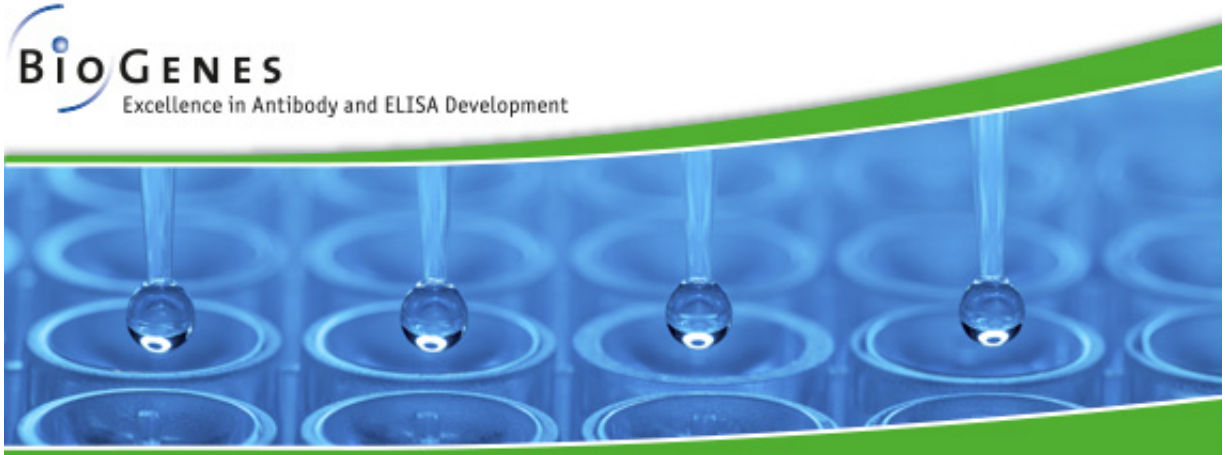
BioGenes
Excellence in Antibody and ELISA Development



Science and Services around anti-idiotypic Antibodies, Part III

October 2020

Dear Valued Customer,

This is the third and final newsletter in our series on anti-idiotypic antibodies (anti-ID). Thank you for staying tuned and being curious to learn more about the multifaceted nature of anti-IDs and how they support research, diagnostics and drug development. If you missed out on our first two episodes of the anti-ID series, no problem! They are available for you on our webpage, take a look here.

In this last newsletter, we will go into more detail about how anti-IDs may be used as positive controls in immunogenicity assays and the regulatory requirements on the subject. We will continue with the discussion about which assay format (direct, indirect or bridging assay) is the most suitable for immunogenicity assessments. Then, one of our scientists briefly summarizes the latest findings and impressions on the Immunogenicity and Bioassay Summit, concluding with the BioGenes approach to custom ELISA development.

Anti-IDs as Positive Controls in Immunogenicity Assays

Positive controls in immunogenicity assays should represent the whole spectrum of ADAs to a biotherapeutic product, as they occur during unwanted immune reactions after treatment in patients.
In general, as a positive control, a drug-specific antibody preparation is recommended for the development and validation of system suitability during the routine assessment of assay performance characteristics, such as sensitivity, selectivity, specificity, drug tolerance, and reproducibility. In some cases, the sponsor may be able to generate such a positive control antibody directly from subjects' samples. However, such patient-derived positive controls are not generally available in sufficient quantities at early clinical phases.
Alternatively, animal serum raised against the biotherapeutic product or monoclonal anti-IDs can be used. In either case, for therapeutic monoclonal antibodies (mAbs), the sponsor should select a positive control antibody that binds to the variable region.

The sponsor first should consider the characteristics of the biotherapeutic drug substance and of the assay format to select a positive control antibody preparation; e. g. a direct/indirect ELISA format using an anti-human IgG as a secondary reagent requires a positive control antibody that is detectable by that same reagent. This set-up would thus exclude the use of a non-primate animal serum or require the extension of assay controls. Monoclonal anti-IDs can be generated in fully human antibody formats and have the advantage of the time-consuming affinity purification being redundant. In addition, monoclonal anti-IDs provide reproducible results, and can be produced unlimited. However, individual monoclonal anti-IDs might not adequately represent the entire range of ADAs as occurring during unwanted immune response.

Polyclonal anti-ID preparations generated by immunizing animals should be affinity purified using the therapeutic mAb. In addition, a depletion of anti-human-IgG antibodies should be applied to enrich with ADA. These important steps are recommended because the hyperimmunization of non-primate animal species may lead to substantial antibody generation against epitopes of the constant regions. This may not correspond to the actual immune response in patients which is mainly directed against epitopes within the variable regions of the mAb.

The Development of ADA Assays: Direct, indirect or bridging assay?

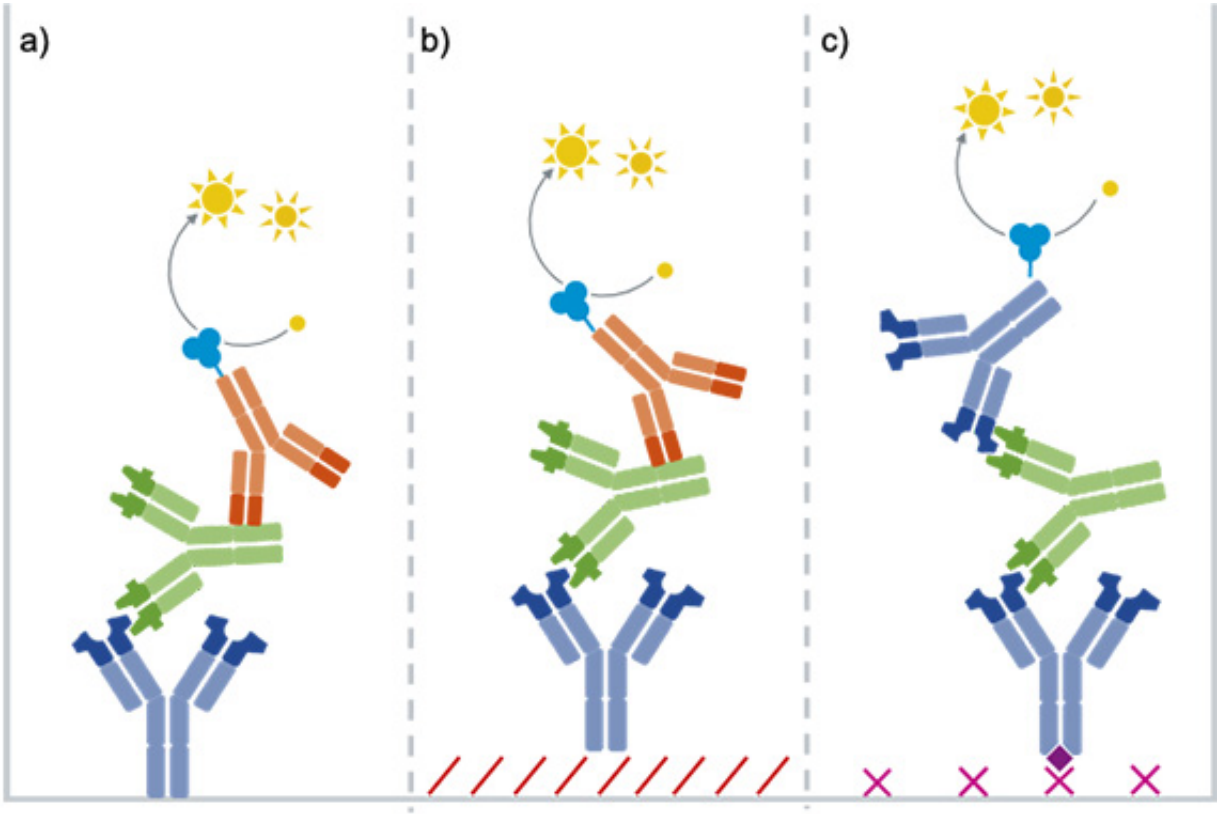


Fig. 1: Different assay formats for immunogenicity assessments. a) direct ADA assay, b) indirect ADA assay, c) bridging ADA assay. blue: antigen (therapeutic antibody), green: ADA, orange: labeled species specific secondary antibody, red coating: capture agent (e.g. monoclonal antibody or streptavidin), purple coating: streptavidin

The enzyme-linked immunosorbent assay (ELISA) is the most commonly used assay platform for immunogenicity assessment. Different assay formats can be considered for the detection of ADAs, such as direct, indirect or bridging assays. In the direct assay, the antigen (which is the therapeutic monoclonal antibody in this case) is directly immobilized onto the ELISA plate, which is then incubated with serum or plasma samples. Due to the antigen immobilization on the plastic surface, a change in antigen conformation and epitope masking can occur. As a result, antibodies specific to the masked epitopes may not be recognized. In the indirect ELISA, a capturing agent (such as an antigen-specific monoclonal antibody, or streptavidin for a biotinylated antigen) is immobilized on the ELISA plate, and the antigen binds in a naïve state. The epitope availability of the antigen is thus significantly increased when compared to the direct ELISA format. However, both assay formats are only conditionally suit-

able for the immunogenicity assessment, as species-specific secondary antibodies are required for detection. This poses a problem when animal serum is used as a positive antibody control for the evaluation of human sera. Secondary antibody cross-reactivity must also be considered.

To overcome this issue, a bridging assay can be applied. In a bridging ELISA, the antigen (the therapeutic monoclonal antibody) is used for both antibody detection and antibody capture. The antigen is therefore labeled differently to 1) bind the antigen to the ELISA plate (e.g. via biotin-streptavidin binding) and 2) allow the calorimetric detection after binding of the ADA (e.g. via horseradish-peroxidase). The principle of the bridging ELISA was briefly outlined in our first newsletter (please find here). Bridging ELISAs benefit from a high specificity (dual arm binding) and the absence of species-specific reagents. As mentioned in our last newsletter (here), bridging ELISAs are applied as the method of choice for the screening assay within the multi-tiered test approach.

Review of the Immunogenicity and Bioassay Summit 2020

In the beginning of October, the 12th Summit on Immunogenicity and Bioassay took place, for the first time in a fully virtual format. The conference program included presentations on immune-oncology, development and assessment of ADA assays and regulatory requirements.

One of our scientists attended the conference and brought her impressions with her:

ADA assays were one of the main topics in the conference which were heavily debated and frequently taken up. Many talks dealt with the optimization of ADA assays and most of the short courses were intended to deal with possible trouble-shootings in the set-up of ADA assays.

Furthermore, the influence and the detection of neutralizing antibodies (NAbs) was also a very important topic during the conference. To date, cell-based detection methods are the most common methods for ADA detection, but various strategies have already been applied to perform ADA assays as a competitive, non-cell-based format. However, the soluble format is not applicable for all drugs and must be adapted to the individual case.

The conclusion is that the design of ADA assays and their implementation into immunogenicity assessments remain a major concern among scientists and executives. The set-up of an ADA assay is challenging, and every drug substance must be thoroughly evaluated, as many case studies have shown. A "one for all" assay is usually not sufficient.

The BioGenes Approach for ELISA Development

BioGenes has 25+ years of experience in the development of robust immunoassays for leading pharmaceutical and biotech companies worldwide. We offer ELISA development for quality control (custom host cell protein assays and assays for other residuals), drug discovery and drug development, biomarker detection, diagnostic kits (human and veterinary) and detection of small molecules, e. g. TNT and cytostatic agents. Additionally, BioGenes provides a wide range of GMP relevant services, including immunoassay development, optimization, qualification/pre-validation, and kit production.

Immunoassays are designed as competitive or sandwich assays. Furthermore, customers can order single services or full-service packages.

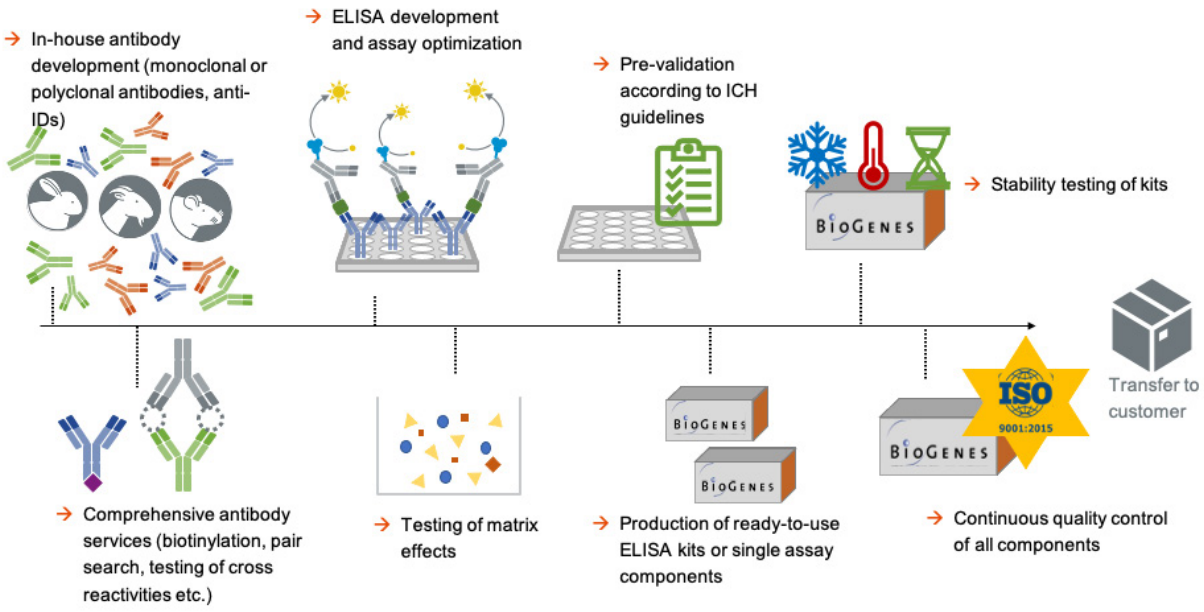


Fig. 2: Workflow of ELISA development at BioGenes

Did you enjoy this? – Give us your feedback!
See contact details below.

Stay tuned!

Contact us

Did you know that BioGenes will participate in the following virtual event?

- Festival of Biologics: November 2-6, 2020

We are looking forward to discussing upcoming projects, renewing contact and talking about the various aspects of anti-IDs and Host Cell Protein (HCP) analysis!

Kind regards,
Your BioGenes Team

Contact: Dagmar Schwertner-Knoll, Head of Marketing/Sales

dagmar.schwertner@biogenes.de

www.biogenes.de

BioGenes GmbH
Koepenicker Str. 325, 12555 Berlin, Germany, Tel.: +49-30-65762396

HR Berlin Charlottenburg 45983
Managing Directors: Dr. Alexander Knoll, Dr. Sergej Ovodov