ENVIGO

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Antibody optimization

Key opinions on the production of antibodies to support the development of reagents for basic research, diagnostics, therapeutic products

This is the first in a series of three white papers reviewing the use antibodies in the development of therapeutic products. Here, we provide an overview of the common applications of antibodies in drug development. We explore some of the potential applications of antibodies for use as reagents. Then, we complete our overview with a short discussion on the current state of antibody quality in the marketplace.

A deeper analysis of key considerations for the generation and large scale production of high quality, optimized polyclonal (pAbs) and monoclonal (mAbs) antibodies will be presented in subsequent articles.

Polyclonal antibodies have been used for many applications by researchers throughout recent history, and in 1975, Georges Köhler and César Milstein developed a process for creating monoclonal antibodies. In many respects, this was the beginning of a technology to generate highly specific immunoreagents that has now become an integral part of the global healthcare industry.

Monoclonal antibodies are now well-recognized as either therapeutic agents in their own right, or critical tools used routinely from early stage research through to clinical diagnostics. They enable the assessment of specific pathways and cell:cell interactions that can provide insight into defined cell populations, the individual markers they express and their function. They are also critical reagents for assessing the performance and characteristics of novel pharmaceuticals, both large and small molecules. However, in all cases their utility is determined by both their purity and specificity and a host of characterization and purification assessments can be applied to identify and recover sufficient quantities of a specific monoclonal antibody that fulfills these requirements.

For those developing therapeutic products, a commonly encountered problem is the absence of suitable commercially available reagents specific for their product, which can impact or delay development programs. In 1975, Georges Köhler and César Milstein developed a process for creating monoclonal antibodies. In many respects, this was the beginning of a technology to generate highly specific immunoreagents that has now become an integral part of the global healthcare industry. Early implementation of high quality antibody reagent production enables the establishment of suitable assays which can be utilized throughout the drug development pathway for a number of purposes. The measurement of binding to candidate targets to sensitive pharmacokinetic (PK) assays that can be modified to accommodate a range of biological matrices, quantification of active drug substances to assessment of anti-drug antibody responses are some of the benefits experienced during the drug development process.

Envigo offers a range of services to generate such custom-made reagents that are manufactured to research grade or cGMP standard as required by our customers.

Introduction

Antibodies, both polyclonal and monoclonal, are well established and versatile reagent tools for the study of a wide variety of biological functions and quantification of a host of analytes. They are also used as therapeutic drugs in their own right and are amongst the biggest selling therapeutics making significant difference to patients' lives.

They are extensively used for a number of purposes including diagnosis of disease, detection and quantification of active drug substances for Chemistry, Manufacturing, and Controls (CMC) and bioanalytical purposes, measurement of biomarkers to measure drug safety or response to drug intervention as well as more basic research activities with the goal of understanding biological pathways.

Antibodies are used to detect a wide range of biochemical determinants, including proteins, carbohydrates, lipids, nucleic acids and even some low-molecular-weight haptens. This versatility has allowed scientists to monitor biological responses, cell surface protein expression, the make-up and location of intra and extracellular proteins, as well as to detect other analytes and pathogens.

As reagents for biomarker and diagnostic assays, antibodies are used in a wide array of analytical methodologies, including immunoblots, ligand binding assays, enzyme-linked immunospot (ELISpot) assays, fluorescent microscopy, flow cytometry, and immunohistochemistry. Although it is not the focus of this series of articles, the remarkable success that mAbs have achieved in the treatment of disease, should be acknowledged. In this context, they are used to modulate the immune response, target antigens associated with disease, and switch cells on or off.

There are currently over 40 marketed monoclonal antibody therapies and it is estimated that at least 70 new monoclonal antibody therapies will be approved by 2020 (Ecker, 2015). Notably, antibody-based assays have played an important role in the development of these biopharmaceutical products. For example, anti-idiotype antibodies are used in PK studies to measure drug levels in patient samples, as well as to monitor for potential anti-drug antibody responses (immunogenicity), which are a clinical safety concern for some classes of biopharmaceutical products.

Moreover, antibodies are increasingly relied on to test the safety of both food and water supplies. For example, to minimize potential health risks from bacterial, viral, and fungal pathogens and chemical contaminants or toxins, food and water are commonly screened with antibody-based methodologies (Gehring, 2014; Medina, 2006).[1, 2] Allergen testing in the food manufacturing industry also relies heavily on the use of antibodies to evaluate the presence of allergens that pose potential health risks for consumers (Schubert-Ullrich, 2009).

This paper, the first in a series on optimizing the production of antibodies as reagents, provides a broad overview of antibody quality and outlines the key considerations researchers should take into account when selecting and preparing an antigen prior to antibody generation. In general, our experience has shown that carefully considering your antigen ahead of time increases the chances of yielding a high-quality antibody that will produce reliable data in the long run, which translates to valuable savings in time and money.

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1. USDA Aflatoxin Testing in Corn: https://www.gipsa.usda.gov/fgis/aflatoxin.aspx
2. EPA Cyanobacteria and Cyanotoxin Testing in Drinking Water: https://www.epa.gov/sites/production/files/2014-08/documents/cyanobacteria_factsheet.pdf.

Current state of antibody quality in the marketplace

The production of antibodies for use in research and development and in medicine has a long history. Although there is no doubt that the use of antibodies has made a significant contribution to our basic understanding of biology, they have also been singled out as a major factor in the poor reproducibility of many biology-based studies (Baker, 2015; Bradbury et al., 2015).

In a study by the Human Protein Atlas, a Swedish consortium whose goal is to generate antibodies directed against every protein in the human genome, the functionality of approximately 9,000 internally generated antibodies was evaluated. The consortium found that less than 50% were effective for examining protein distribution in preserved slices of tissue (failures were detected on the basis of a staining pattern not consistent with experimental and/or bioinformatics data, Berglund et al., 2008). In another study, comprising assessment of 246 antibodies widely used in the field of epigenetics, researchers revealed that 25% of reagents failed tests for specificity, with some actually displaying high specificities for incorrect molecular targets (Egelhofer et al., 2011). The issue of antibody quality continues to be a pressing challenge for scientists (Prassas et al., 2014). Accordingly, numerous current initiatives aim to facilitate the availability of data regarding antibody quality and to develop validation standards for such reagents; a prominent example is the recently formed International Working Group on Antibody Validation.[3]

Researchers continue to investigate why some antibodies purchased from commercial sources or generated in-house exhibit poor performance in downstream applications. This problem can often be traced back to technical oversights or shortcuts that result in suboptimal functionality. The generation of antibodies is complex; a variety of factors can impact each phase of the production process. Careful consideration of each step involved in the production of antibodies for use as reagents often represents the difference between obtaining an exceptional antibody that features high specificity, high avidity, high sensitivity, proper glycosylation, stability, correct Fc domains, intended biological function, or other desired traits and one of poor performance characteristics.

3. https://www.genomeweb.com/proteomics-protein-research/new-international-working-group-formed-tackle-questions-antibody

Poor quality antibodies that display one or more of the following undesirable characteristics: non-specificity, low avidity, contamination with adventitious agents such as viruses and mycoplasma, and low expression levels will be far less useful as a reagent and it should be noted that the last two characteristics mentioned have a direct relationship to the cost of producing the antibody.

The generation of a poor-quality antibody intended for use as a reagent often requires the re-optimization of its protocol, but starting the process from scratch is hardly desirable, due to the high manufacturing costs and significant delays associated with developing new antibodies. An up-front investment in the optimization process gives researchers the best chance of generating an antibody that will meet their needs in the long term.

Although this may require a higher initial investment and could extend the pre-production timeline, it can substantially reduce the financial, regulatory, and/or scientific consequences resulting from the production of poor-quality antibodies.

Academic and industry researchers often find themselves relying on poorly performing commercially available antibodies. However, with careful planning, high-quality antibodies can be generated before embarking on studies that require the generation of consistent and reproducible data.

An up-front investment in the optimization process gives researchers the best chance of generating an antibody that will meet their needs in the long term

One of the first questions is therefore: 'How do you intend to use your antibody?'

Selection and preparation of the antigen

Optimal selection and preparation of the antigen is crucial to obtaining high-quality antibodies in both monoclonal and polyclonal antibody production. Although the importance of antigen selection and preparation is not immediately apparent to some in the field, Envigo's experience has shown that it is a critical consideration.

The first step in this process is to select the antigen of interest. Features that require consideration include immunogenicity potential; many antigens are not highly immunogenic as they are self-antigens. The antigen type, source, purity, 3-D structure, and the intended final use of the antibody product are also important up front considerations since some applications are more suitable for polyclonal rather than monoclonal production or vice-versa. One of the first questions is therefore: "How do you intend to use your antibody?"

After the appropriate antigen is selected, the next step is preparing the antigen for immunization and subsequent antibody collection. Because access to the native antigen is usually limited, recombinant protein expression is often used to produce sufficient quantities of antigen. This process employs both eukaryotic and prokaryotic expression systems (Greenfield, 2013; Magistrelli et al., 2014).

Consideration when using non-mammalian hosts is their possible impact on the proper folding and post-translational modifications of the antigen, which may influence whether the epitope is presented properly when antigen presentation occurs. On the other hand, the presence of post-translational modifications may not be important for the presentation of some antigens, such as small antigens or peptides in which the 3-D structure is not an issue.

In addition to recombinant proteins, synthetic peptides are employed as an antigen source (Hancock et al., 2005a). Part of their appeal is that they are readily available and inexpensive to generate and can be used to produce antibodies against different protein isoforms or site-specific phosphorylated proteins (Hancock et al., 2005a; Lateef et al., 2007). Although, because most peptides lack secondary structures, it is important both to avoid epitopes that may be unavailable due to a protein's tertiary structure (because the antibody may fail to recognize the native protein) and to assess diligently the possibility for cross-reactivity with antigens of similar structure. Another consideration is whether the antigen is naturally found as a solitary molecule or as part of a larger complex of proteins. Insights regarding whether the native protein undergoes changes like cleavage and post-translational modification, or how it folds can be immensely helpful; these changes can affect the availability of epitopes for detection in the real-world setting (Hancock et al., 2005a).

To induce an immune response in animals and generate anti-peptide antibodies, synthetic peptides are usually coupled to carrier proteins like keyhole limpet hemocyanin (KLH), thyroglobulin (Tg), ovalbumin (OVA), or bovine serum albumin (BSA). These proteins are themselves immunogenic which leads to the generation of misdirected antibodies so antibody characterization is important.

The synthesis procedure can also be a source of impurities. For example, reaction byproducts or contamination with other peptides is possible; these impurities can have a dramatic effect on the immune response following immunization. Alternative approaches to using standard peptide-carrier conjugates have also been developed. One example is the multiple antigenic peptide system, which uses the epsilon group of lysine residues to generate a branched core matrix that is then employed as a scaffold for peptide synthesis (Hancock et al., 2005a; Tam, 1988).

Full-length (native) protein antigens are often considered a superior choice relative to other antigen strategies, based largely on empirical data indicating that they tend to perform better at eliciting antibodies that recognize the native target protein. In one study, investigators raised antibodies against 10 serum proteins using three immunization methods: peptide antigens, DNA immunization, and full-length proteins. The authors reported that overall, immunization with full-length proteins consistently yielded the best results (Brown et al., 2011).

Another consideration is whether the antigen is naturally found as a solitary molecule or as part of a larger complex of proteins

However, generating full-length proteins in the quantities needed for successful immunization is often a limiting factor and thus not appropriate in all cases.
In addition, the processes necessary to prepare full-length protein antigens may introduce impurities such as detergents, denaturants, solvents, or non-neutral pH conditions that can affect the health of animals and result in poor titer antiserum.

Once an appropriate antigen has been generated in sufficient quantities, further steps in its preparation must be optimized to provide the best opportunity for inducing a potent and appropriate immune response. In particular, the purity of the antigen can have significant effects on antibody yield; if the raw material used for the antibody production process is contaminated or inferior in other ways, such as the presence of bacterial antigens or inappropriate buffer composition (which can affect the solubility and structure of the antigen), then the end product is at an increased risk of performing poorly (Leenaars et al., 2005).

Although obtaining antigens of high purity can be an arduous process, implementing protocols that minimize contamination of the antigen is a best practice that helps ensure antibody specificity. The immune system can mount a significant response to impurities, which can muddy antibody preparation with misdirected antibodies.

Increasing the chances of developing an exceptional antibody requires careful planning from the earliest stages, including selection and preparation of the antigen. This up-front investment will position the antibody for success, saving time, effort, and money over the long run.

The immune system can mount a significant response to impurities, which can muddy antibody preparation with misdirected antibodies

Conclusions

In research, diagnosis, and clinical development, the utility of antibodies as reagents is of paramount importance, and they will continue to play an important role in the research laboratory and clinic. While the processes associated with the production of antibodies have not changed dramatically over the past several decades, inferior quality antibodies is an everyday reality. With an upfront investment in careful planning and due consideration, scientists can maximize their chances for obtaining a high-quality antibody.

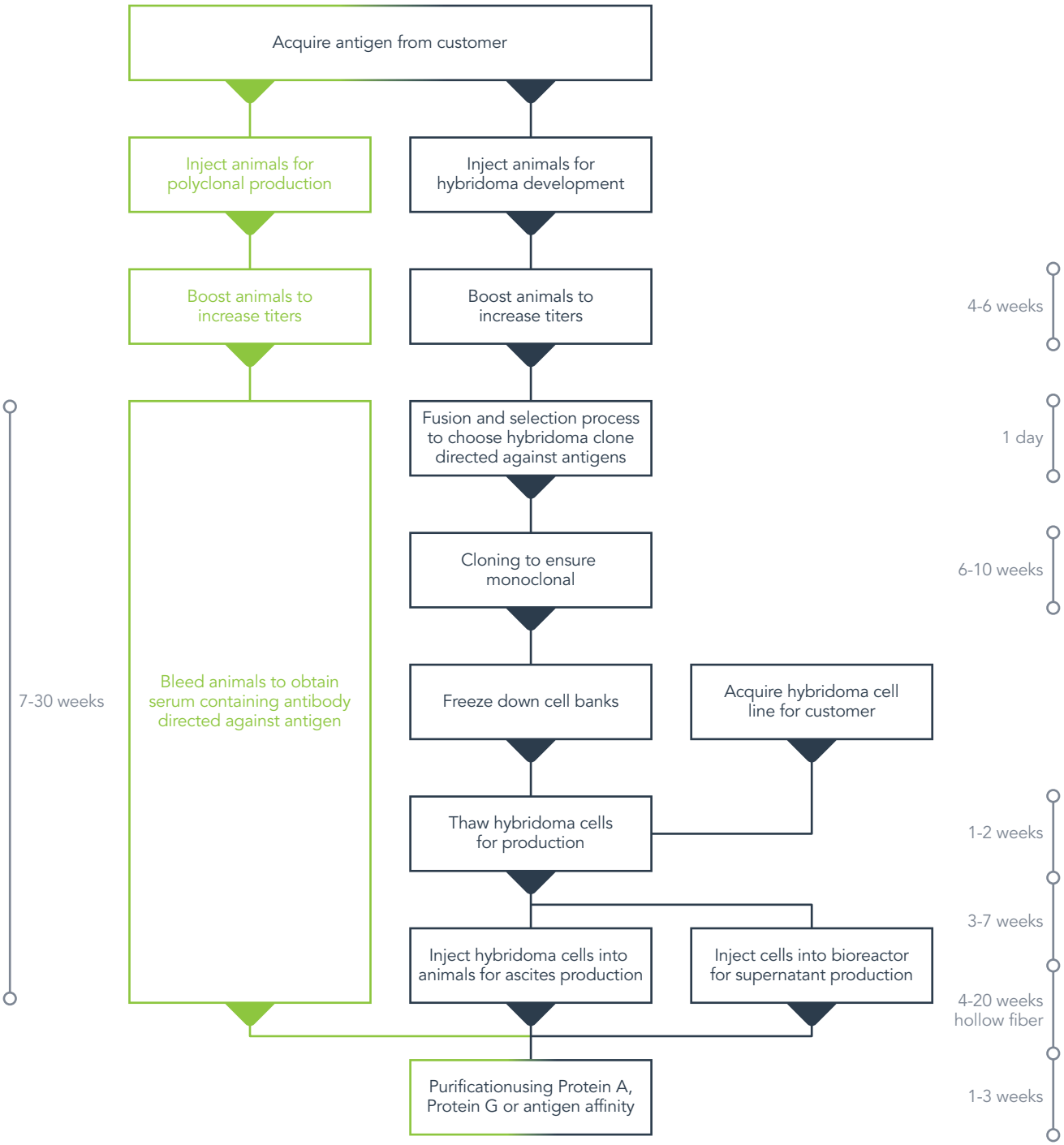
Depending on the ultimate application of your antibody, one should begin with an open-mind and give consideration whether a pAb or mAb is the best fit for your application. As depicted overleaf (Figure 1), the development of a custom pAb is not necessarily faster than generating a monoclonal antibody producing cell line if extended protocols are considered.

Of course, if a stable and well-characterized cell line already exists, then the time required to obtain an acceptable mAb is significantly reduced relative to a pAb.

As explained in this paper, selection and preparation of the antigen is an important step in the production of high quality antibody reagents. But there are many other factors that can affect the performance of antibodies. In this article, we have provided the basics for embarking on a new antibody production project.

In upcoming articles, we will take a more in-depth look at specific steps that should be considered for the optimal production of pAbs and mAbs for use as reagents.

Conclusions



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Authors

Richard J Drucker

In possession of more than 30 years of experience in the field of antibodies development and production, he started his career in 1983 by working in hybridoma development and technical transfer. Over the course of his career, Richard gained specializations as a scientist in both industry and research institutes in the areas of hybridoma development, cell line optimization, product development, oncology, immune modulation, ascites production, and hollow fiber antibody production. He has been employed by Envigo (formerly Harlan Bioproducts for Science) since 2000 as Production Manager of In Vitro Production and Purification.

Richard graduated with a MS degree in Virology from University of South Dakota Medical School in 1982.

Buddy Curlee

Has spent over twenty years in laboratory animal medicine and managed numerous special projects for Envigo (formerly Harlan Laboratories). His expertise in project management and animal welfare earned him a promotion within the Envigo ranks seven years ago. He is currently the Operations Manager for Envigo Bioproducts.

Buddy received his under graduate and doctorate of veterinary medicine from Auburn University.

Manuel Vazquez

Since joining Envigo (formerly Harlan Laboratories) in 2004, Manuel Vazquez has gained valuable experience and knowledge across the breadth of services within the RMS industry. During this time Manuel has maintained an important role in the optimization and development of the Immunization strategies used for antibody generation and in doing so has gained valuable experience with a wide range of species, from rodent to camelid (and everything in between) and non-human primates.

As a UK Home Office Project License holder, Manuel has a very strong focus on all aspects of animal welfare and has worked with a number of external facilities on best practice and the use of animal models in antibody generation projects, and the use of adjuvant systems in immunization strategies across all species. This strong focus on animal welfare was recently rewarded with an industry recognized award.

Editor

Lee Coney

Recruited by Huntingdon Life Sciences at the end of 2004, Lee Coney took a leading role in handling inquiries from customers developing biopharmaceutical products. He brought to the role hands on experience of biopharmaceutical drug development gained in companies such as; Cantab Pharmaceuticals, Xenova and CellFactors. During his time in the biotechnology industry, Lee was involved in developing therapies including: vaccines, recombinant proteins; monoclonal antibodies; immunomodulatory molecules; viral and non-viral gene delivery systems; and cell-based therapies.

He has a well-rounded knowledge of the regulatory environment for biologics and particular expertise in the analysis of protein and viral products. At Envigo, Lee currently serves as Chief Scientific Officer. He is also a serving committee member on the Monoclonal Antibody Expert Working Group of the National Centre for the 3Rs, The BIA Manufacturing Advisory Committee and the RSC / RPSGB Joint Pharmaceutical Analysis Group.