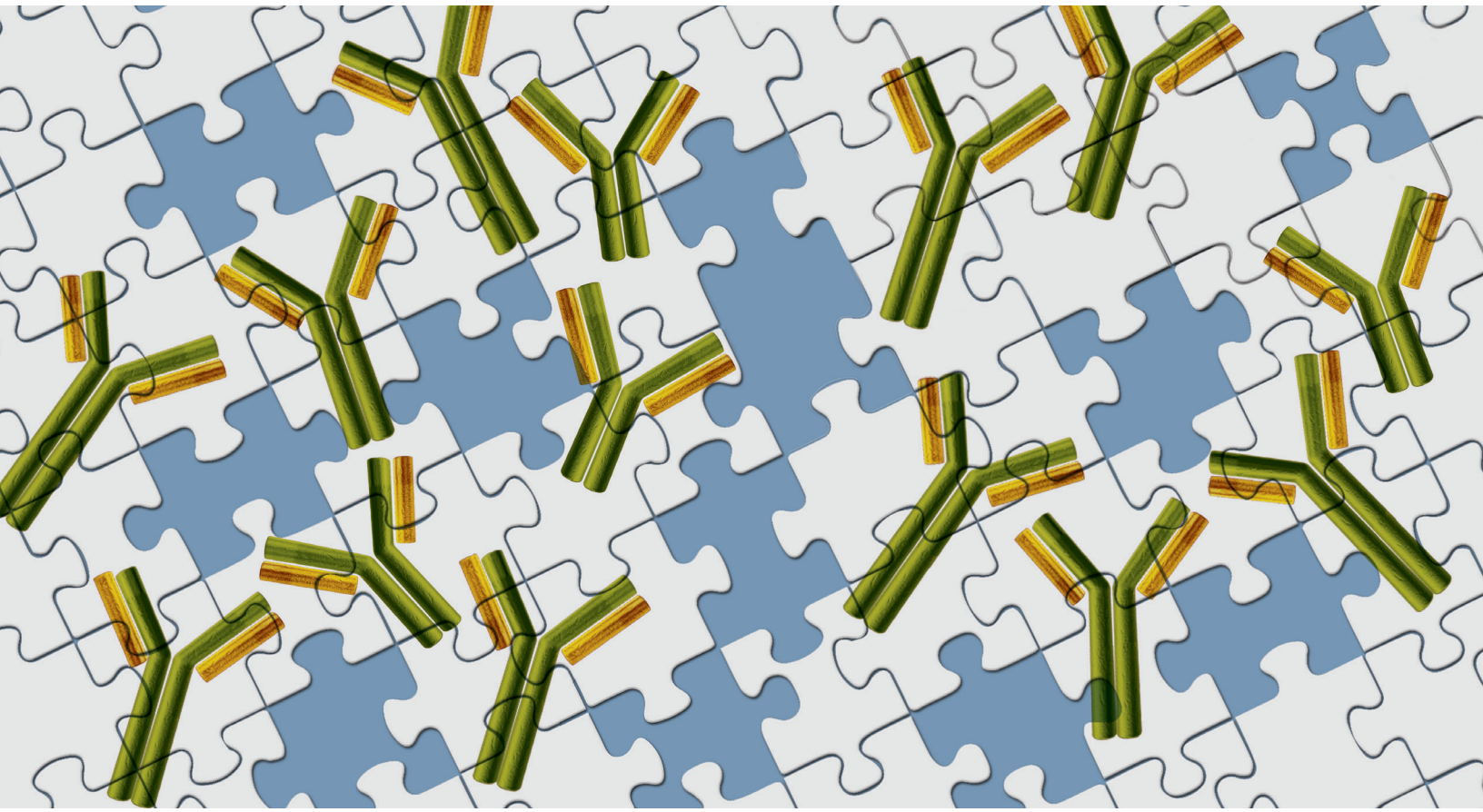
Genalyte

Are you sure your ELISA isn't missing something?



*This document refers to the Maverick™ Detection System in a Research Use Only application.

The generation of IgG4 anti-drug antibodies has been shown to coincide with repeated exposure to therapeutic proteins such as Factor VIII, IFN-β, and particularly TNFα inhibitors for autoimmune disease. [2,4-6]

Studies have shown that up to 45% of psoriasis patients taking infliximab or adalimumab exhibit detectable levels of ADA.5 Additionally, anti-adalimumab antibody formation has been linked to diminished clinical response in RA patients with IgG4 contributing a significant part of the ADA response.[3,4] This presents significant concerns todevelopers of new biologics and biosimilars as the FDA demands more thorough characterization of biologic therapeutics.[6]

FDA guidance for immunogenicity testing of therapeutic proteins calls for using a risk-based multi-tiered approach for anti-drug antibody (ADA) testing beginning with a screening assay that…

> "…should be able to detect all isotypes (particularly immunoglobulin M (IgM) and the different immunoglobulin G (IgG) isotypes)."[6]

Purported positive samples should be confirmed by a secondary assay and then…

> "… in some circumstances the applicant should develop assays that discriminate between antibodies of specific isotypes."[6]

Bridging ELISAs are the most commonly applied assay technology for ADA detection and require that a bivalent ADA cross-link two differentially labeled drug molecules in order to be detected. While it is an efficient and sensitive assay platform, it alone cannot satisfy the above requirements making development of multiple assays necessary.

Bridging ELISAs cannot detect all ADA isotypes

Most IgG4 ADA are monovalent due to Fab arm exchange.[2] A bridging assay's requirement for bivalency causes an understatement of immunogenicity since most IgG4 ADA will not be detected.

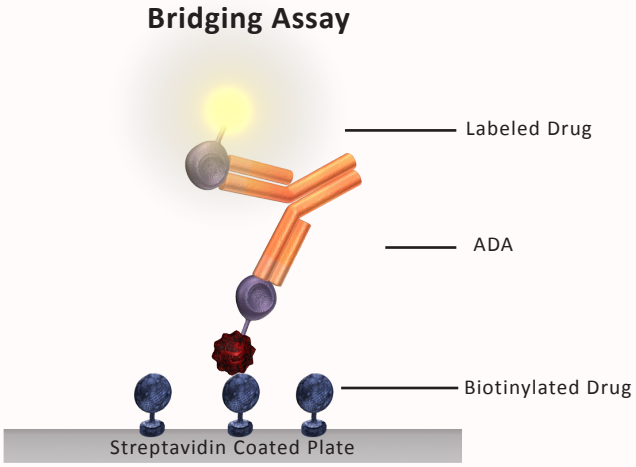
In 2011, Hart and colleagues reported that during the testing of 216 samples from rheumatoid arthritis patients (who were treated with adalimumab) for anti-adalimumab antibodies that the bridging ELISA lacked sensitivity to IgG4 ADA and was more susceptible to drug interference. [2]

When results from the anti-adalimumab bridging ELISA were compared to those from an antibody binding test (ABT) specifically designed to detect IgG4 there was a significant difference in the number of positives samples detected, demonstrating a failure of the bridging ELISA to detect IgG4 ADA.

- 7% of samples were positive for ADA by bridging ELISA.[2]
- 22% of samples were positive for ADA by the IgG4-specific ABT.[2]

Bridging ELISAs cannot discriminate between ADA isotypes in a single assay

Figure 1. *Bridging ELISAs are prone to free drug interference because both Fabs mustbe available for drug binding in order to cross-link and generate a signal.*[2]



Maverick MT-ADA Isotyped Immunogenicity Assay *Detect and Isotype Anti-Drug Antibodies in One Assay*

The Maverick™ MT-ADA assay detects ADA of all isotypes, including monovalent IgG4, and discriminates between the isotypes and subclasses in a single assay.

Photonic Ring Sensors on the assay chip are functionalized with anti-isotype capture antibodies that capture ADA by the Fc region. A drug-specific result is generated through the binding of streptavidin coated beads to biotinylated drug - ADA complexes.

This assay format offers two distinct advantages over bridging ELISAs and antibody binding tests.

i. **Multiplexing** - The inherent multiplexing capability of the Maverick assay chip detects and differentiates between all isotypes and subclasses simultaneously without additional assays or resources.

ii. **Fc Region Capture** - Both ADA Fab arms are available for drug binding, yet only a single labeled drug molecule is required to bind to the ADA inorder to generate a signal making detection of monovalent IgG4 possible.

The Maverick generates and displays data in real-time for each ADA isotype. The visual representation of the kinetics of binding events provides insight into ADA affinities and their impact on the final result, in contrast to endpoint assays that only provide a final result.

Multiple configurations of ADA - Biotinylated Drug binding result in a positive signal providing more opportunities for the assay to detect positive samples that may be missed in other assay formats.

Figure 2. *Maverick sensorgram generated by anti-adalimumab IgG4-positive sample BRH866102 shown in Figure 7.*

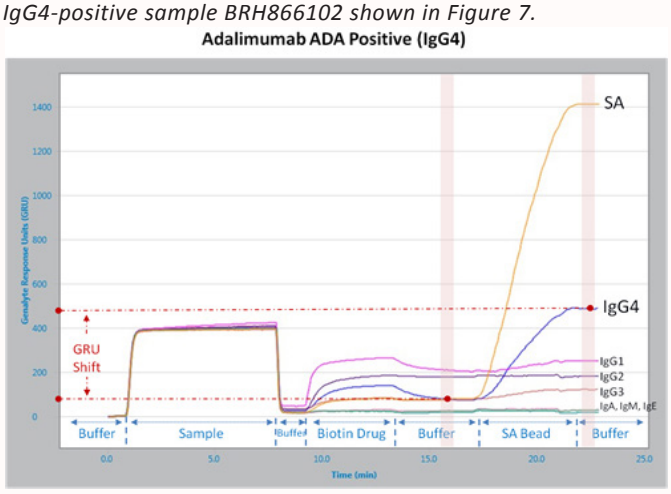
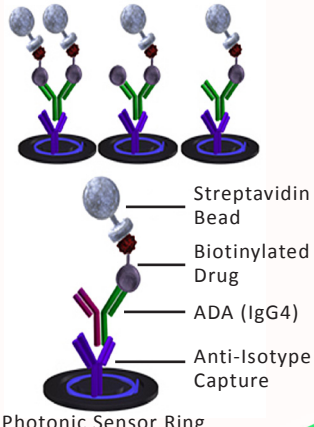


Figure 3. *Binding configurations causing a positive result.*



Photonic Sensor Ring

Figure 4. *Maverick sensorgram generated by anti-adalimumab negative sample BRH866098 shown in Figure 7.*

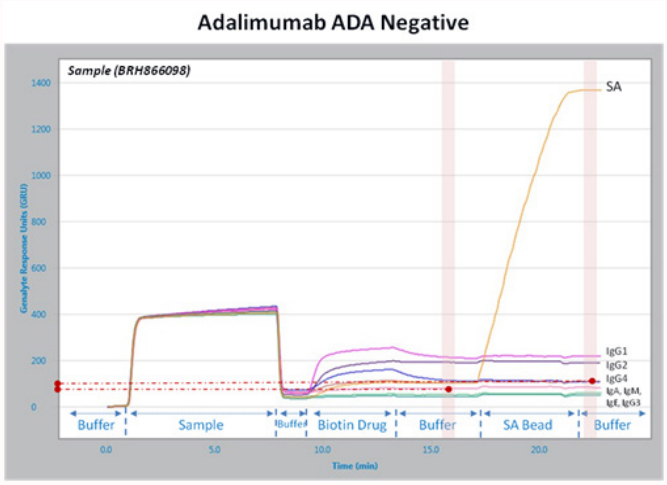
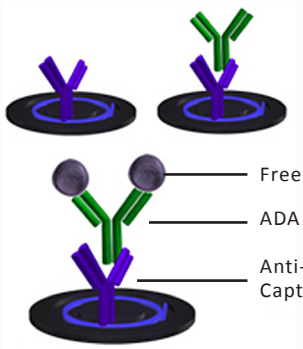


Figure 5. *Binding configurations causing a negative result.*



Photonic Sensor Ring

Maverick MT-ADA Assay Demonstrated in Multiplex Isotyped Detection of Anti-Drug Antibodies to TNFα Inhibitors - Adalimumab & Infliximab

Genalyte scientists developed multiplex isotyped anti-drug antibody assays for Adalimumab and Infliximab using commercially available monoclonal antibodies and the Maverick Human MT-ADA Assay Kit. Assay sensitivity and free drug tolerance we determined. Commercially sourced serum samples from patients taking one of the two drugs were tested on the developed assays and the multiplex isotyped ADA profile reported.

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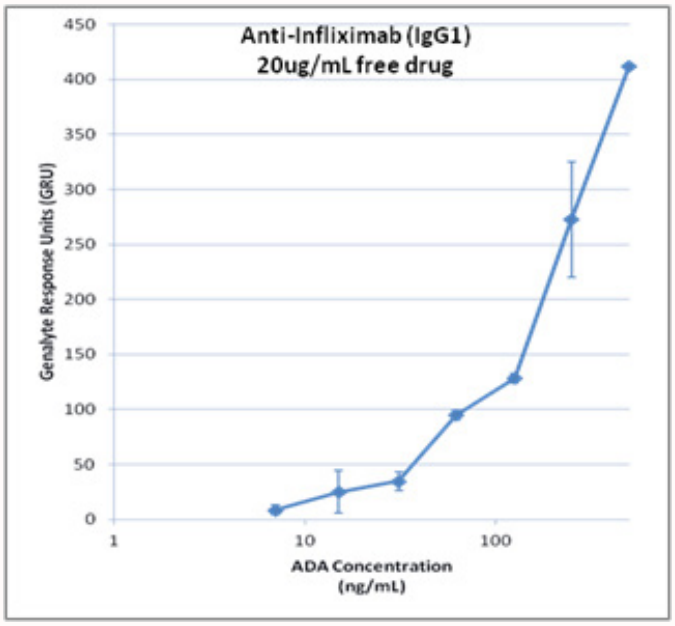
Adalimumab

An anti-adalimumab assay was developed using a commercially available anti-adalimumab monoclonal IgG1 antibody spiked into normal human serum. A titration was performed to determine assay sensitivity. Assay sensitivity of 62ng/mL ADA at 20µg/mL free drug was achieved (Figure 6).

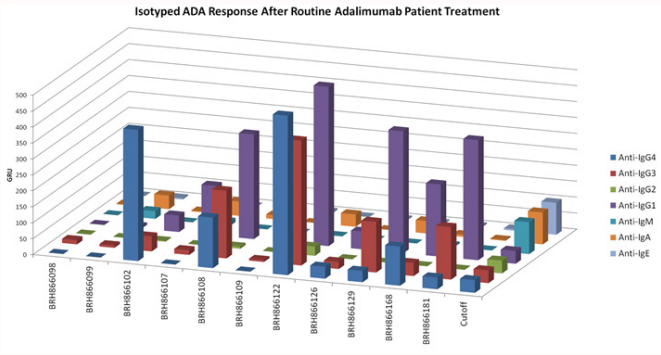
Figure 6. *Anti-adalimumab (human IgG1) sensitivity and free drug tolerance.*



Eleven (11) serum samples from patients on routine adalimumab treatment were obtained from a commercial source and tested on the multiplex isotyped ADA assay developed at Genalyte. Cut points for each isotype were set at 2x mean GRU shift from a set of normal samples tested with the same assay.

Multiple positive samples were detected (63%) with strong IgG4 reactivity in 4 of 7 positive samples.

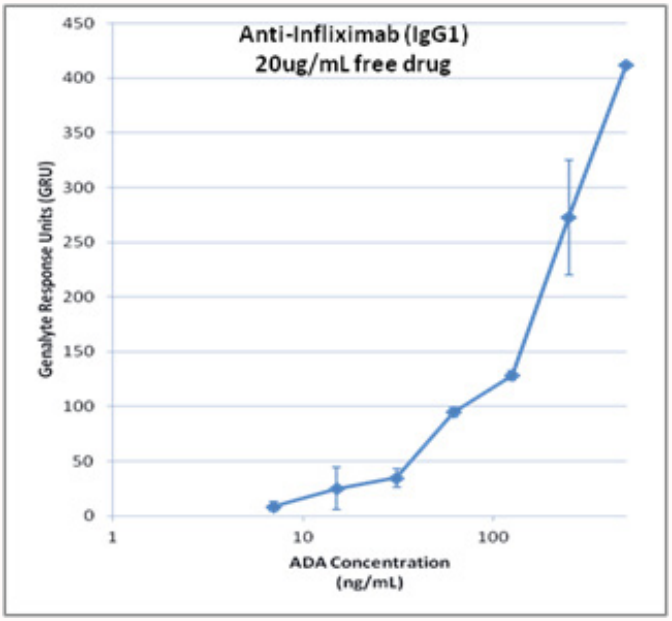
Figure 7. *Anti-adalimumab ADA isotype profile for 11 commercially sourced patient samples.*



Infliximab

An anti-infliximab assay was developed using a commercially available anti-infliximab monoclonal IgG1 antibody spiked into normal human serum. A titration was performed to determine assay sensitivity. Assay sensitivity of 62ngmL ADA at 20µg/mL free drug was achieved (Figure 8).

Figure 8. *Anti-infliximab (human IgG1) sensitivity and free drug tolerance.*



Forty-five (45) serum samples from patients on routine infliximab treatment were obtained from a commercial source and tested on the multiplex isotyped ADA assay developed at Genalyte. Cut points for each isotype were set at 2x mean GRU shift from a set of normal samples tested with the same assay. Multiple positive samples were detected (31%) with strong IgG4 reactivity in 10 of 14 positive samples.

Figure 9. *Anti-infliximab ADA isotype profile for 45 commercially sourced patient samples.*

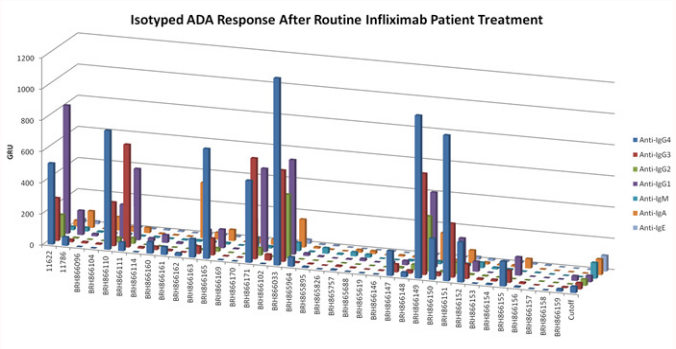
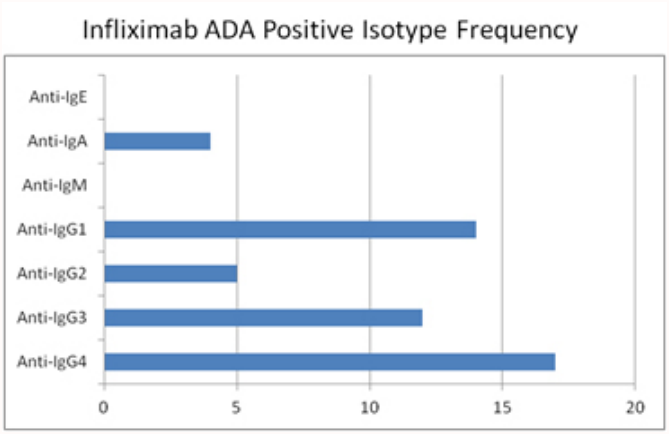


Figure 10. *Frequency of positive ADA isotypes for tested infliximab patient samples.*



CITATIONS

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2. Hart MH, et al., Differential Effect of Drug Interference in Immunogenicity Assays, Journal of Immunological Methods 372 2011, 196-203.
3. Bartelds G, Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up, JAMA, April 13, 2011, vol. 305, no. 14.
4. Schouwenburg A, et al., IgG4 Production Against Adalimumab During Long Term Treatment of RA Patients, J Clin Immunol. 2012, 32:1000-1006.
5. Hsu L, Snodgrass B, Armstrong A, Antidrug Antibodies in Psoriasis: A Systemic Review, Br J Dermatol., 2014 Feb; 170(2):261-73.
6. FDA Guidance for Industry – Assay Development for Immunogenicity Testing of Therapeutic Proteins, 2009.

Part No.	Description
85000	Maverick M24 Detection System
85023	Maverick Human MT-ADA Assay Kit
85060	Maverick Rabbit MT-ADA Assay Kit

Please inquire for other species

Genalyte

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