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Review

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

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“White Paper” Purpose:

- To provide scientific recommendations for the validation and performance of anti-drug antibody (ADA) immunoassays.
- These recommendations are supplemental to:
 - Regulatory documents (ICH, EMEA, FDA,...)
 - Previous “Recommendations...” documents published by the AAPS Ligand-Binding Focus Group .

The **recommendations** are intended to:

- facilitate a standardized approach to antibody detection and characterization
- serve as a basis for further advancement of testing methodologies
- provide regulatory agencies with industry's science-based position on immunogenicity testing

Immunogenicity Working Groups

- Review and discuss methods used across the industry
- Strive towards an agreement on the most important aspects of immunogenicity testing
- Recommend “best practices”

As a result, five recommendations white papers for immunogenicity testing have been published or drafted so far.

The **recommendations** are **not intended to mandate** a standardized approach to antibody detection and characterization.

It is the responsibility of the sponsor to determine a scientifically sound immunogenicity assessment for each biotherapeutic.

Consult regulatory advisors as early as possible.

Guidance “White Paper” References

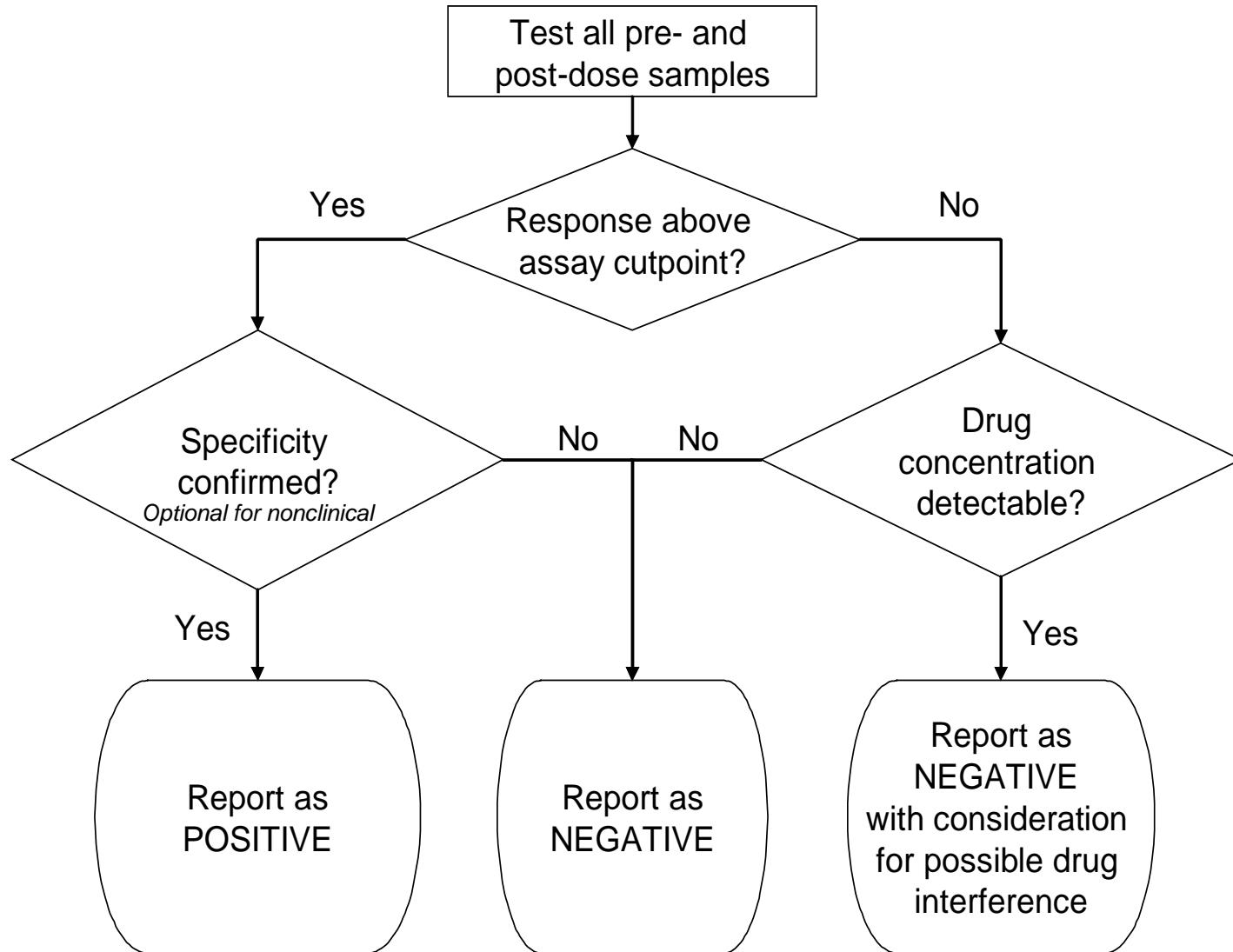
- Recommendations for the [Design and Optimization of Immunoassays](#) used in the detection of host antibodies against biotechnology products. A.R. Mire-Sluis, et al., 2004. J. Immunol. Meth. 289: 1-16.
- Recommendations on Risk-Based [Strategies](#) for Detection and Characterization of Antibodies against Biotechnology Products. E. Koren, et al. 2008. J. Immunol. Meth. 333:1-9
- Recommendations for the [Validation of Immunoassays](#) Used for Detection of Host Antibodies Against Biotechnology Products. G. Shankar, et al. 2008. J. Pharmaceutical and Biomedical Analysis 48:1267–1281.
- Recommendations for the [design, optimization and qualification of cell-based assays](#) used for the detection of neutralizing antibody responses elicited to biological therapeutics. S. Gupta, et al. 2007. J Immunol Methods. 321(1-2):1-18
- Immunogenicity of biologically-derived therapeutics: [Assessment and interpretation of non-clinical safety studies](#). R. Ponce, et al. 2009 (accepted). Regulatory Toxicology and Pharmacology.
- Recommendations for the [validation of Nab assays](#)... In progress

Regulatory References

- ICH-S6 Proceedings of the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Tripartite Guideline, S6: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. July, 1997.
- Guidance for Industry - Immunotoxicology Evaluation of Investigational New Drugs: Section IV. Immunogenicity. CDER-FDA. Oct 2002
- Guidance for Industry - Nonclinical Safety Evaluation of Drug or Biologic Combinations. CDER-FDA. March 2006.
- Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. CBER – FDA 1997. (<http://www.fda.gov/cder/guidance/index.htm>)
- EMEA, Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins. Doc. Ref. EMEA/CHMP/BMWP/14327/2006
- EMEA, Guideline on Requirements for First-in-Man Clinical Trials for Potential High-Risk Medicinal Products. Doc. Ref. EMEA/CHMP/SWP/28367/2007
- International Conference On Harmonization (ICH) Of Technical Requirements For Registration Of Pharmaceuticals For Human Use ICH Harmonized Tripartite Guideline, Q2A – *Text on validation of analytical procedures*, March 1995.
- EMEA, Concept Paper on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use. EMEA/CHMP/BMWP/114720/2009 (in DRAFT)

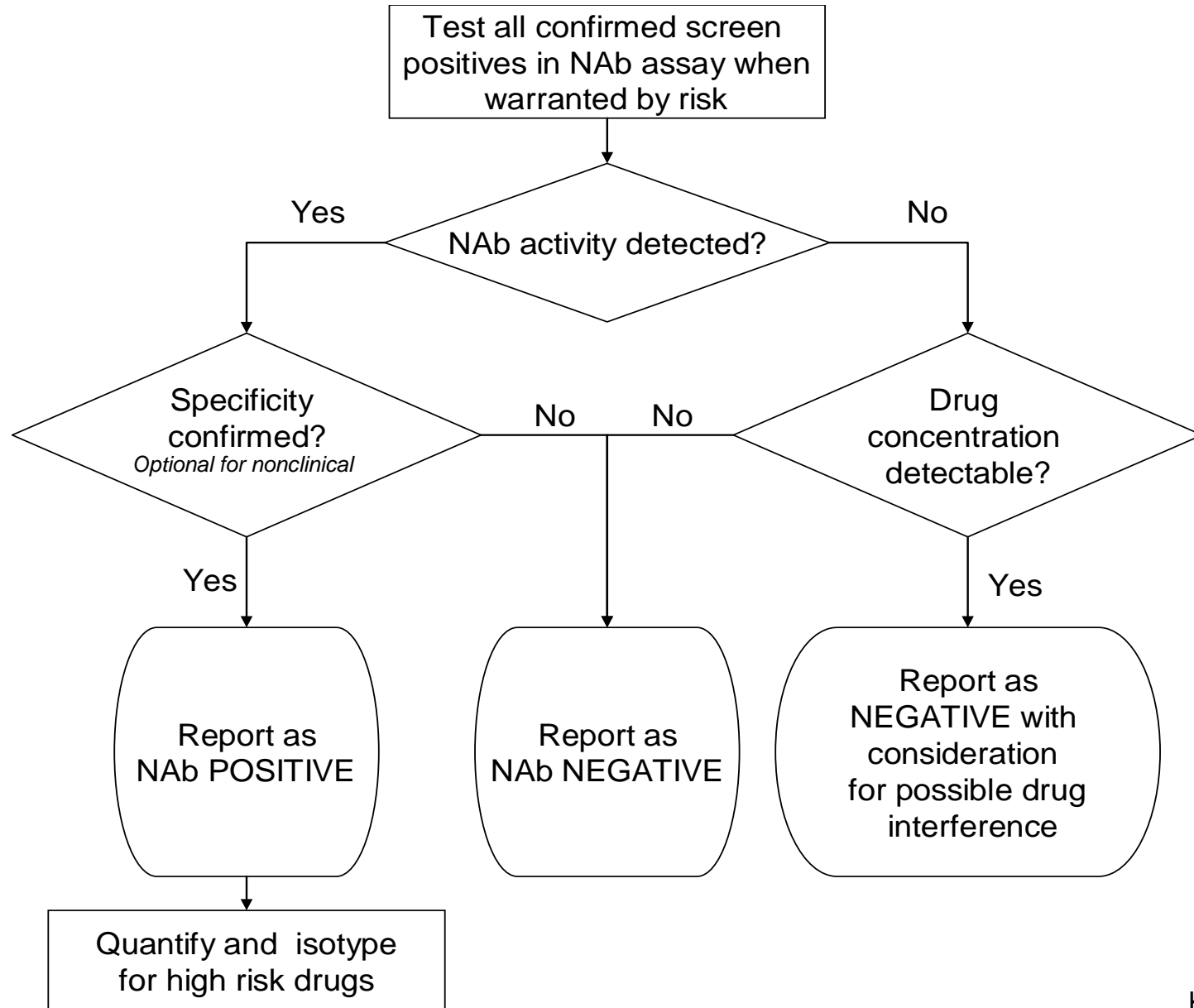
Strategies

Step 1: Recommended testing strategy for detection of anti-drug antibodies



Strategies

Step 2: Recommended strategy for characterization of anti-drug antibodies



Recommendations for Validation of Immunoassays for ADA

Topics covered:

- ADA Detection Methods
- Pre-Study Validation
- In-Study Monitoring & Re-Validation
- Appendices
 - Statistical Examples

ADA Detection Methods

- Formats (ELISA, RIA, ECL, SPR, other)
- Types (screening, confirmation, titration, other)

Pre-Study Validation

- Bioanalytical performance characteristics with ADA-specific parameters:
 - Screening Cut Point
 - Dilutional Linearity
 - Sensitivity
 - System Suitability Controls (QCs)
 - Selectivity (recovery)
 - Specificity Cut Point for Confirmation Assay
 - Precision
 - Robustness
 - Stability

Screening Cut Point

Sample Selection

- drug naïve healthy donors
- target disease population (if feasible)
- study specific baseline samples
- individual samples, not pooled
 - ≥ 25 individuals recommended

Balanced experimental design

- 3 independent assay runs
- Include relevant potential sources of variability
 - Multiple analysts
 - Multiple days
 - Number of replicate tests within a run (e.g. duplicate vs triplicate)

Types of Screening Cut Point

- Fixed

 - Determined in pre-study validation and the value remains constant during the in-study phase

- Floating

 - Calculated by adding or multiplying a specific normalization factor, determined from the pre-study validation data, to the biological background during the in-study phase

- Dynamic

 - A plate-specific cut point that does not use the variation estimates from pre-study validation

Assay Sensitivity

- Highly dependent upon the affinity characteristics of the positive control calibrator reagents used to define it.
- “Relative” sensitivity - No positive control can be expected to represent the spectrum of the immune response observed in individuals.
- Positive Control antibodies from the species being tested is optimal, but not always feasible.
- Polyclonal is preferred over monoclonal.
- If multiple calibrators evaluated, report range of sensitivities.

Sensitivity, *continued*

- Definition: The lowest concentration at which a positive control antibody “consistently” provides a positive signal (\geq cut point).
- Positive control prepared in undiluted pooled matrix, and then prepared according to the assay design. Repeat $\geq 3x$.
- Sensitivity is reported after factoring in the assay Minimum Required Dilution (MRD)
- Recommended Sensitivity:

Clinical	250 – 500 ng/ml
Nonclinical	500 – 1000 ng/ml

Study Drug Interference

- Residual drug in a sample may interfere with the assay- competition for product specific antibodies.
- Important to understand how much drug the assay can tolerate while reliably detecting ADA.
- Interference testing is not necessary if ADA testing is performed on samples without residual drug, such as after a washout period, as indicated by toxicokinetic analysis, or if assay methodology is not affected by presence of drug.

Study Drug Interference, *continued*

- Low positive control matrix spiked with varying concentrations of drug. Compare to low positive control matrix without drug.
- The drug tolerance limit is interpreted as the drug level at which the sample is consistently detected as positive (\geq cut point).

Note: It is advised that this assay characteristic not be used for interpreting assay results. The negative or positive results of a sample containing drug should include a comment indicating that the result may be incorrect due to the possible interference of drug.

Specificity Confirmation

- A specificity confirmation assay is usually a **competitive inhibition test** where the sample is pre-incubated with study drug and monitored for a reduction in signal as compared to the same sample without the drug.
- This test is performed to identify “true positives”.
- The specificity cut point is the reduction of signal or titer statistically determined to confirm specificity.
- Alternative formats? Immunodepletion or no-antigen binding may be performed for additional characterization.

Sample Stability

From a stability standpoint, ADA are polyclonal antibodies, regardless of specificity. It is not stipulated that sample stability for each drug-specific ADA be separately validated.

- literature references are available to support the stability of antibodies at temperatures of $\leq 20^{\circ}\text{C}$ for 2 years or longer, provided that the sample integrity is retained (no freeze/thaw, no drying out, etc...).
- Sample stability testing conditions should mimic the expected study sample and reagent handling conditions:
 - storage and handling temperatures, with varying lengths of time, compared to freshly prepared samples.

Dilutional Linearity

- Traditional approach to linearity testing in analytical assays may not apply in bioanalytical assays for ADA.
- When ADA is evaluated as a titer, demonstrate that there is a concentration-related response (decrease) with dilutions of a positive control sample(s).

System Suitability Controls

- “Ensure that the validity of the analytical procedure is maintained whenever used” [ICH-S6]
- Quality control samples – minimum of two positive levels, low and high, and a negative matrix control.
 - Performance range of the QC samples determined during assay validation (signal or titer)
 - Performance of the QC samples monitored with acceptance criteria during in-study phase.

Selectivity (Recovery)

Selectivity is the ability of an assay to measure the analyte of interest in the presence of other constituents in the sample. [DeSilva 2003 PharmRes20:11]

- It is important to evaluate selectivity in the sample matrix:
 - Identify potential interfering factors such as study drug, endogenous protein, rheumatoid factor, etc...
- Caution: Selectivity in an ADA assay is relative to the selection of the positive control and may not be a true reflection of the specificity of the assay for a study sample.

Other Bioanalytical Parameters

Precision is the quantitative measure of the random variation between a series of measurements from a method. Report intra-run and inter-run %CVs.

Robustness is an indication of the reliability of an assay, assessed by the capacity of the assay to remain unaffected by small, but deliberate, variations in the method parameters.

Ruggedness is usually the cross-validation across laboratories.

Statistical Evaluation

- Suggestions and Examples for statistical approaches are included in the Appendices
 - Balanced Experimental Design
 - Distribution of Results and Excluding Outliers
 - Comparison of Means and Variances for Cut Point
 - Relevance of the Cut Point
 - Calculation of Cut Point
 - System Suitability Controls (QCs)
 - Competitive Inhibition Specificity Cut Point
- One statistical approach does not fit all data sets...if the suggested statistical approaches are not appropriate, work with a statistician to apply appropriate models.

Take Home Message(s)

Can't see the forest for the corn?

- There are lot of details
 - Recommendations...white papers...regulatory guidances
 - Presentations on how sponsors addressed immunogenicity assessment
- Goal: Immunogenicity Assessment
 - Does the assay reliably detect the presence of ADA under the appropriate circumstances for the drug and study?
 - Apply scientific and statistical judgment, in addition to the Recommendations and Regulatory Guidances, to determine what is “appropriate”.
 - The assay is only a part of the immunogenicity assessment. Drug exposure, Drug Activity, and Clinical Observations are also good indicators of the presence of ADA.