

# An Alternative to Traditional Workflows For Immunogenicity Studies: xMAP® Multiplex Assays Save Time and Resources



*In the study summarized here, scientists used bead-based multiplexing technology to streamline an immunogenicity testing workflow into a single assay, preserving sample volume and integrity.*

## Introduction

In drug discovery and development pipelines, measuring immunogenicity—or the body’s ability to produce an immune response—is a critical step. During vaccine development, a strong immune response indicates that protection against an infectious disease has been achieved. In biotherapeutic development, however, signs of immunogenicity suggest the production of anti-drug antibodies and may lead to anti-drug-antibody (ADA)-mediated adverse events, including reduced treatment efficacy and treatment failure.<sup>1</sup>

As a result, immunogenicity studies must be able to detect the presence of anti-drug specific antibodies, determine the titer of those antibodies, and evaluate antibody isotypes. Traditional immunogenicity characterization methods involve ELISAs and electrochemiluminescence assays, and often, these assays are deployed in a tiered strategy, which tends to be time-consuming and expensive. The use of multiple assays also increases the likelihood of unreliable results due to repeated freeze/thaw cycles and frequent sample handling.

Multiplex immunoassays offer an attractive alternative to overcome these obstacles—xMAP multiplexing in particular can simultaneously detect multiple antibodies and their isotypes (up to 500 analytes per well), providing comprehensive immune response data while saving time, reagents, and sample volumes.

In the study summarized here, scientists at Immunologix Laboratories consolidated a multi-step screening assay and a confirmatory assay into a single multiplex immunoassay to comprehensively characterize anti-drug antibody responses in a simple overnight procedure.

## Methods

The team at Immunologix sought to develop and optimize a multiplex immunoassay to assess the immunogenicity of Humira®, a recombinant anti-TNF $\alpha$  monoclonal antibody, due to its well-documented immunogenicity profile.<sup>2</sup> Because commercially available isotyping kits don’t support immunogenicity evaluation of specific biologics, they designed their xMAP assay to detect ADAs reactive to Humira®, which resulted in highly specific detection.

To evaluate the assay, the researchers screened and isotyped 50 serum samples from healthy individuals and 20 rheumatoid arthritis serum samples from people who had never received Humira®. For screening, a bead set was coupled to Humira® and was then used to capture anti-drug antibodies from the samples. For isotyping, human anti-drug antibody isotypes were evaluated by coupling mouse isotype-specific capture antibodies to a unique bead set. The coupling confirmation test showed a high level of Humira® on the bead surface—with a median fluorescent intensity (MFI) of 26,923—and high MFI values for the isotype-specific bead sets and their respective human control antibodies as well.

Their results also indicated that the assay, which was designed with the goal of meeting FDA guidelines for selectivity and sensitivity in immunogenicity testing, generated results comparable to those from conventional testing methods. Importantly, although the assay did not reach the 100 ng/mL FDA-recommended threshold for sensitivity, the researchers noted there are several strategies that could be used to improve the sensitivity and further optimize the assay. Additionally, they noted that using a multiplex assay offered several benefits—it made it possible to avoid repeated use of precious clinical samples, it decreased the volume needed for testing, and it eliminated iterative freeze/thaw cycles which can damage samples.

“This study should encourage labs to explore the use of multiplexing immunogenicity assays to characterize anti-drug antibody responses quickly,” the scientists reported. They also noted that xMAP Technology could be valuable for immunogenicity assessment in clinical settings.

## Discussion

As they shared in their original publication, multiplexing immunogenicity assays presents an important opportunity for further exploration due to its potential for generating comprehensive information on ADA responses in addition to the time, resource, and cost-saving benefits.

The multiplexing capacity of xMAP in particular makes it ideal for assays that need to detect multiple isotypes of antibodies and anti-drug antibodies simultaneously. xMAP bead-based assays deliver optimal throughput while minimizing reagent and sample volumes required.

Additionally, unlike other immunoassays, xMAP assays are run entirely in a liquid medium, which facilitates rapid reaction times and eliminates detection challenges associated with assays run on solid-phase substrates. It also contributes to the high sensitivity and specificity characteristics of xMAP assays.

## Conclusions

As the Immunologix study demonstrates, xMAP Technology offers a cost-effective, resource-preserving alternative to traditional immunogenicity testing. xMAP-based assays have the potential to meet all the standards for immunogenicity studies while minimizing sample use and streamlining the testing process into a single, easy-to-run assay. As biologic-based therapies become more routine in drug discovery and development, it will be increasingly important to implement multiplex testing to manage potential anti-drug immune responses.

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## REFERENCES

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2. Alleyn M, Closson K, Gentile A, et al. Design and Evaluation of a Multiplexed Assay to Assess Human Immunogenicity Against Humira®. *AAPS J.* 2020;22(5):104. Published 2020 Aug 3. doi:10.1208/s12248-020-00487-4.