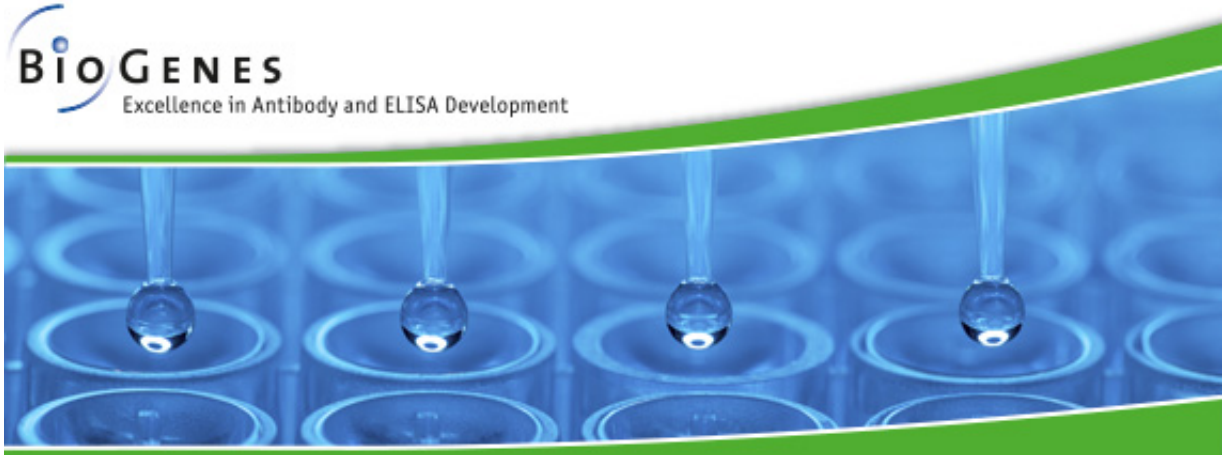
BioGenes
Excellence in Antibody and ELISA Development



Science and Services around anti-idiotypic Antibodies Part I

August 2020

Dear Valued Customer,

Anti-idiotypic antibodies (anti-IDs) are optimal tools for supporting the development of new therapeutic antibodies and biosimilars.

In order to provide you with more detail about the various anti-ID applications, we will publish a newsletter series on the subject of anti-IDs, based on our long-standing expertise in anti-ID development, and including a brief summary of the scientific background information. This will allow you to learn more about the multifaceted nature of anti-IDs, their application in preclinical and clinical research and how they might be integrated in your drug development and post-marketing processes.

Starting with our first newsletter, we will highlight the science background of anti-IDs, their modern *in vitro* diagnostic application, and conclude with Bio-Genes' approach to polyclonal anti-ID development.

Scientific Background

The Idiotype of an Antibody

Ever since the first findings on antibodies to have a specificity for the idiotype of a target antibody were published in the 1950s, anti-idiotypic antibodies (anti-ID) have become more and more important for preclinical and clinical research, and made their way into specific forms of therapy.

What is an idiotype?

The idiotype is the collective set of idiotopes which are the antigen determinants, formed by the V_L and V_H chain of the hypervariable region within the Fab fragment of an immunoglobulin. Idiotopes can occur either in close proximity to the paratope or be partially overlapping. In general, idiotopes are clonally unique to antibodies, and therefore anti-IDs are specific to only one antibody.

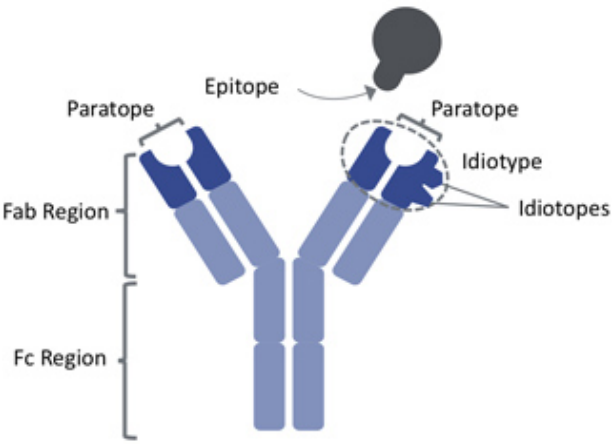


Fig 1: Antibody model with a description of functional domains (blue) and a cognate antigen (grey)

Types of anti-IDs

Anti-IDs can be divided into three subgroups, according to their primary target of binding. Non-inhibitory anti-IDs bind the target antibody by any idiotype on the V_L or V_H chain situated outside the paratope, preserving the original antigen-binding properties. Inhibitory anti-IDs bind the target antibody via the paratope and thus inhibit the binding to its antigen. Complex-binding anti-IDs show affinity to the target antibody upon its prior binding to the antigen as a prerequisite, thus also having a non-inhibitory nature.

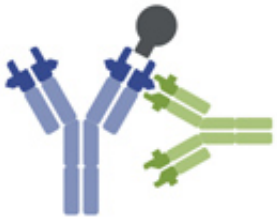
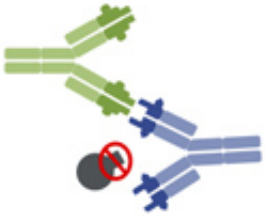
Non-inhibitory anti-IDs	Inhibitory anti-IDs	Complex binding anti-IDs
		
• Binding to any idiotope except the paratope • Detect the total amount of target antibodies	• Binding to paratope • Neutralizing • Detect the amount of unbound target antibodies	• Binding to target antibody-antigen complexes • Non-inhibitory

Table 1: Description of the three anti-ID subtypes considering their respective properties, green: anti-ID, blue: target antibody, grey: antigen

Brief History of anti-ID Discovery

In 1963, Henry Kunkel and Jacques Oudin proposed the existence of anti-IDs based on their observation that the immunization of animals with antibodies from another animal led to the production of specific anti-antibodies. Based on these findings, Niels Jerne predicted the existence of an immune network in 1974. According to the network hypothesis, the immune system is regulated by an interplay of idiotypic interactions from antibodies and T cell receptors.

Application of anti-IDs in *in vitro* Assays

The assessment of drug safety is a major concern of biologics drug development. Regulatory authorities have released a number of guidelines for how these issues should be addressed using *in vitro* studies. In particular, pharmacokinetic studies (PK) and immunogenicity assessments largely depend on the use of anti-idiotypic antibodies (anti-ID). With monoclonal antibodies becoming increasingly relevant for therapeutic use, anti-idiotypic antibodies are required to answer questions of product efficacy using PK assays and drug safety using anti-drug antibody (ADA) assays. The latter is used to detect potentially unwanted immunological reactions to the administered therapeutic mAb.

Anti-IDs in Pharmacokinetic (PK) Assays

Pharmacological drug characterization involves the assessment of the interaction of the molecule with the human body. As suggested by the "LADME" scheme, the distribution, metabolization and elimination of a pharmacologically active substance needs to be monitored (also known as a pharmacokinetic (PK) study). Furthermore, during the development of biosimilar drugs, the assessment of potential differences in biological activity to the reference drug is required, which employs comparative PK studies.
PK assays are usually designed in a Sandwich ELISA approach. This means that the therapeutic drug antibody is sequestered by one capture and one detector antibody in a layered fashion. The capture Ab is a monoclonal anti-ID specific for the targeted drug antibody.

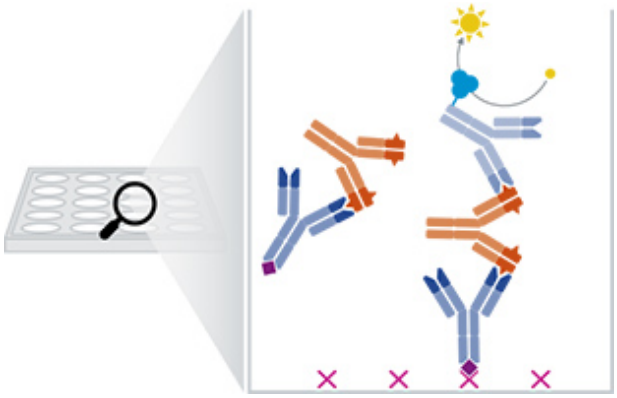


Fig 2: Sandwich ELISA principle used for PK assay; blue: monoclonal anti-ID (biotinylated or enzyme-conjugated), orange: target drug antibody (analyte); anti-ID conjugation enables assay readout

Anti-IDs in Immunogenicity/Anti-Drug Antibody (ADA) Assays

The immunogenicity assessment of a drug antibody is based on a three-tiered approach, and involves the use of anti-IDs. In a first Screening Assay, the labeled drug antibody is incubated with sample serum to test for the occurrence of ADA produced by the organism. The ADA establishes a molecular bridge between the labeled drug antibodies, and this method of detection is therefore called Bridging ELISA. As a comparative positive control, polyclonal anti-IDs specific for the drug antibody are used. To eliminate false-positive results, a Confirmation Assay and a Neutralization Assay are required to reliably assess the presence of ADA.

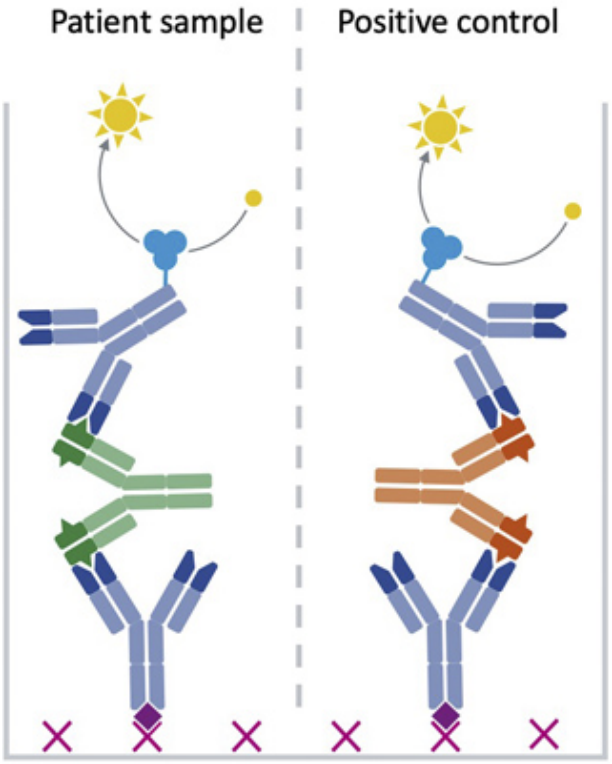


Fig. 3: Bridging ELISA principle used for Screening Assay; blue: labeled drug antibody, green: ADA from patient sample, orange: polyclonal anti-ID serving as assay positive control

The BioGenes Approach to anti-ID Development

Polyclonal anti-ID Development

BioGenes offers the development of specific anti-idiotypic polyclonal antibodies in rabbits and goats. Throughout the project, all the results 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.

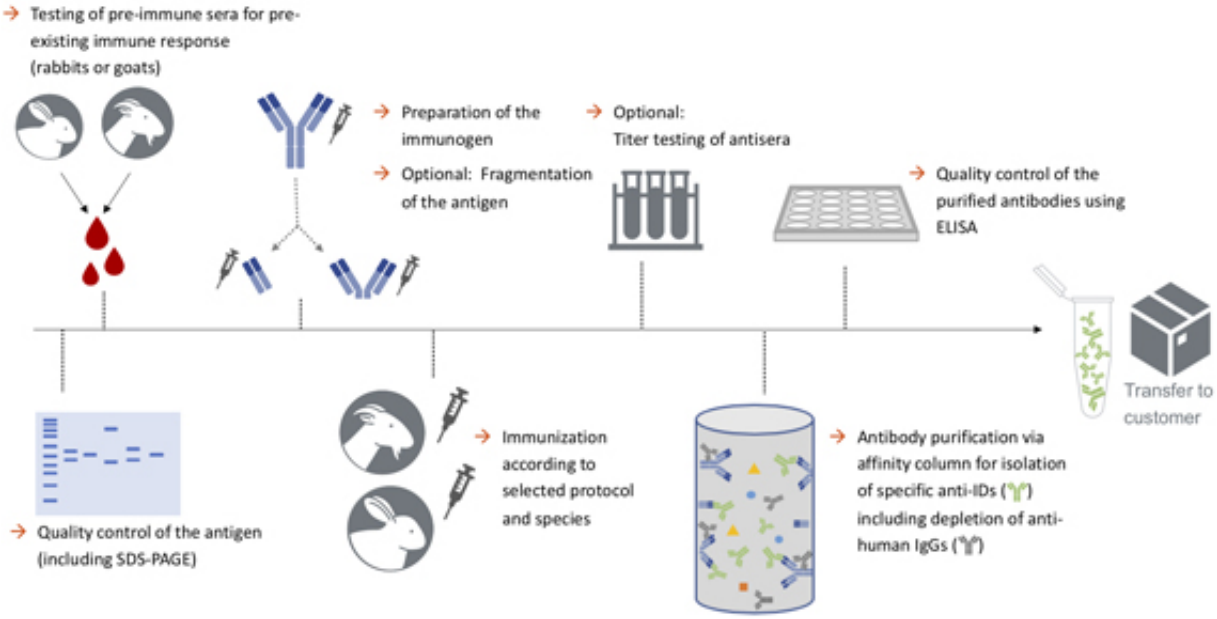


Fig 4: Workflow of anti-ID development at BioGenes
Depending on the quantity of purified polyclonal anti-IDs required, the species, the number of animals and the immunization schedule will be suggested.
Time frame – 4-8 months

Get your specific offer

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

BioGenes' newsletter series will continue, with specific coverage of:

- Regulatory recommendations and assays for ADA detection
- The use of anti-IDs as therapeutic agents
- The BioGenes approach to monoclonal anti-ID development

Stay tuned!

Contact us

Kind regards,
Your BioGenes Team

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