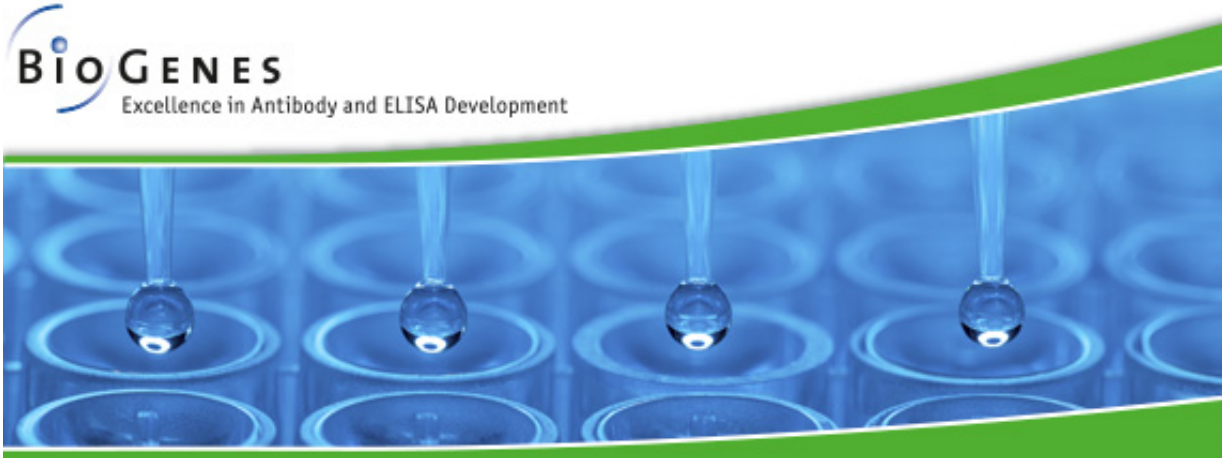
BioGenes
Excellence in Antibody and ELISA Development



Science and Services around anti-idiotypic Antibodies, Part II

September 2020

Dear Valued Customer,

Thank you for reading our first newsletter about the development and the scientific background of anti-idiotypic antibodies (anti-IDs). We hope you have enjoyed this. You missed out on our first newsletter of the anti-ID series – no problem, it's available for you on our webpage (have a look here)!

In our second newsletter, we interview Dr. Detlef Schmechel, Scientist at BioGenes GmbH, who talks about challenges and pitfalls in monoclonal anti-ID development, concluding with the BioGenes approach to monoclonal anti-ID development. Furthermore, we continue with the application of anti-IDs in *in-vitro* assays with an overview on the stepwise methodology of an ADA testing approach. Today's newsletter ends with specific spotlights on each of the different assays.

The BioGenes approach to monoclonal anti-ID development

Expert interview with Dr. Detlef Schmechel,
Scientist at BioGenes GmbH

Hello Dr. Schmechel, thank you for your time today. Our first question is: What are the advantages and disadvantages of using the full-length antibody for the immunization of mice, instead of immunization with antibody fragments (Fab, $F(ab)_2$)?

There are both advantages and disadvantages. One advantage is that full-length antibodies induce an improved immune response due to their multivalent properties. Additionally, the screening of hybridomas should be performed against the full-length antibody anyway, in order to reduce unwanted denaturation on the ELISA plate, which can occur with antibody fragments as a result of conformational instabilities.
One disadvantage of using full-length antibodies as antigens is that many antibody-producing B cells against the constant parts (such as against isotypic or allotypic epitopes) of antibodies can be generated. However, in order to avoid the generation of antibodies against these constant parts, the appropriate screening on cross-reactants should be performed.

How many positive clones do you usually obtain in a standard anti-ID project?
The number of positive clones is greatly variable. It depends on the relative immunogenicity of the actual antibody-binding site (paratope) in comparison to all other epitopes present in the antibody to be immunized with. An increased immunogenicity can usually be observed within glycosylation patterns, xenotopes (remaining determinants from other species which might be present in chimeric or humanized antibodies) as well as unique antibody tags, such as affinity tags or drug conjugates.

How can one distinguish between the generation of inhibitory and non-inhibitory anti-IDs?
We perform a competitive ELISA with the antigen to determine whether the monoclonal anti-ID clones are directed against the binding site (inhibitory antibodies) or against any other idiotope outside the binding site (non-inhibitory).

What is the time frame for a monoclonal anti-ID development?
It usually takes 5 to 8 months to develop a stable, anti-ID producing hybridoma cell line.

And finally, what should be considered when developing anti-IDs?
The design of a proper screening ELISA on cross-reactants for the generation of highly specific monoclonal anti-IDs is very important. Cross-reacting antibodies may be directed against allotopes (determinants unique to antibodies from different individuals), xenotopes or neoepitopes (epitopes formed during fragmentation of the antibody). A thorough understanding of the antibody which was used as an antigen, such as knowledge of the IgG subclass, the type of light chain (κ or λ) or the pattern of glycosylation (host cells of different species may use different carbohydrate types and patterns) are also important parameters for a successful cross-reactant screening to avoid the generation of false-positive hybridomas.

Monoclonal anti-ID development at BioGenes

BioGenes offers the development of specific anti-idiotypic monoclonal antibodies according to the optimized hybridoma technology in mice. Throughout the project, all the results (hybridomas and intellectual properties) are provided for the customer. The customer has the opportunity to monitor the overall progress, modify, repeat and stop individual work packages, depending on the results and the customers' specific requests.

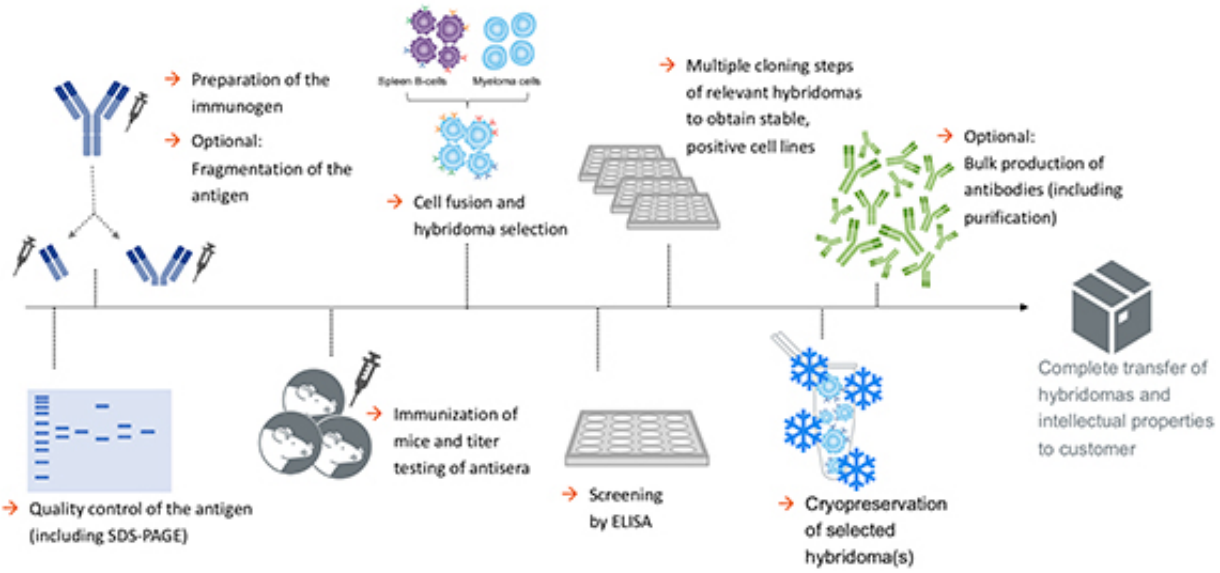


Fig. 1: Workflow of monoclonal anti-ID development at BioGenes

Application of anti-IDs in *in-vitro* assays

Brief overview of the stepwise methodology in ADA assays

Anti-drug antibodies (ADAs) may occur as the response of an organism to a therapeutic protein drug during treatment, a process also known as unwanted immunogenicity. ADAs harbor the risk of mediating unwanted biological or physiological effects, either by inhibiting the therapeutic effect of the treatment itself or by eliciting adverse events.

As briefly outlined in our recent newsletter, the immunogenicity assessment of a therapeutic antibody drug also involves the use of anti-IDs as a positive assay control. Since the thorough examination of the occurrence of ADAs in patient samples is particularly challenging, a multi-tiered testing approach is currently recommended by regulatory authorities.

In the following, we would like to give our readers a brief overview of the current recommendations for a stepwise methodological approach. Moreover, you will learn about the main differences between the individual assays.

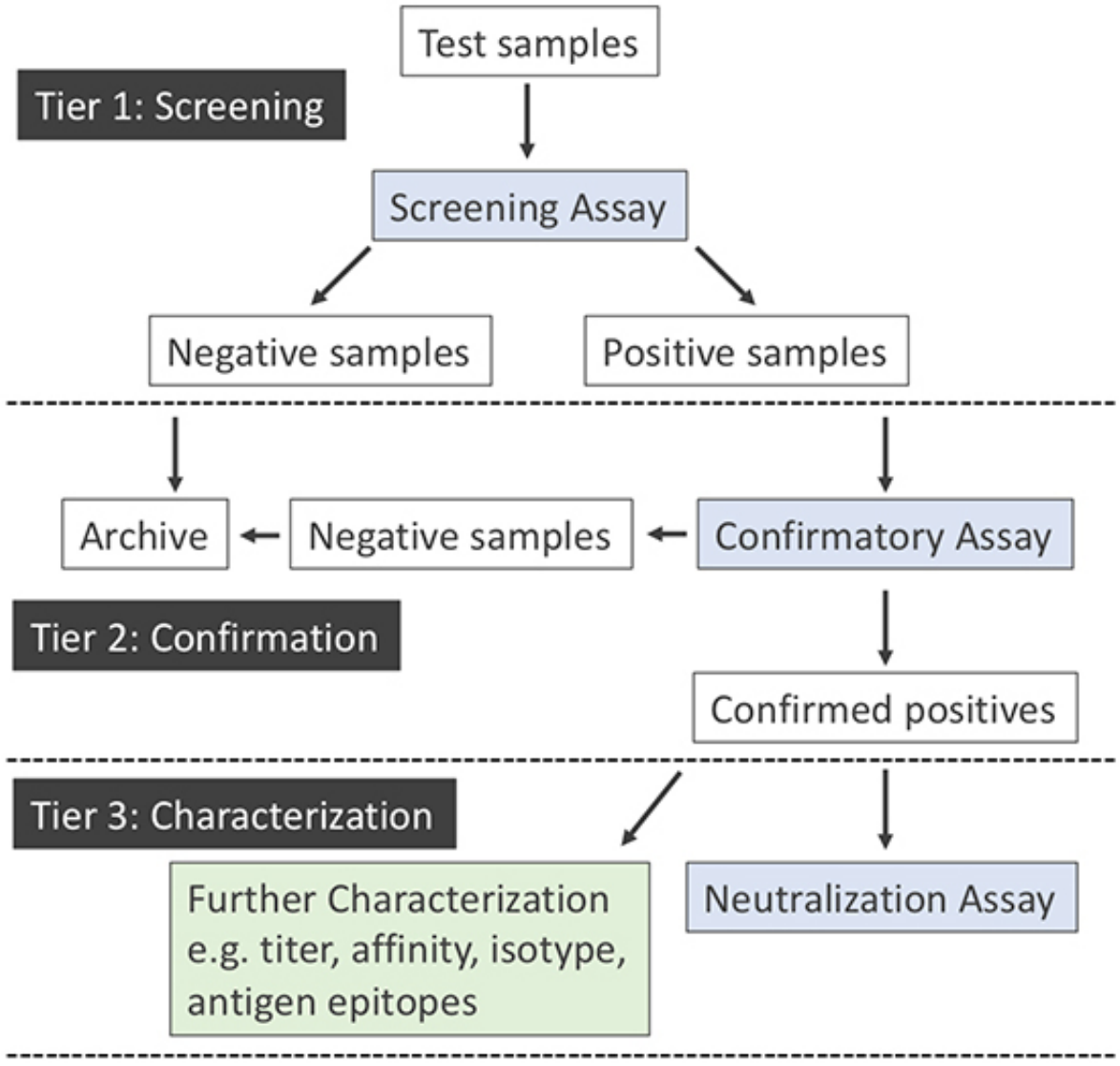


Fig. 2: **Multi-tiered testing approach** for immunogenicity assessment of therapeutic protein drugs.
Adapted from "Guideline on Immunogenicity assessment of therapeutic proteins" EMEA/CHMP/BMWP/14327/2006 Rev 1, 18 May 2017.

Screening Assay

A screening assay is first applied, in order to detect antibodies that are directed against the therapeutic protein drug. In general, screening assays are immunoassays which can be based on a variety of suitable formats and detection systems. We have already described the principle and assembly of a bridging ELISA that is frequently used for screening assays in our recent newsletter (have a look here). Screening assays should be highly sensitive and are primarily designed to detect low levels of high- and low-affinity ADAs. Whether or not a sample is considered positive is defined by cut-point estimation. The cut-point should be statistically determined using samples from treatment-naïve patients early in assay development. Moreover, screening assays should be able to detect all clinically relevant immunoglobulin isotypes, depending on the route of drug administration.

Confirmation Assay

To confirm that samples tested positive in the screening assay contain ADAs that are truly directed against the therapeutic protein drug, a confirmatory assay is required.
Confirmatory assays should be designed to demonstrate specific ADA binding to the therapeutic drug protein, in order to reliably eliminate false positive samples. The format and detection system may be equal or different from those used for the screening assay. One option would be the implementation of a competitive inhibition assay, in which the positive samples from the screening assay are preincubated with the therapeutic protein prior to assessment in a binding assay. Confirmation is considered to be given by a subsequent inhibition of the signal.

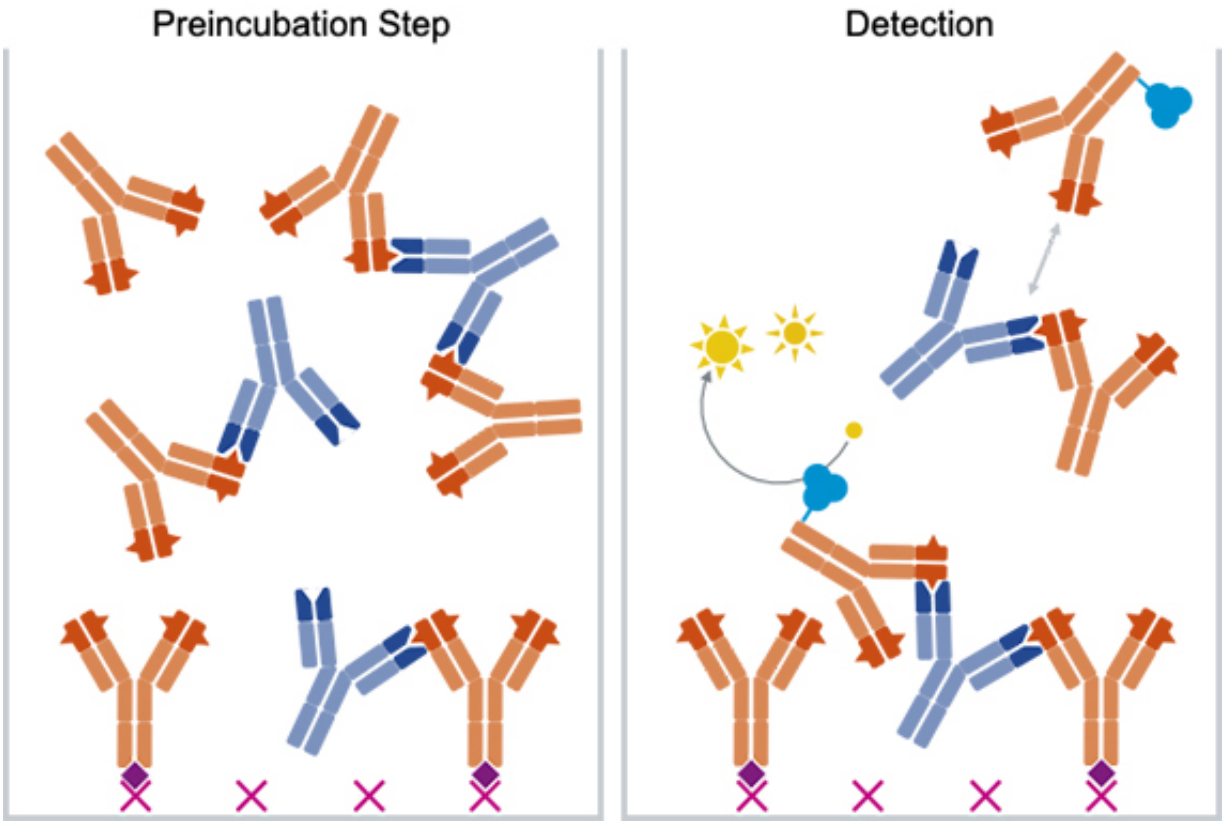


Fig. 3: **Competitive ELISA** principle used for the Confirmation Assay. Preincubation with the therapeutic antibody drug prior to assessment in the bridging assay should result in the inhibition of the initial binding of the ADAs to the antigen, and in the reduction of the positive signal. Orange: unlabeled or labeled therapeutic antibody drug, blue: ADA from patient sample.

Characterization and Neutralization Assay

Finally, the functionality of ADAs in samples confirmed as positive in the confirmatory assay should be further investigated by means of titration or neutralization assays. Additional ADA characterization, such as isotype determination, may also be considered.

A semi-quantitative assay, such as a titration assay, may be applied to assess the quantity and quality of ADAs, e.g. when pre-existing antibodies are present. Testing samples for a post-treatment increase in ADA titer may provide further information on the immunogenic impact of a therapeutic protein drug.

Neutralization assays are implemented to investigate the potential of ADAs to interfere with the therapeutic activity of the protein drug. The selected testing method to assess neutralizing potential for ADA-positive samples should be based on the mechanism of action of the therapeutic protein product. Generally, cell-based bioassays and non-cell-based competitive ligand binding assays are used to measure NAb activity *in-vitro*.

Our next newsletter will go into more detail on how anti-IDs may be used as positive controls in immunogenicity assays, and you will learn more about the regulatory requirements which surround the subject.

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

In October, BioGenes' last episode of the anti-ID newsletter series will be about:

- Regulatory requirements
- Anti-IDs as positive controls in immunogenicity assays
- ELISA formats @ BioGenes
- The BioGenes approach for ELISA development

Stay tuned!

Contact us

Kind regards,
Your BioGenes Team

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