic proteins during clinical trials
 - binding assays
 - confirmatory assays
 - neutralizing assays
- Does not specifically discuss the development of immune assays for animal studies, however the concepts discussed are relevant
- Does not discuss the product and patient risk factors that may contribute to immune response rates.



I. Introduction

- This guidance does not pertain to immunogenicity assays for assessment of immune response to preventative and therapeutic vaccines for infectious disease indications.
- In addition, this document does not specifically discuss how results obtained from immunoassays relate to follow-on biologic therapeutic proteins.

General Discussion

- Assays are critical when immunogenicity poses a high-risk therefore real time data concerning patient responses are needed
- Preliminary validated assays should be implemented early (preclinical and phase 1).

General Discussion

- Therapeutic proteins are frequently immunogenic in animals.
 - Immunogenicity in animal models is not predictive of immunogenicity in humans.
 - Assessment of immunogenicity in animals may be useful to interpret nonclinical toxicology and pharmacology data.
 - Immunogenicity in animal models may reveal potential antibody related toxicities that could be monitored in clinical trials.

Immunogenicity Testing During Product Development

- Assay differences can make immunogenicity comparisons across products in the same class invalid.
- Therefore, in the product labeling, FDA does not recommend comparing the incidence of antibody formation between products when different assays are used.
- A comparison of immunogenicity across different products in the same class can best be obtained by conducting head-to-head patient trials using a standardized assay that has equivalent sensitivity and specificity for both products.

Overview of Design Elements

Multi-tiered Approach

- FDA recommends a multi-tiered approach to the testing of patient samples to allow for culling of samples for further testing.
 - A sensitive (250 – 500 ng/ml) screening assay with a low but defined false positive rate (5%) should initially be used
 - The screening assay should be able to detect all isotypes but particularly IgM and IgG
 - Positive samples should be tested in a confirmatory assay.
 - Confirmed samples should be tested in a neutralizing Ab assay.
 - Tests to assess the isotype and epitope specificity may also be recommended

Obtaining Patient Samples

- Pre-exposure samples should be obtained from all patients.
- Subsequent samples should be obtained with timing depending on the frequency of dosing.
- Samples should be obtained when there will be minimal interference from product present in the serum.
- If drug-free samples cannot be obtained during the treatment phase of the trial, then additional samples should be obtained after an appropriate washout period (e.g., five drug half-lives).
- If the product in is an immune suppressant samples should be obtained from patients who have undergone a washout period

Reporting Results

- Results of patient sample testing are often reported as positive vs. negative
- An assessment of antibody levels may be informative. FDA, therefore, recommends that positive antibody responses be reported as a titer (e.g., the reciprocal of the highest dilution that gives a value equivalent to the cut point of the assay).

Reporting Results

- Values may also be reported as amount of drug (in mass units) neutralized per volume serum with the caveat that these are arbitrary in vitro assay units and cannot be used to directly assess drug availability in vivo.
- Antibody levels reported in mass units based on interpolation of data from standard curves generated with a positive control standard antibody are generally less informative because interpretation is based on the specific control antibody.

Screening Assay Selection of Format

- The FDA does not recommend a specific format
- A number of different formats are available
- Each format has its advantages and disadvantages

Assay Validation: Cut Point

- The cut point of the assay is the level of response of the assay at or above which a sample is defined to be positive and below which it is defined to be negative.
- The cut point should be statistically determined by using negative control samples (e.g., samples from patients not exposed to product).
- A small number of samples (5-10 samples from untreated individuals) may be used during assay development.
- Assay validation with a sample size of 50-100 is statistically more reliable
- It may also be necessary to determine the cut point for different populations of patients.

Assay Validation: Cut Point

- The removal of statistically determined outlier values should be considered.
- Using immunodepletion approaches, the applicant should identify those samples with pre-existing antibodies and remove them from the analysis.
- If the presence of pre-existing antibodies is a confounding factor, it may be necessary to assign positive responses or a cut point based on the difference between individual patient results before and after exposure.

Assay Validation: Cut Point

- FDA recommends that the cut point have an upper negative limit of approximately 95 percent, particularly in the initial screening assay
- Possible approaches:
 - parametric approaches using the mean plus 1.645 standard deviation (SD), where 1.645 is the 95th percentile of the normal distribution may be appropriate.
 - median and median absolute deviation value instead of mean and SD.
- Whatever approach is used, data must be presented to support the conclusion and the conclusion statistically justified.

Screening Assay Validation: Specificity

- When the therapeutic protein represents an endogenous human protein, the applicant should assess cross reactivity with the native human protein.
- When the therapeutic protein is a member of a family of homologous proteins, the applicant should assess cross reactivity with multiple family members.
- For monoclonal antibodies and Ig-fusion proteins it should be clearly demonstrated that the assay method specifically detects anti-monoclonal antibodies and not the monoclonal antibody product itself, non-specific endogenous antibodies, or antibody reagents used in the assay.



Assay Validation: Specificity

- Interference by drug present in the serum should be assessed by testing the effect of various concentrations of study drug on the high, medium, and low QC positive controls.
- For patient populations with a high incidence of RF, the demonstrate that RF does not interfere with the detection method.

Additional Validation Parameters

- Selectivity – assay performance in the target matrix
- Precision – Includes intra- and inter-assay precision
- Sensitivity – the lowest concentration at which the Ab preparation consistently produces either a positive result or a readout equal to the cut point. Should be expressed in mass units.
- Ruggedness (inter-laboratory) if applicable

Additional Parameters

- The following are parameters that should be established but may not need to be confirmed in a validation exercise. [However supporting data should be provided to the Agency to establish assay suitability.]
 - Minimum required dilution
 - Robustness
 - Reagent stability
 - Positional effects

Validation of Confirmatory Assays

- Immunodepletion/competition assays are most commonly used for confirming screening results.
- Confirmatory assays need to be fully validated in a manner similar to binding and neutralizing assays
- The most difficult issue is identifying the degree of inhibition or depletion that will be used to ascribe positivity to a sample.
- FDA recommends that sponsors carefully address this issue during assay development and base determinations on meaningful data.

Neutralizing Assay: Selection of Format

- Types of assays generally used: cell-based biologic assays and non cell-based competitive ligand-binding assays.
- FDA considers that bioassays are more reflective of the in vivo situation and are recommended
- The bioassay should be related to product mechanism of action to be informative as to the effect of NAB on clinical results
- Competitive ligand-binding assays may be the only alternative in some situations
- Assays may use direct (inhibition of stimulation) or indirect (inhibition of inhibition)

Neutralizing Assay: Activity Curve

- Most commonly the neutralization assay employs a single concentration of product with different concentrations of antibody samples added to determine neutralizing capability.
- A product concentration whose activity readout is sensitive to inhibition should be used

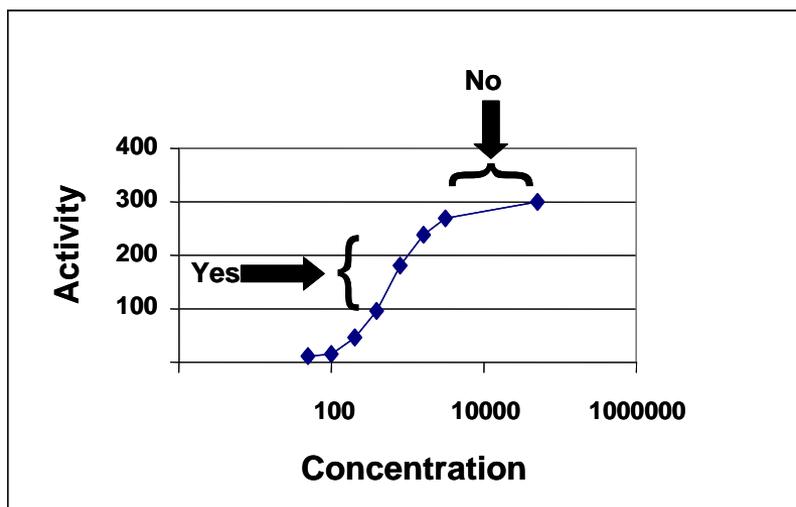


Figure 1. Activity Curve for a Representative Therapeutic Protein



Neutralizing Assay Validation: Cut Point

- The determination should be statistically based and derived from assays using samples from patients not exposed to the product.
- If variation makes it difficult to assess neutralizing activity, other approaches may be considered but should be discussed with FDA
- Alternatively, exploring other assay formats that lead to less variability and provide a more accurate assignment of cut point may be necessary.

Neutralizing Assay Validation: Specificity

- Assay specificity should be demonstrated.
- For cells that may be responsive to stimuli other than the specific therapeutic protein, the ability to demonstrate that NAB only inhibit the response to product and not to other stimuli is a good indication of assay specificity.
- The applicant should also confirm the absence of alternative stimuli in patient serum.



Additional Validation Parameters

- Sensitivity - Similar to the binding assay sensitivity should be reported in mass units.
- Precision
- Ruggedness (if applicable)

Additional Parameters

- The following are parameters that should be established but may not need to be confirmed in a validation exercise. [However supporting data should be provided to the Agency to establish assay suitability.]
 - Amount of drug used if applicable
 - MRD
 - Robustness
 - Cell passage number
 - Incubation times
 - Media components
 - Others as applicable
 - Reagent stability
 - Positional effects

In-study performance: Concurrent Positive and Negative Quality Controls

- Positive control or QC samples are critical and should be run concurrently with patient samples.
- QC samples should have known negative, low, medium, and high reactivity in the assay.
- More importantly, the samples should be diluted in the assay matrix
- Low positive control samples should be selected based upon statistical analysis that would lead to the rejection of an assay run 1 percent of the time.
- Should be detected by the secondary detecting reagent, to ensure that negative results that might be observed

Next Steps

- Collate comments to FR publication, white paper recommendations and EMEA recommendations
- Review comments with Immunogenicity Guidance Committee
- Amend Guidance
- Publish