Algorithme Pharma
AN ALTASCIENCES COMPANY

TRADITIONAL & NOVEL PLATFORMS FOR IMMUNOGENICITY ASSESSMENT OF BIOPHARMACEUTICAL PRODUCTS

Determining the Immunogenicity Profile of Biologics

Human administration of recombinant proteins or biological substances can elicit an immune response leading to the production of antibodies, which in some cases may neutralize their activity. This makes the assessment of immunogenicity an essential component of biologic safety evaluation. While bioavailability, pharmacokinetics and pharmacodynamics (PK/PD) can be effected, leading to impaired efficacy, such immunogenic responses can also induce allergic reactions where in some cases severe autoimmune reactions may occur.

Immunogenicity Platforms

Algorithme Pharma provides immunogenicity services by developing and applying methods commonly required in safety and efficacy studies, such as PK/PD, bridging immunogenicity, drug tolerance and neutralization assays.

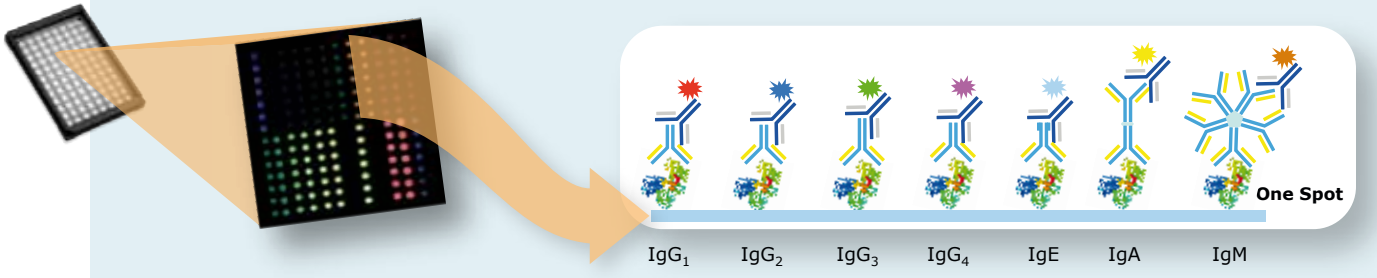
We currently offer screening assays, antibody characterization and quasi-quantitative titers that describe anti-drug-antibody (ADA) immune responses. These services are available on multiple platforms including the Mesoscale Discovery (MSD) Imager (ECL), Alpha-Lisa bead-based assays with luminescence or fluorescence detection, ELISA and our newest immunogenicity platform, the SQI Diagnostics' SQiDlite™ multiplexing system.

SQI Diagnostics' SQiDlite™

The SQI Diagnostics' SQiDlite™ system combines ADA screening and quantitative capabilities while simultaneously characterizing antibody isotypes (e.g. IgG, IgA, IgE or IgM), and subclasses (e.g. IgG1, IgG2, IgG3 and IgG4). This is achieved by using combinations of patterned microarrays and differentially labeled secondary antibodies. The SQiDlite™ demonstrates a very competitive platform for biomarker monitoring of human cytokines and chemokines in multiplexed (8 to 20 plex) modes in one well. (see figure 1)

This system allows for a reduction in the total number of tests performed by decreasing the number of wells needed to run a data set. This is made possible as ADAs are tested against their therapeutic compound and simultaneously tested against multiple proteins in the same well. The immune response can then be compared directly to multiple biosimilars or downstream metabolites all within the same serum sample. This novel technology drastically reduces overall cost and time.

Figure 1. Multiplexed Antibody detection in a single well.



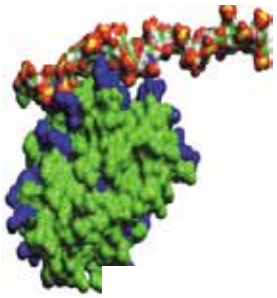
Case Study Using SQiDlite™ :
Detection of Heparin-Platelet Factor 4 (Hep-PF4) Autoantibodies Isotypes

Exposure to Heparin or Low Molecular Weight Heparin (LMWH) can result in the rapid formation of antibodies of any or all of the three major immunoglobulin classes IgG, IgA and IgM. This immune reaction is induced by the formation of Heparin/Platelet Factor 4 (Hep-PF4) complexes which bind with Hep-PF4 (AHP) autoantibodies. The resulting immune cluster activates platelets, and the formation of platelet microparticles, which initiate the formation of blood clots. As a result, the platelet count falls, leading to thrombocytopenia.

Hep-PF4 autoantibody subtypes (IgG, IgA, IgM) can be induced by any of the LMWHs with their relevance ranging from 12% to 18% in patients treated with different branded LMWHs. As seen with Heparin-induced thrombocytopenia (HIT) patients, there is a higher proportion of IgG type antibodies present. *(see Table 1)*

In collaboration with SQI Diagnostics, Algorithme Pharma has created a unique multiplexed immunoassay to characterize, (in a single well) the whole profile of antibody anti-Hep-PF4 isotypes in subjects treated with Heparin or LMWH products.

The assay developed has a limit detection of 2 ng/mL, a precision in the 9% to 15% range and is Heparin-dependent. Its sensitivity and specificity outperform that of typical ELISA based assays. *(see Figure 2)*



Heparin – PF4 Complex

The novel SQiDlite™ platform can be used to detect, characterize and simultaneously quantitate multiple ADAs related to a large variety of biologics including therapeutic monoclonal antibodies, proteins and peptides. Because of its high throughput and multiplexing capabilities, this platform is ideal for characterising ADAs in biologic and biosimilar development programs involving multiple clinical studies.

Table 1. Multiplexed HIT (Anti-PF4/Heparin) Assay

Sample Category	Laboratory-Defined (IgG Positive)			Clinically-Defined (HIT Positive)		
	IgG	**IgA**	**IgM**	**IgG**	**IgA**	**IgM**
% Positive Agreement (Sensitivity)	**86.4 (38/44)**	**72.7 (32/44)**	**52.3 (23/44)**	**100 (8/8)**	**100 (6/6)**	**83.3 (5/6)**
% Negative Agreement (Specificity)	**86.4 (57/66)**	**95.5 (63/66)**	**98.5 (65/66)**	**100 (0/8)**	**100 (2/2)**	**100 (2/2)**
% Overall Agreement	**86.4**	**NA**	**NA**	**100**	**100**	**87.5**

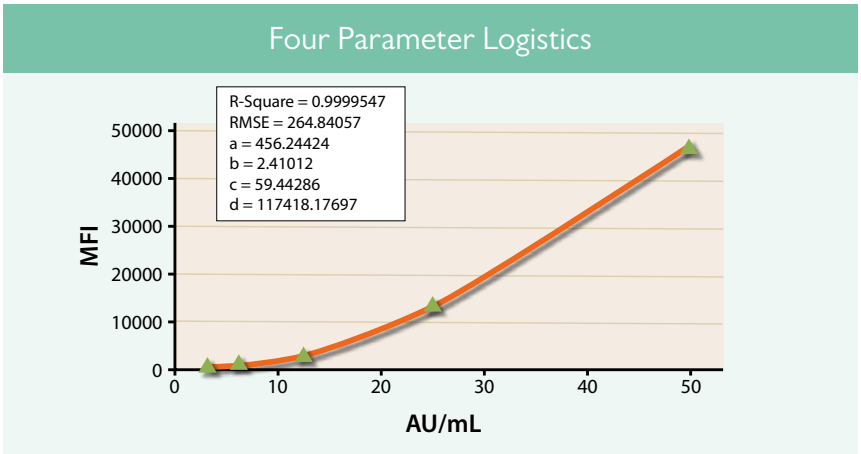


Figure 2. HIT Assay: Semi-Quantitative Standard Curve

Traditional and novel platforms that are used for immunogenicity of biopharmaceutical products, provide multiple options for selecting the ideal assay format. This selection is ultimately dependant upon the biologic under investigation and the specific study objectives.

Partnering with Algorithme Pharma has benefited many pharmaceutical and biotechnology companies by using the latest immunogenicity assessment platforms in developing methods commonly required in safety and efficacy studies.

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