SQI Diagnostics Ig_PLEX Assay: Aligned with Immunogenicity Testing FDA and EMA Guidance

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SQI Diagnostics, Toronto ON Canada, 9-Oct-2012

In the past 20 years there has been a steady rise in biopharmaceutical drug offerings and diversity. The surge of new therapeutic proteins has widened awareness of the extreme risks to some patients. Patient immune response to new therapeutic proteins varies greatly and inherent individual risk factors are not well understood. Methods to determine anti-drug immune response more clearly continue to evolve in order to meet the demand for sensitive and robust methods that remain efficient and cost effective.

Historical Perspective

Methods to identify and characterize antidrug antibody (ADA) response during drug development are quickly advancing. While new devices promise to increase sensitivity and precision, regulatory bodies are struggling to standardize immunogenicity testing and understand the implications of evolving technologies (1). It is understood that patient health is paramount and a "harmonized" process is necessary to ensure risk factors are mitigated. In an effort to standardize this growing field, several regulatory bodies have issued guidance documents that outline the critical components of immunogenicity testing (2). The intent of these documents is to focus the industry's endeavors on key assay outcomes and analytical performance standards. It is well understood that the variability of immune responses make a singular method of ADA assay development difficult to conceive. With this in mind, market stakeholders and regulatory bodies are motivated to determine a set of parameters that ensure testing effectiveness while allowing flexibility in an evolving market.

Statistical and analytical performance characteristics generally discussed in guidance documents are variability, precision, selection of controls, and cut point value (or cut-off). Current detection methods have their own strengths and weaknesses in addressing each step of ADA detection design (3). Presently ADA assay design includes screening, specificity confirmation, and quantitation.

Introduction to SQI Diagnostics Ig_PLEX

SQI's Ig_PLEX technology is the world's first and only multiplexing methodology that allows simultaneous quantitation of immunoglobulin isotype and subclass for multiple proteins.

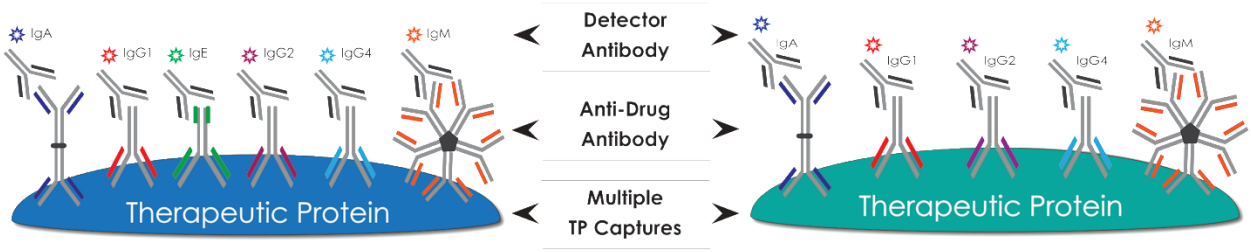
By incorporating planar microarrays printed in each of 96 wells on a functionalized glass plate, and a detector cocktail of anti-human secondary antibodies differentially labeled with fluorescent

dyes the quantification and classification of a patient immune response can be measured in a single test.

Applying this technology to bioanalytic analysis involves printing the therapeutic protein as multiple 200 micron spots on our covalent binding surface. Biosimilar drugs or metabolites can also be printed to assess specificity or cross-reactivity of anti-drug antibodies (ADA) from patient serum.

Following incubation with patient sample, the captured ADAs can be probed for immunoglobulin isotype (G, A, E or M) and/or IgG subclasses (IgG1, 2, 3, 4).

The customizable reporter cocktail is comprised of labeled secondary antibodies each with a fluorophore of distinct excitation and emission spectra (e.g. Anti-IgG1-RPE, anti-IgG2-FITC, anti-IgE-Cy3). Specific reporter signals are detected and quantitated by a multi-wave length scanner. Assay conditions are optimized to achieve the required sensitivity and precision and minimize cross reactive or non-specific binding of each reporter.



Alignment with Regulatory Guidance and Industry Needs

Prominent throughout regulatory guidance and industry "white papers" is method harmonization (4,5). Current trends show a diverse use of technologies during various phases of biopharmaceutical drug development. SQI Diagnostics' Ig_PLEX technology is uniquely designed to meet or exceed expected performance standards across the testing spectrum, while accelerating the immunogenicity testing process. We have leveraged the technology underpinning commercially launched *in-vitro* diagnostic multiplexed automated microarray assay system to satisfy the high standards and requirements for statistical and analytical performance. SQI's strict adherence to clinical grade assay development provides multiple alignments within regulatory guidance.

Harmony

SQI Diagnostics' Ig_PLEX technology provides a unified approach across the immunogenicity testing spectrum and promotes harmony on two levels. SQI's advanced traditional method is ideal for multiple testing stages: PK, ADA screen, specificity confirmation, titer, bridging assays, drug tolerance, drug on board, etc. Ig_PLEX is uniquely designed for within assay harmonization as well. Simultaneous detection of ADAs to multiple therapeutic proteins at the isotype / subclass level is optimal for unified results. By performing sample screen and isotype / subclass quantitation simultaneously, the patient samples are treated and analyzed uniformly.

Concurrent homogeneous sample analysis offers a single uniform result across screening and quantitation.

Equivalence

Utilizing standard immunoassay capture techniques, SQI's Ig_PLEX technology is equivalent to the inherent technology of the industry's most common ADA detection method, ELISA (6). This similarity has been proven by SQI with excellent predicate agreement during device equivalency studies (7).

Sensitivity

Due to inherently low background interference, SQI's Ig_PLEX technology has shown consistency in attaining sensitivities in the pg/mL range (8). SQI's planar capture techniques also provide dynamic analytical sensitivities across a wide range of antibody affinities commonly seen in traditional ELISA assays. Fixed / bound captures reduce protein-protein interactions and reduces non-specific binding seen in some bead-based methods.

Precision

SQI understands the complexity of protein epitopes and how to print and present them consistently. Multiple tiers of quality control are used to monitor spot alignment, morphology and spot consistency. SQI utilizes state of the art printing technology that provides <2% CV that provides completed plates of <10% CV. Rigorous cGMP requirements dictate that strict lot-to-lot equivalency standards are maintained.

Efficient Optimization

SQI's extensive assay development experience offers multiple optimization models. Testing protocols can be created for bench or automated runs and then validated at the user's facility. The automated load & go SQiDlite™ System offers an open assay development tool kit to accelerate automation optimization while reducing variability.

Method

Screening and Specificity Confirmation

In assays, targets of interest are printed as microarrays in a 96 well format. Following incubation with patient sample, a reporter cocktail containing multiple anti-human secondary antibodies, each with a different fluorescent tag are added to each well. The signals are detected and quantitated by a multi-wave length scanner. A primary screen for ADAs and specificity confirmation can be run simultaneously on the instrument, even as the assay can be configured to differentiate each of the Ig classes. In this case, each patient sample would be pipetted into two wells of a dilution plate and be incubated in the presence and absence of free drug. The diluted serum (+/- drug) is then transferred to two separate wells of the assay plate and the remaining assay steps are carried out through signal detection. Analysis algorithms

report the quantitation of each of the included Ig classes and any reduction in signal resulting from preincubation with free drug.

Titer

SQI currently produces assays for the IVD market and assay development protocols regularly include establishing a starting sample dilution and a cut off range for each isotype or subclass. These dilutions and definitions will be used for the screening assay. Negatives and low positives (no further dilution needed) will be reported at the established dilution (titer). For mid to high positive samples, 4-8 wells of a second plate can be run with a dilution series to get the signal to the cut off range. The advantage remains that all of the Ig response(s) (isotype or subclass) can be titered simultaneously in those 4 wells.

Additional Assay value

Because SQI prints multiple capture spots on the arrays, the response to any therapeutic protein can also be compared to biosimilars, metabolites, or dissected into subunit contribution of recombinant /chimeric constructs within one well. Each specific fluorescent signal is quantitated separately for each capture by our analysis software.

Conclusion

SQI Diagnostics' Ig_PLEX technology has been shown to exceed performance standards for detection sensitivity, precision, and efficiency while meeting the needs of regulatory and industry bodies. Ideal for multiple stages of therapeutic protein drug development, the Ig_PLEX advanced traditional method provides a unified development technology and harmonized multiplexed results.

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