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aaps White Papers

Recommendations for immunoassay methods used for the detection of host antibodies against biotechnology products

Paper #3: Immunossay Validation

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2003: The LBABFG Steering Committee tasked a newly created Action Program Committee (APC) with the goal to prepare consensus "best practice" recommendations on immunoassay development and optimization, bioanalytical testing strategy, and method validation.

Thanks to the subcommittee members (co-authors), FDA reviewers, and feedback from numerous Round Table attendees at the 2007 and 2008 AAPS NBC annual meetings

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Standardization

Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products

Anthony R. Mire-Sluis[a,*], Yu Chen Barrett[b], Viswanath Devanarayan[c], Eugen Koren[d], Hank Liu[e], Mauricio Maia[f], Thomas Parish[g], George Scott[h], Gopi Shankar[i], Elizabeth Shores[j], Steven J. Swanson[d], Gary Taniguchi[k,†], Daniel Wierda[l], Linda A. Zuckerman[m]

KEY MESSAGES:

- ADA immunoassays qualitative (screening) or quasi-quantitative (titration)
- Assay quality controls: positive and negative controls should be used
  - When possible, polyclonal antibody positive controls are preferred
  - Depending on the assay type, species–specific assays should preferably have species specific controls
- Use a risk-based approach to assure that low positive samples can be identified, and false-negative samples are limited. The screening assay cut point should be computed to allow (theoretically) the selection of of 5% false-positive samples
- Immunoassay sensitivity recommendations:
  clinical – 250 to 500 n/mL
  non-clinical – 500 to 1000 ng/mL

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Paper #2 2008

Review

Recommendations on risk-based strategies for detection and characterization of antibodies against biotechnology products

Eugen Koren [a,*], Holly W. Smith [b], Elizabeth Shores [c], Gopi Shankar [d], Deborah Finco-Kent [e], Bonita Rup [f], Yu-Chen Barrett [g], Viswanath Devanarayan [h], Boris Gorovits [i], Shalini Gupta [j], Thomas Parish [k], Valerie Quarmby [l], Michael Moxness [j], Steven J. Swanson [j], Gary Taniguchi [m], Linda A. Zuckerman [n], Christopher C. Stebbins [l], Anthony Mire-Sluis [o]

- **A risk-based, tiered, bioanalytical strategy for the testing and characterization of ADA**

- **Separate testing strategies were recommended for non-clinical studies and the 4 Phases of clinical drug development**

KEY MESSAGES:

- Test all pre-dose (baseline) and post-dose samples
- ADA specificity confirmation by competitive drug inhibition
- Titer preferred; additional characterization may be needed for higher risk drugs
- Due to the possibility of assay interference, binding ADA or NAb negative samples containing quantitative levels of drug should be reported as Negative with "a statement of possible drug interference" accompanying the result.
- Declining PK, or changing PD, values may indicate the presence of ADA that was undetectable in the immunoassays

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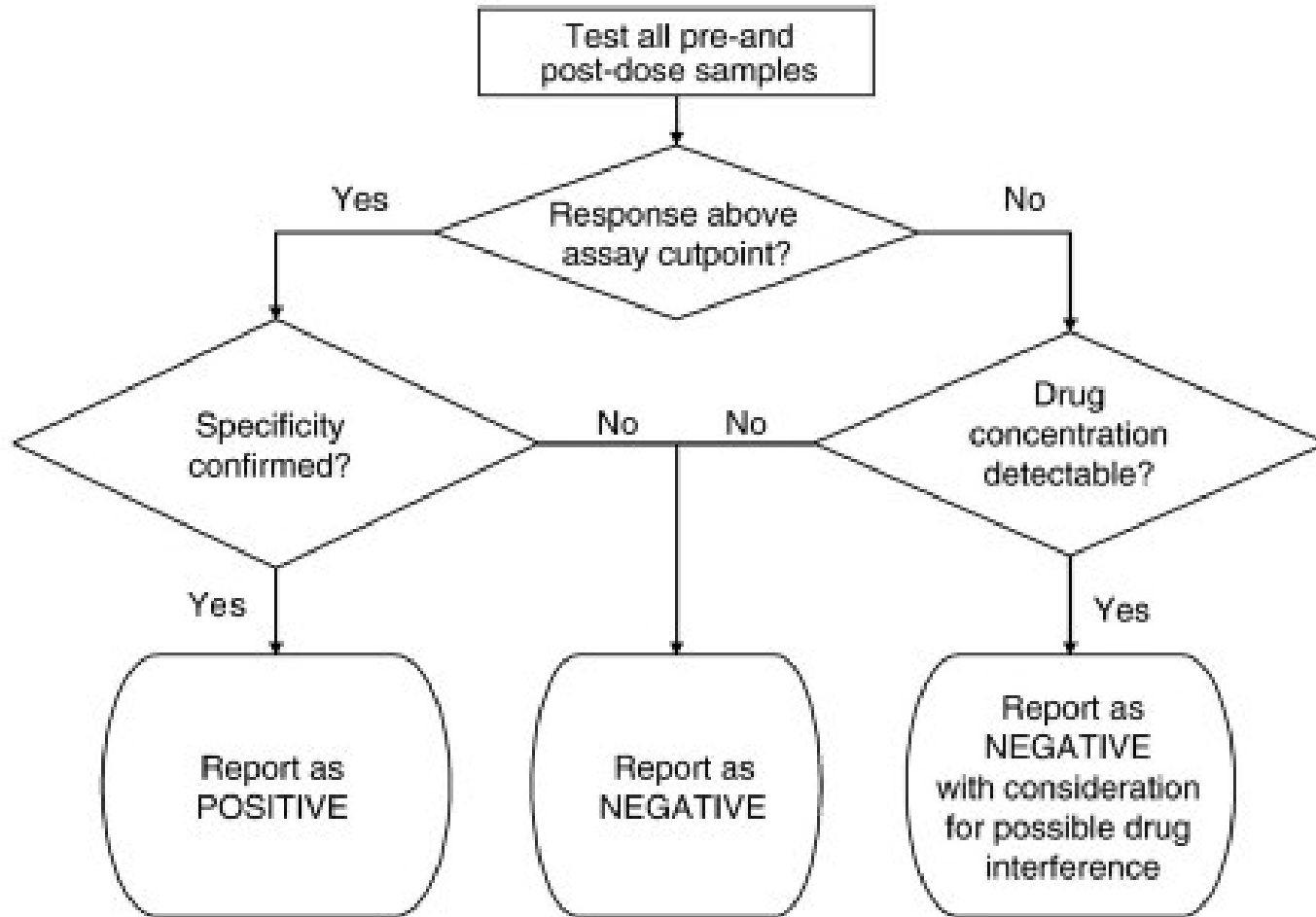
ADA Detection Strategy

Fig. 1. STEP 1: Recommended testing strategy for detection of anti-drug antibodies.

From: Koren, E., *et al*. (2008) Recommendations on risk-based strategies for detection and characterization of antibodies against biotechnology products. *J Immunol Meth*, 333: 1-9

Review

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

Gopi Shankar[a], Viswanath Devanarayan[b], Lakshmi Amaravadi[c], Yu Chen Barrett[d], Ronald Bowsher[e], Deborah Finco-Kent[f], Michele Fiscella[g], Boris Gorovits[h], Susan Kirschner[i,1], Michael Moxness[j], Thomas Parish[k], Valerie Quarmby[l], Holly Smith[m], Wendell Smith[n], Linda A. Zuckerman[o], Eugen Koren[p,*]

• Consensus recommendations *for pre-study validation of* ADA screening, specificity confirmation, and titration immunoassays were provided in detail, with advice also provided for *in-study validation* and *revalidation*

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Why validate?

• To be able to detect the analyte reliably. Assay should remain in control long after development and produce same results when performed by multiple analysts, when transferred to other laboratories, etc.

• It s a regulatory requirement for GLP non-clinical and pivotal clinical studies. You cannot get a drug approved without adequate bioanalytical method validation.

Definition*: *Validation is the process of demonstrating, through the use of specific laboratory investigations, that the performance characteristics of an analytical method are suitable for its intended analytical use*

*Source: V.P. Shah, et al., J. Pharm. Sci. 81 (1992) 309-312

Guidance from regulatory documents

ICH Q2A (Mar 1995)

ICH Q2B (Nov 1996)

Specificity

Accuracy

Precision

Detection Limit

Quantitation Limit

Linearity

Range

Robustness

Stability

System Suitability testing

Bioanalytical Method Validation,U.S. DHHS, FDA, CDER, CVM. Guidance for Industry (May 2001)

Accuracy

Precision

Selectivity

Sensitivity

Reproducibility

Stability

Standard curve

Linearity

Quantitation Limits (LLOQ and ULOQ)

EMEA/CHMP guidance on immunogenicity assessment (Apr 2008)

Linearity

Accuracy

Precision

Sensitivity

Specificity

Robustness

Inter-laboratory variance

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Assay performance characteristics for validation:

1. Screening cut-point

2. Specificity cut-point

3. Sensitivity

4. System suitability control (QCs) acceptance criteria

5. Interference

6. Precision

7. Robustness

8. Stability

9. Ruggedness, when applicable

Note:

- To ensure objective criteria, use of statistics is important

  - need not be complicated; can do without an expert statistician

- Analytical variation and biological variation:

  - Pooled matrix sample ≠ individual matrix samples

- Capturing analytical + biological variation is critical for certain assay performance characteristics

Screening cut-point

- "A screening assay that does not identify any reactive samples whatsoever can cast doubt on the ability of the assay to detect low positive samples. A screening assay that picks up some (e.g., ≥5%) positives that can subsequently show to be non-specific in a confirmatory assay provides assurance that true low positives can be detected." *Mire-Sluis et al, 2004.*

- To distinguish negative samples from 'potentially positive' samples a cut-point is determined by parametric or non-parametric approaches to allow for a theoretical 5% false-positive rate.

- Usually a floating cut-point, but sometimes a fixed or even dynamic cut-point may be used.

- Samples from drug-naïve individuals are evaluated (□15 animal samples or □ 50 human samples, when possible)

  - Typically evaluated at 2 times by at least 2 analysts (at least 4 runs)

  - Use a balanced design in order to minimize confounding influences

Types of Screening Cut-Point

- Determine the type of cut-point (fixed, floating, or dynamic):

    - ✓ A **fixed cut-point** is appropriate whenever the mean and variance are similar between runs

    - ✓ A **floating cut-point** should be considered when the variance is similar but the means are not similar between repeated runs

    - ✗ A **dynamic cut-point** should be considered when the variance is dissimilar between repeated runs (irrespective of means from repeated runs)

- If there are significant differences between subject subpopulations, determine a different cut-point f or each subpopulation

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Specificity cut-point

- Expressed as the % inhibition of signal due to drug

  % inhibition = 100*[1-(signal of sample + drug)/(signal of sample alone)]

- At least 25 naïve individual samples pre-incubated with or

  without drug

    - Typically evaluated at 2 times by at least 2 analysts (at least 4 runs)

    - Balanced design

- % "inhibition" of signal due to drug calculated for each sample

- Cut point = Mean inhibition + 3.09 SD or 2.33 SD
  (i.e., allowing a false-positive rate of 0.1% or 1%)

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Cut-point issues…

- Very low screening cut points

- May need "titration cut point"

- Very low specificity cut-points

Sensitivity

- Caveats

    - Positive control antibody may not represent ADA in subjects

    - Assay will appear more sensitive for high affinity ADA versus low affinity ADA

- Positive control antibody is serially diluted in pooled matrix (spanning the cut-point) and tested in multiple runs of the assay (preferably more than 1 operator).

- Sensitivity =

    - The lowest conc of antibody that is consistently (in all runs) above the screening cut-point, OR

    - Mean of the antibody concentrations interpolated at the cut-point (note: a sample with this level of antibody would be detectable in the assay only 50% of the time), OR

    - Mean of the antibody concentrations interpolated at the cut-point + 95th or 99th confidence interval

System Suitability Controls

- "Consistency" controls

  - Matrix negative controls

  - Diluent negative control (optional, but useful)

  - Low positive ADA

  - High positive ADA (optional, but useful)

- Specificity controls

  - Uninhibited control (ADA – drug)

  - Inhibited control (ADA + drug)

- At least 2 analysts x 3 runs each (use all available data)

- Acceptance criteria for consistency controls **for in-study phase**:

  - For negative controls, < screening cut-point

  - For low positive control, based on a predicted 1% failure rate with respect to high and low extremes

  - For high positive control, based on a predicted 1% failure rate with respect to the low extreme

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Interference

- Drug Interference: ability of drug or other relevant factors (i.e. drug target) to impact assay results

  The low positive control is preincubated with serial dilutions of drug, and then tested in the assay. The lowest concentration of drug that prevents the detection of the low positive control is the "drug tolerance" of the assay

- Target interference in bridging assays: negative interference by monomeric targets or positive interference by multimeric drug targets (not described in detail in the white paper)

  Negative interference: low positive control is preincubated with serial dilutions of drug target, and then tested in the assay. The lowest concentration of drug target that inhibits the detection of the low positive control is the "drug target tolerance" of the assay

  Positive interference: drug target is serially diluted in matrix control and tested in the assay. The highest concentration of drug target that produces assay signals equal or above the screening assay cut point is the "drug target tolerance" of the assay

Precision

Precision is a quantitative measure of the random variation between a series of measurements from a method.

- All controls are evaluated for precision. Data is omitted from precision only when 1) a deviation from the assay method or an error occurs, or 2) the assay method is intentionally altered.

- Screening assay precision: intra-assay and inter-assay

- Specificity assay precision: inter-assay precision

Robustness

- Deliberate alterations in assay conditions (incubations, temperature, batches of critical reagents, etc.)

Stability

- Stability of drug-specific ADA determined in matrix at -70, -20°C, 4°C, 25°C.

- Stability may be performed for ADA against a "class" of drugs

Ruggedness

- Relevant when method is transferred across laboratories.

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GLP Considerations

- Prepare a validation plan
- Check that equipment calibrations are current
- Validate new equipment and new data capture systems (LIMS)
- Validate data analysis spread sheets
- Prepare an assay checklist
- Draft SOPs for any new reagents and screen, titer, and specificity assays
- Maintain reagent tracking log (freezer inventory & traceability)
- Document analyst training on the method
- Assemble a validation binder (validation plan, experiments, any paper calculations/data/metadata, certificates of analysis, email, deviations, etc)
- Prepare a validation report
- Document method transfers to other groups and trainings on the method

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CONCLUSION

Review

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

Gopi Shankar[a], Viswanath Devanarayan[b], Lakshmi Amaravadi[c], Yu Chen Barrett[d], Ronald Bowsher[e], Deborah Finco-Kent[f], Michele Fiscella[g], Boris Gorovits[h], Susan Kirschner[i,1], Michael Moxness[j], Thomas Parish[k], Valerie Quarmby[l], Holly Smith[m], Wendell Smith[n], Linda A. Zuckerman[o], Eugen Koren[p,*]

KEY MESSAGES:

- Objective decision criteria are critical; subjective approaches should be eliminated/minimized
- Alternate objective approaches may also be applied
- Application of statistics is important (balanced experimental design, outlier exclusion, etc)
- Assay means and variability across runs drives choice of screening assay cut point: fixed, floating or dynamic
- Specificity cut point should be based on analytical and biological variation (like screening cut point)
- Positive controls used to validate methods may not represent the analyte (ADA)
- CAUTION: over-reliance or dependence on quantitative data (sensitivity, drug tolerance) generated using positive control "standard" reagents
  - Sensitivity and drug tolerance limit can vary widely based upon the analyte (ADA) avidity/affinity and are therefore "soft estimates"

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