

Immunogenicity at a Glance

- Biotherapeutics are large molecules such as monoclonal antibodies or peptides and can cause an adverse immune response. (1)(2)
- Immunogenicity testing requires complex validation schemes and keen scientific expertise and collaboration with each sponsor. (3)
- The 2004 Mire-Sluis et al. publication provides scientific recommendations for validating and characterizing ADA assays. (4)
- Generally, a non-GLP environment is acceptable for ADA testing in clinical samples, but GLP is often requested when there is also a need for PK testing.
- A risk-based approach is recommended for determining frequency of testing. (5)

Related Information

- Anti-drug antibody assays are highly recommended for large-molecule drugs; biologics with high risk are nonhuman or chimeric proteins, or frequent dosing
- Even generic biologics (bio-similars) will most likely need immunogenicity testing during clinical trials.
- ELISA and ECL on the MSD platform are most frequently used for ADA assays
- Immunogenicity Testing is recommended for biotherapeutics such as:
 - Enzymes and regulatory proteins
 - Hormones
 - Modified natural enzymes
 - Monoclonal antibodies
 - Natural Interferons
 - Peptides (e.g. GLP-1)
 - Targeted proteins (e.g. immunoadhesions)

Available at PBI Lab

Immunogenicity Testing

- Quantitative ligand binding assays for ADA
- ELISA and ECL on MSD
- Full regulatory compliant validation
- EP Evaluator™ Validation Report

Clinical Biomarker Services

- Development of novel assays
- Validation of quantitative ligand binding methods
- Transfer & validation of a Sponsor initiated method
- Validation/Verification of commercial kit assays
- Platforms for novel biomarkers:
 - Bio Plex-Luminex™ 200
 - Elecys® Automated Analyzer
 - ELISA, EIA
 - Gradient Gel Electrophoresis
 - Hitachi Mod P® Chemistry Analyzer
 - HPLC
 - Meso Scale Discovery (MSD®)
 - Radiometric Immunoassay (RIA)
 - Siemens Immulite®
 - Ultracentrifugation

Regulatory Compliant Analytical Validation

- Preparation of validation protocol details
- Preparation of validation reagents, standards, QC
- Full regulatory compliant assay validation:
 - Minimum dilution
 - Sensitivity
 - Precision
 - Specificity
 - Cut-point determination for immunogenicity
 - Performance with normal human serum samples
 - Free drug interference
 - Robustness
 - Ruggedness
- Preparation of bioanalytical validation report

Go to www.pacbio.com for more information or call us at 800.767.9151

FEATURED:

Immunogenicity

Guiding development and improving the safety and efficacy of novel drugs.

SAMPLE REQUIREMENTS

Optimum volume: 0.5 mL

Sample Type: Serum or Plasma

Method: ELISA, MSD, others

PBI

Pacific **Biomarkers**

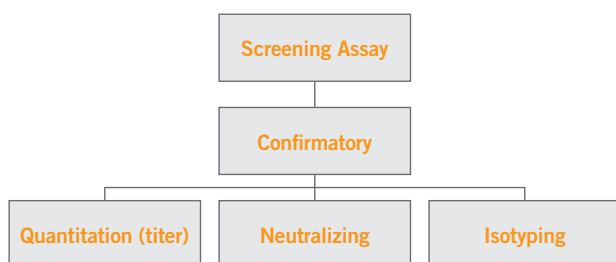
The Value of Expertise

Background Information

Immunogenicity is a measure of the immune response to a biotherapeutic drug. It is a very relevant problem affecting not only the use of therapeutic protein drugs such as monoclonal antibodies but also peptides, enzymes, cytokines, growth factors, engineered proteins and other biological products. The development of anti-drug antibodies can cause allergic or anaphylactic reactions, reduction of efficacy, and/or induction of autoimmunity. In the wake of such effects observed in the clinical trial of earlier protein therapies, the FDA and responsible pharmaceutical companies are insisting that immunogenicity testing become an integral part of protein development. (1)(2)

During the next decade, biopharmaceutical companies hope to introduce a new generation of antibody and biologic drugs that is safer and more effective. Many members of this new category are fragments of antibodies that can reach targets that whole antibodies cannot. Some are proving useful for treating diseases once thought to be beyond the reach of antibodies.

Immunogenicity Testing Strategy



Clinical consequences of immunogenicity include altered clearance and assay interference for pharmacokinetics. Factors contributing to immunogenicity include: genotype of the patient, therapeutic protein sequences, uptake by immune cells, and modification in formulation (glycosylation, chemical modification, PEGylation). Other factors that contribute include: pre-and co-medications, route of administration (IM, IV, etc), formulation, dose, and frequency of dosing.

Most biologics elicit some level of antibody response. Since this antibody response could lead to potentially serious side effects, it is necessary not only to screen for immunogenicity, but also to quantify and characterize the antibody response. Two publications provide recommendations based on the experience of the consortium of authors for the development of anti-product antibody immunoassays intended for clinical studies. (3)(4)

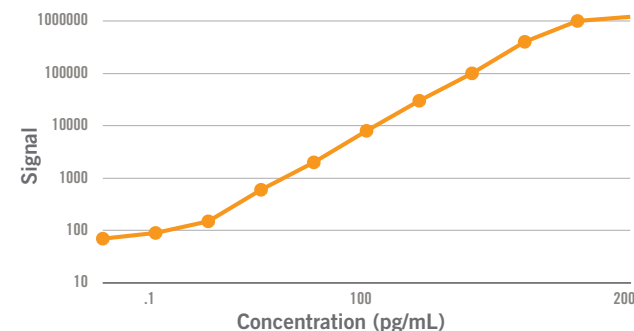
Many anti-drug antibody (ADA) assays are performed by ELISA, but electrochemiluminescence (ECL) has several advantages and so is increasingly being used for this application. Compared to ELISA, advantages of ECL on the Meso Scale Discovery (MSD) platform include: better free drug tolerance, detection of low-affinity antibodies, higher throughput, improved sensitivity, increased dynamic range, and higher binding capacity. In addition to immunogenicity testing, the MSD platform has the advantage of multiplexing several assays for biomarker analyses simultaneously.

While immunogenicity is a clear concern with monoclonal antibodies, it may be an even more important issue with certain biological therapies that are not monoclonal antibodies. ADAs to proteins that are endogenous in the body offer the potential for causing severe side effects. It can be expected that higher-risk emerging therapies will be evaluated for immunogenicity more frequently than lower-risk therapies. (5)

| | TYPICAL ELISA | HIGH SENSITIVITY ELISA | MSD ULTRASENSITIVE |
|-----------------------|---------------|------------------------|--------------------|
| Detection Limit pg/mL | 15 - 30 | 0.2 | 0.25 |
| LLOQ | 20 - 50 | 0.5 - 1 | 0.5 - 2 |
| Upper Limit | 200 - 1000 | 32 | 2500 |
| Sample Vol (uL) | 200 | 50 | 25 |

Supporting outsourced clinical development services for biotherapeutics requires unique capabilities and expertise because clinical development and regulatory approval are very complex processes. There is often a reluctance to outsource because of the extraordinarily complex nature of these quasi-quantitative

MSD: Large Dynamic Range Assays with High Sensitivity



MSD Ultrasensitive TNF-alpha

- Detection Limits (pg/mL): 0.25
- LLOQ (pg/mL): 1-2
- Upper End (pg/mL): 2500
- Sample (uL): 25

Most ELISA assay formats do not allow for measurement of normal and disease populations with a single dilution.

immunoassays, and because guidelines by regulatory agencies are constantly evolving. Expert scientific experience is required to collect and interpret data to make decisions on the efficacy and safety of new drugs. PBI is in a unique position in this regard since the organization has been specifically built to address this need, with a high proportion of Ph.D. scientists on staff.

References: 1. E. Check, *Nature* 26 April 2007, pp 964-966. Antibody Therapy: Clinical Trials and Tribulations. 2. B. Leader, Q.J. Baca, D.E. Golan, *Nature Reviews: Drug Discovery* Vol. 7, January 2008, pp 21-39. Protein Therapeutics: A summary and Pharmacological Classification. 3. A.R. Mire-Sluis et al. *Journal of Immunological Methods* Vol 289, 2004, pp 1-16. Recommendations for the Design and Optimization of Immunoassays used in the Detection of Host Antibodies Against Biotechnology Products. 4. G. Shanker, E. Shores, C. Wagner, A.R. Mire-Sluis, *Trends in Biotechnology* Vol. 24 (6) pp 273-280, Scientific and Regulatory Considerations on the Immunogenicity of Biologics. 5. G. Shanker, G. et al. *Nature Biotechnology* Vol. 25 (5), pp 555-561. A Risk-Based Bioanalytical Strategy for the Assessment of Antibody Immune Responses against Biological Drugs.