Towards Immunoassay Platform Convergence:
From ELISA to Multiplex Immunoassays

by Anteo Technologies

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Why ELISA has stood the test of time

Today, the enzyme-linked immunosorbent assay (ELISA) performed in a 96-well plate remains the mainstay of the clinical laboratory.

The ELISA technique, originally published in 1971 [1] [2], allows highly sensitive, qualitative and quantitative analyte detection within complex samples including plasma and serum. As a result, this singleplex immunoassay can be found in hundreds of FDA approved commercial products for the diagnosis of a broad spectrum of diseases.

The robust ELISA method, together with the introduction of hybridoma technology in 1975 [3], resulting in monoclonal antibody generation, further embedded the ELISA as the gold standard technique. Available in a number of different formats, the "sandwich" ELISA, in which a capture antibody is bound onto the plate substrate capturing the target antigen in the test sample, followed by addition of detection antibody, is by far the most common. Over the years, countless monoclonal antibodies have been specifically screened and selected for their ability to withstand passive adsorption onto ELISA plates resulting in high performance ELISAs further entrenching the method in the laboratory.

Multiplex immunoassays are increasingly being used

However, increasingly complex multifactorial diagnosis of cancers, neurodegenerative and autoimmune diseases together with monitoring of complex disorders and individual patient stratification in the new era of personalised healthcare, are stretching the ELISA beyond its limits.

To this end, multiplex assays in which multiple analytes can be quantitated within a single patient sample have been gaining market acceptance. Multiplex immunoassays can be either planar based, as in microscope slide and chip based arrays, or suspension based utilising microspheres. The former faces considerable hurdles as the successful immobilisation of many different proteins in their native conformation onto planar surfaces is technically challenging [4]. As a result the microsphere based multiplex immunoassays are the preferred technology with a number of FDA approved multiplex assays finding utility in clinical settings. Even so, a key challenge associated with these is in achieving fully functional antibody immobilisation. This is a crucial element in reducing problems associated with cross-reactivity between unrelated proteins, often observed in multiplex formats [5] and in particular when utilising antibodies validated for the ELISA singleplex method.

The difficulty in direct assay transfer from ELISA to Multiplex Immunoassays

Issues associated with cross-reactivity highlights a need for application specific monoclonal antibodies that are selected for optimal performance in the multiplex platform [5].

Unfortunately, the availability of well defined monoclonal antibodies with known cross-reactivity, affinity, specificity and kinetic parameters are limited. In order to achieve high assay performance in the multiplex platform, selection of the correct chemistry for antibody immobilisation must be considered in order to achieve high binding capacity, retention of immunologic activity, high signal-to-noise ratio, and low variability between batches [6].

To date the predominant chemistry relies on EDC/NHS covalent coupling, which is considerably different to passive adsorption techniques typically employed with ELISA. Ideally, when researchers adapt ELISA reagents to the multiplex platform it would be beneficial to use the same or similar antibody coupling method in order to retain the same antibody functionality regardless of the assay format.

The ways to overcome the barriers with Mix&Go™

Antibody immobilisation is critical to assay performance and whilst antibodies are less prone to denaturation than other proteins, even minimal perturbation of the tertiary structure, may expose hydrophobic regions, increasing non-specific protein binding and potentially affecting assay sensitivity.

Mix&Go technology helps overcome these issues by creating an activated surface that gently yet strongly binds proteins using metal chelation rather than covalent chemistry (Figure 1). In particular, scientists using the Luminex® xMAP® platform may encounter problems associated with coupling certain proteins to microspheres. There are many reasons why this happens, from protein incompatibility with covalent attachment to sub-optimal conditions during the coupling process. By using Mix&Go in combination with the Luminex xMAP technology, researchers will ensure gentle and secure antibody binding through metal chelation resulting in, less damage to antibodies, decreased background and less sample volume required to achieve comparable results to those obtained using ELISA.

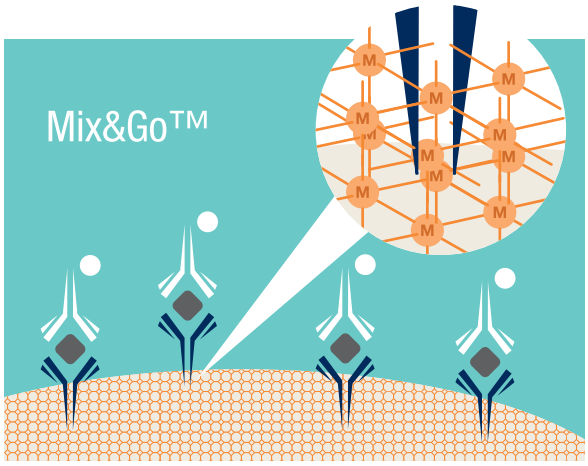

Figure 1 *Binding a protein via Mix&Go utilises multiple chelation points.*

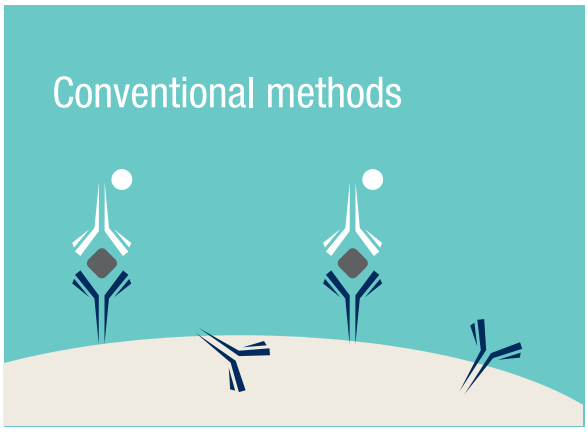

Figure 2 *Conventional covalent attachment chemistries, as well as passive adsorption to surfaces, tend to damage the attached biomolecules.*

What is Mix&Go and how does it work

Mix&Go is a series of reagents that contain hydrolytic oligomers of metal ions in aqueous solution. This polymer of metal ions, chelate and bind by avidity to both the surface and to biomolecules, acting as a molecular velcro`.

A single chelation point is not strong enough to bind a metal ion to a synthetic surface or to bind a biomolecule to the metal ion. Mix&Go technology overcomes this limitation by using polymeric metal ions that form multiple chelation points with both the underlying surface and the biomolecule of interest (Figure 3). Together many such interactions form very strong, yet flexible bonds that are as secure as a conventional covalent bond.

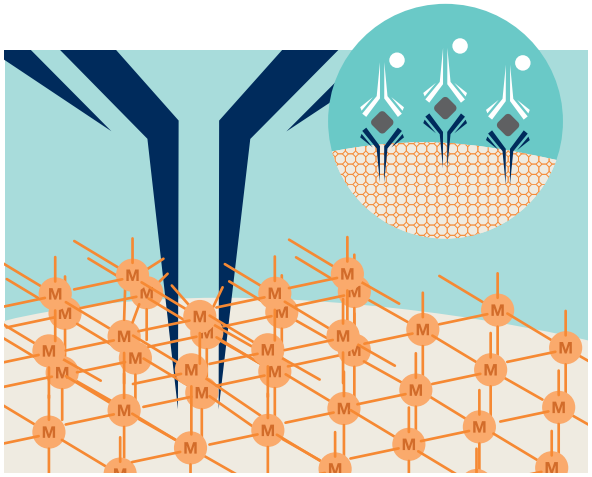

Figure 3 *Mix&Go is a molecular glue comprised of polymeric metal ions that chelate to available electron donating groups on synthetic surfaces and biomolecules.*

Numerous applications can benefit from utilising Mix&Go, in particular, those researchers looking to apply their ELISA antibodies directly onto the Luminex multiplexing format. Using the Anteo Mix&Go™ (AMG) Activation Kit for Multiplex Microspheres, we have easily transferred four individual cytokine sandwich assays, developed for ELISA, directly onto the xMAP platform in the form of a multiplex assay. This has been done without the need to select different antibody pairs compatible with microsphere-based assays and without extensive assay optimisation.

Mix&Go brings together the best of both worlds: *Allowing users to benefit from the handling characteristics reminiscent of passive binding (generally used for ELISA), together with the strength of binding typically achieved with covalent chemistry.*

The Anteo Mix&Go™ (AMG) Activation Kit for Multiplex Microspheres showcases the Mix&Go technology in an easy to use kit format that contains the necessary reagents to transfer your difficult proteins onto the xMAP platform. Luminex MagPlex® Microspheres may be coupled with antibodies specified for use in ELISA using Anteo Technologies AMG Activation Kit [7].

The data shows a multiplex cytokine immunoassay developed using antibodies from BD Biosciences specified for ELISA use. The standard curves generated compare well to the standard curves shown in the BD technical data sheets (Figures 4–7).

The curves generated on the Luminex system show that the limit of quantitation (LOQ) of the assay can be further lowered, showing the improved sensitivity possible using the Luminex platform versus traditional ELISA.

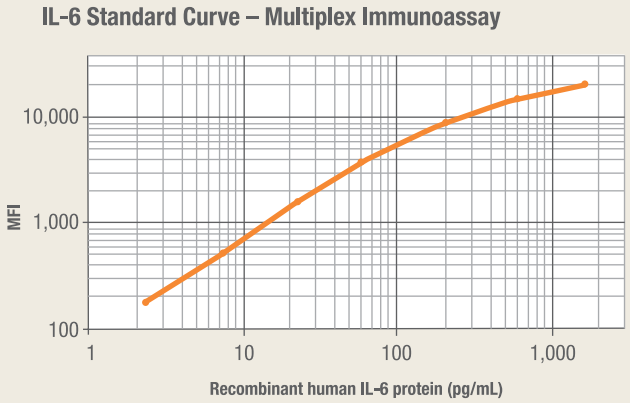

Figure 4 *IL-6 standard curve generated using microspheres coupled with IL-6 antibody using the AMG Activation Kit for Multiplex Microspheres.*

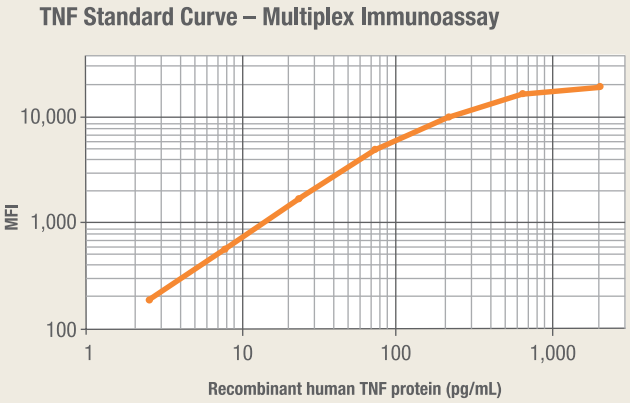

Figure 5 *TNF standard curve generated using the AMG Activation Kit for Multiplex Microspheres.*

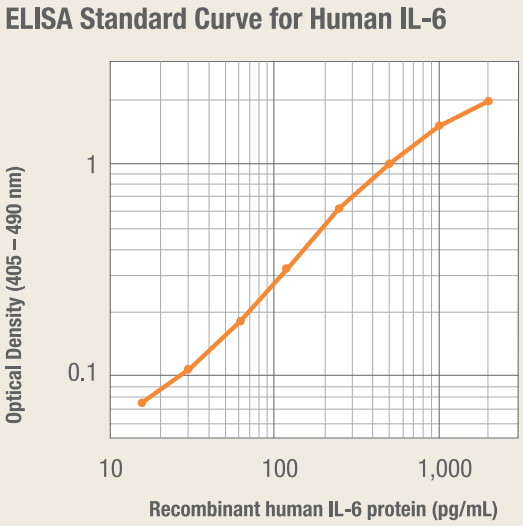

Figure 4 *IL-6 standard curve taken from the BD Biosciences Technical Data Sheet: Purified Rat Anti-Human IL-6 (554543 Rev. 1).*

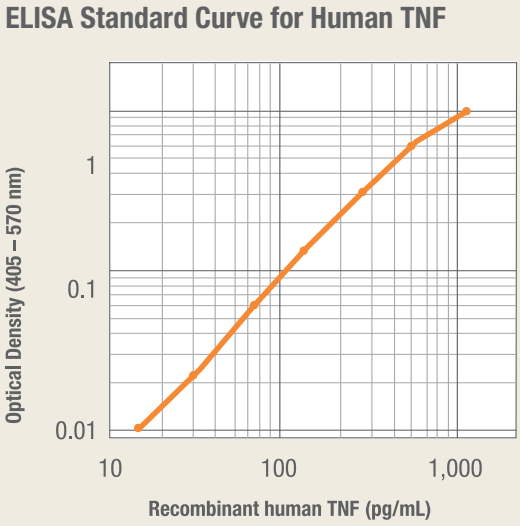

Figure 6 *TNF Standard curve taken from the BD Biosciences Technical Data Sheet: Purified Mouse Anti-Human TNF (551220 Rev. 1).*

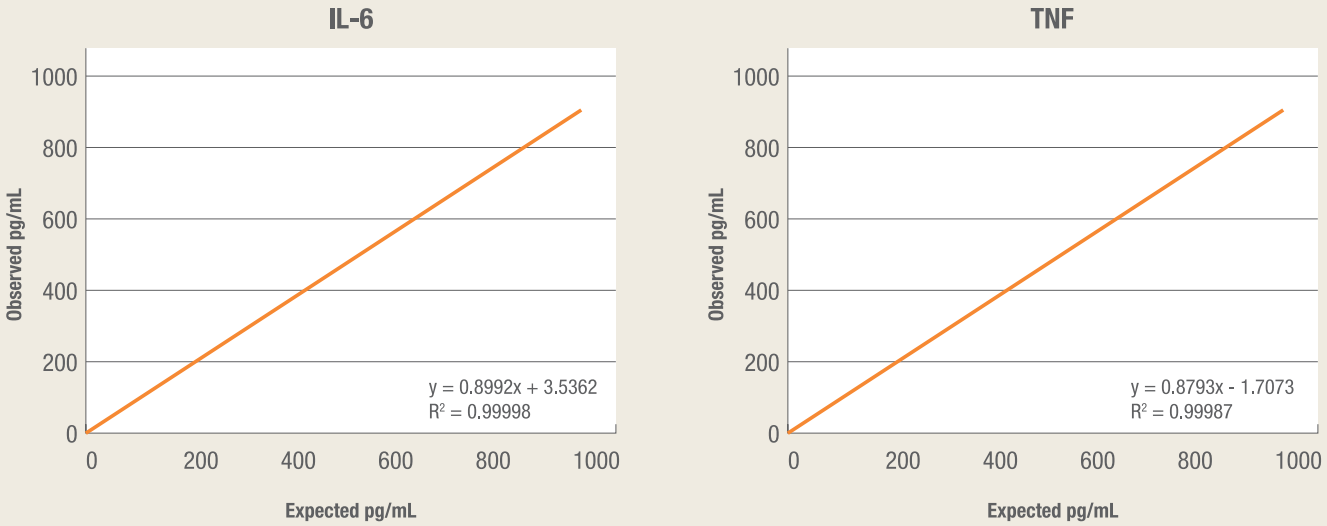

Figure 7 *Observed vs Expected for the two controls. Slope of the line shows good accuracy for IL-6 and TNF.*

High precision and accurate results are obtained when solving controls using the standard curve (Figure 7). In this example, the expected vs obtained slope was almost a perfect correlation, with an R^2 value > 0.999. The AMG Activation Kit can be used to effortlessly transfer ELISA methods onto the Luminex multiplex platform without the need for extensive optimisation.

Being able to harness the benefits that multiplex immunoassays provide is now simpler with the use of the AMG Activation Kit from Anteo Technologies. It enables researchers to easily develop multiplex immunoassays on the Luminex system using the same antibodies typically used for ELISA without the need to specifically test for new antibody pairs that are compatible with the alternative approach of antibody coupling onto Luminex microspheres relying on covalent EDC/NHS chemistry.

We have shown that the AMG Activation Kit allows the user to easily transfer ELISAs to the Luminex multiplex system. Luminex xMAP multiplex assays are now more user friendly with Mix&Go.

[1] Engvall E, Perlman P (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8 (9): 871–4.

[2] Van Weemen BK, Schuurs AH (1971). Immunoassay using antigen-enzyme conjugates. FEBS Letters 15 (3): 232–6.

[3] Köhler G, Milstein C (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256 (5517): 495-7.

[4] Tighe PJ, Ryder RR, Todd I, Fairclough LC (2015). ELISA in the Multiplex Era; Potential and Pitfalls. Proteomics Clinical Applications doi: 10.1002/prca.201400130.

[5] Ellington AA, Kullo IJ, Bailey KR, Klee GG (2010) Antibody-based protein multiplex platforms: technical and operational challenges. Clinical chemistry 56: 186-93.

[6] Seurynck-Servoss SL, White AM, Baird CL, Rodland KD, Zangar RC (2007). Evaluation of surface chemistries for antibody microarrays. Analytical Biochem 371:105–15.

[7] Anteo Technologies Application Note

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About Anteo Technologies

Anteo Technologies is breaking the barriers of traditional coupling chemistry with our patented Mix&Go™ Activation Reagent.

Mix&Go Activation Reagent is designed using coordination chemistry for gentle and secure multipoint binding of biomolecules to synthetic materials. Water based, the reagent activates surfaces in one step and improves uniformity between experiments by giving scientists a consistent substrate to work with. Mix&Go activated surfaces can be stored for up to one year and protein coupling takes one hour.

Cook Medical Australia and **IMRA America Inc** have recently entered into separate partnerships with Anteo Technologies to develop products with broad applications in the medical device, in-vitro diagnostic and global life science markets. A trusted distributor of innovative products, **Veritas Corporation** has signed an agreement with Anteo to own distribution rights to Mix&Go Reagents and the Anteo Mix&Go (AMG) product range in Japan.

From off-the-shelf solutions to developing custom products for our existing and future collaborations, Anteo Technologies is transforming the way scientists work by providing new, powerful tools to significantly advance traditional coupling methods. In turn, helping to improve medical devices and in-vitro diagnostics, leading to the faster detection of disease.