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White Paper

# Considerations in the production of antibodies as reagents:

## MONOCLONAL ANTIBODIES

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It is hard to imagine a world without monoclonal antibodies (mAbs). They are widely used in the scientific community and have assisted in the advancement of research and development. As reagents and therapeutics in their own right, they have played an important role in the advancement of basic research, diagnostics and product development, and they have delivered huge patient benefits across a wide range of therapeutic areas.

# Introduction

**The advent of mAbs in research and medicine followed a major breakthrough in the 1970s where antibodies could be derived from a single B cell clone (Köhler and Milstein, 1975). In contrast to the heterogeneous nature of polyclonal antibodies (pAbs) (discussed in a previous article in this series), mAbs are capable of recognizing only one epitope found on an antigen. They have greater specificity than pAbs and are well suited to scientific applications like examining protein-protein interactions, phosphorylation, 3D structure, and other protein modifications.**

The specificity of mAbs has also made them ideal for medical applications such as diagnostic and therapeutic use. Moreover, technologies such as antibody phage display and humanized mice have expanded the available options to generate novel mAbs, in particular human or humanized mAbs for therapeutic purposes. While a short discussion of each of these technologies is provided later, the primary focus of this paper is the production of antigen specific mouse mAbs - using hybridoma technology - for use as reagents.

Generally, when compared to pAbs, mAbs are considered superior as they are more homogenous and have greater batch-to-batch consistency. But custom mAb production.

This can result in additional costs and time to generate batches. Considering the hybridoma method of generating antigen specific mouse mAbs, both processes begin with the immunization of the animal to generate antibody-producing B-cells. However, when generating mAbs, the B-cells are isolated from the spleen or other lymphoid tissues and fused with a non-secreting, self-replicating myeloma cell line to form a hybridoma (instead of collecting serum, as in the production of pAbs).

# Hybridoma

Individual hybridoma cells have the ability to reproduce and secrete the antibody of interest while continuing to proliferate indefinitely. Clonal cell populations are obtained by limited dilution cloning and secreted antibodies are screened for antigen specificity. Once a hybridoma clone with the required characteristics has been isolated, production is scaled up to obtain large quantities of the antibody needed for use in downstream applications (Figure 1).

The generation of hybridomas and production of mAbs can take several months or even longer depending on the antigen of interest (Figure 2). In the majority of cases, a 9–10 month development time is a reasonable estimate for project planning purposes.

The principal advantages of mAbs over pAbs are homogeneity and consistency. Following isolation of a single hybridoma, these cells can serve as a quick, constant, cost-effective and renewable source of a specific mAb. Yet the production of pAbs can vary from batch to batch due to the variation in the timing and magnitude of immune responses in animals.

The unlimited replicative potential of hybridoma cells allows high-density growth in culture and produces significant levels of mAbs. A hybridoma cell line is essential to the production of a mAb. Each stabilized cell line yields a consistent amount of antibody when cells are cultured. As cell cultures are expanded, the amount of antibody produced by the cells will increase.

Since the advent of monoclonal technology, there have been significant advancements in cell culture techniques and systems. As a result, large quantities of highly concentrated antibody can be produced. Numerous technologies and approaches based on cell culture methods are available utilizing tissue culture flasks, spinners, roller bottles, stirred tank fermenters and hollow fiber bioreactors. Traditional methods of production involved *in vivo* ascites production in animals, although these approaches are less common today due to animal welfare considerations. Indeed, ascites production has been banned outright in Europe.

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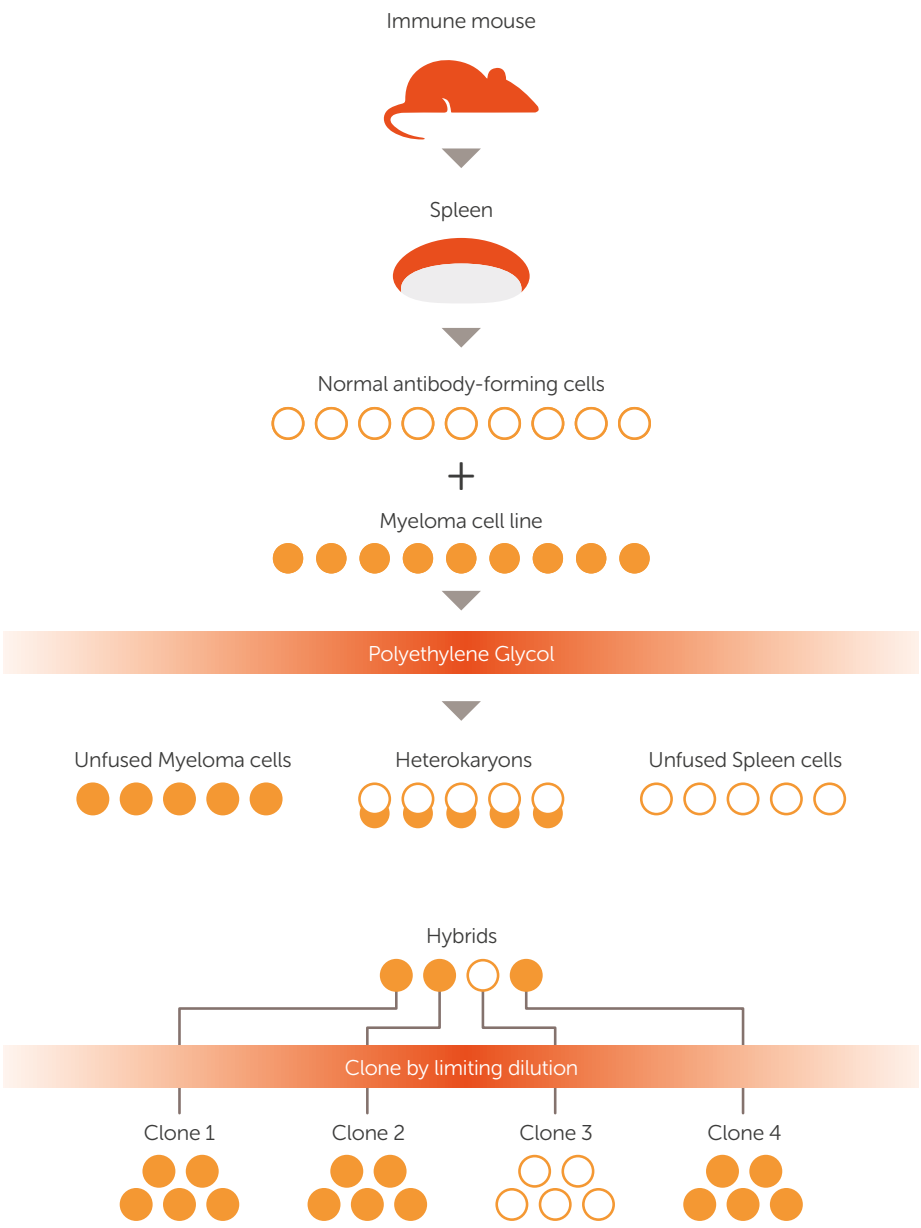
# Hybridoma

## Figure 1: The two major stages of mAb production using hybridoma technology.




### Stage 1:

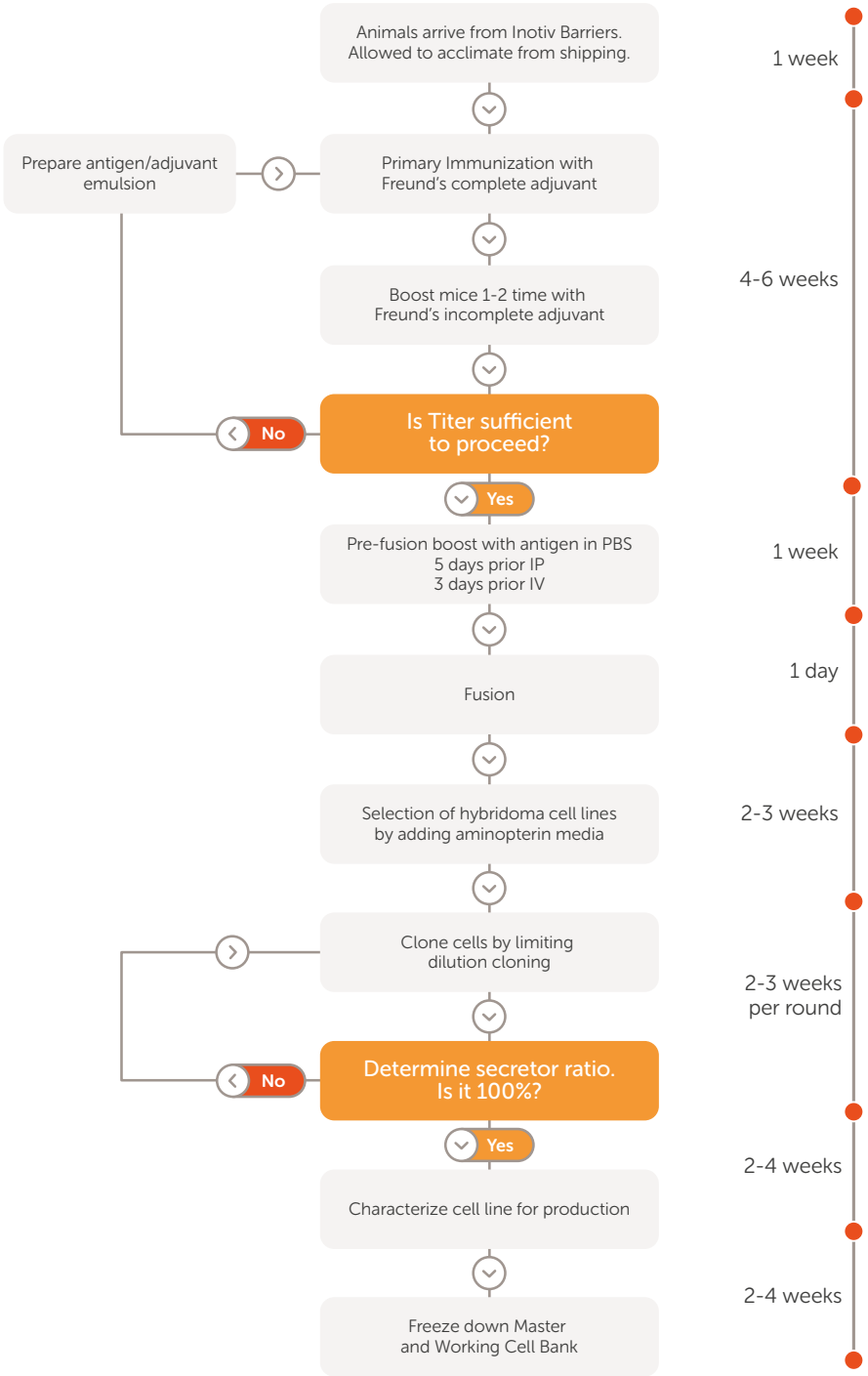
Splenocytes from immunized mice are fused with myeloma cells that have a defective hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) gene that makes them drug-sensitive. When the cell mixture is cultured in the presence of a selective drug, only the hybrid myeloma cells survive, because they obtained a normal HGPRT gene from the splenocytes. These cells are immortal and some will secrete the desired antibody.

### Stage 2:

Clones of cells that secrete the antibody of interest are identified and expanded (figure adapted from Goding, 1986).

# Hybridoma

## Figure 2: Flowchart depicting the typical processes and timelines related to the development of a custom mAb using the hybridoma method.



# Antibody phage display

**Antibody phage display (APD) is a platform used to generate fully human antibodies against a selected antigen. Building upon phage display technology, which was originally described by George Smith in 1985 (Smith, 1985), a number of scientists subsequently demonstrated that antibody fragments could be displayed on the surface of phage particles (McCafferty, 1990; Barbas, 1991).**

This breakthrough meant that human antibodies could now be manufactured with the use of *in vitro* processes, which enable greater opportunities for optimization once such antibodies are generated (e.g., engineered to make mutant proteins of higher affinity or smaller molecular variants) (Lerner, 2016). Furthermore, genetic and functional analyses of the selected mAb have significantly facilitated studies on the mechanisms of the human immune system.

Generally, APD (as well as other large antibody diversity systems) involves three major steps. First, a vector is constructed to allow the incorporation of a large number of different light chain and heavy chain genes, either of natural or synthetic origin (Lerner, 2016). The huge diversity in the library is generated by the combinations of these different antibody chains, whereby each library is constructed from cDNA derived from the B cells of naive and/or immunized human/animal donors (Lee, 2007; Lerner, 2016; Shukra, 2014; Lonberg, 2008). Second, the vector is expressed in a host system – such as the filamentous M13 phage - that allows the coupling of the genotype (i.e., antibody gene) with the phenotype (i.e., antibody molecule expressed outside of the host) (Lee, 2007; Lerner, 2016). For instance, in the case of the phage system, the heavy chain is linked to a coat protein of the phage, resulting in an antibody that is accessible on the surface of the phage particle. Once a large collection of phage-bearing antibody molecules has been generated, a selection process must be applied to isolate those phages that bind to a given antigen. This third step is often referred to as "panning," in which phages are exposed to the antigen (with the use of antigen-conjugated affinity binding columns), and only the antigen-bound phages are replicated via infection of Escherichia coli to amplify the monoclonal antibody construct (Hoogenboom, 2005; Lerner, 2016). Subsequently, the antibody genes in these phage particles are used in expression systems to generate purified antibodies (Shukra, 2014, Lerner, 2016). Antibody phage display has been used to successfully generate research antibodies, as well as a number of antibody-based therapeutics, including adalimumab (Humira; AbbVie) (Lorenz, 2002), belimumab (Benlysta; GlaxoSmithKline) (Stohl, 2012), and many others (Nixon, 2014).

Antibody phage display has enjoyed remarkable successes over the past few decades, and it will likely continue to play a major role in the development of next-generation research and therapeutic mAbs. However, APD remains a technically demanding and time-consuming platform whose potential benefits and risks relative to other available technologies must be carefully weighed.

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# Humanized mice

Approximately two decades ago, a series of publications disclosed the first genetically engineered mice that expressed fully human antibody repertoires (Lonberg, 1994; Green, 1994). These engineered animals had targeted disruptions of the endogenous mouse heavy and light chain genes, together with introduced transgenes comprising un-rearranged human heavy chain and light chain gene segments. In sum, these human immunoglobulin transgenic mice express B cell receptors that are fundamentally hybrids of mouse and human components, and their B cells develop and mature into seemingly normal B cell subtypes (Longberg, 2008). Although the un-rearranged gene segments included in the original transgenes represented only a small fraction of the complete human heavy and light chain repertoire, IgM (Green, 1994) and IgG (Lonberg, 1994) mAbs, which specifically recognized the antigens of interest, were isolated.

The goal to humanize mouse antibodies achieved its apex in September 2006, when the FDA approved the first human mAb generated in a transgenic mouse—Vectibix® (panitumumab; Amgen)—for epidermal growth factor receptor-expressing colorectal cancers (Jakobovits, 2007). Transgenic mice for the development of human mAbs remains an active area of research and development; a variety of additional transgenic rodent strains have been developed and reported in the literature (Bruggemann, 2015).

A prominent example that has garnered much attention is the VelocImmune® mouse developed by scientists at Regeneron Pharmaceuticals (Murphy, 2014). This mouse retains the transcriptional control elements and constant regions of the mouse, but six megabases of mouse DNA that code for the production of every mouse variable region has been replaced with the human heavy and light chain counterparts (Murphy, 2014).

Importantly, because the VelocImmune® mouse still harbors the mouse constant regions, the immune systems of these animals mount a robust, humoral-immune response that is indistinguishable from that of a normal, wild-type mouse (Murphy, 2014). The amazing efficiency of the VelocImmune® approach was confirmed by the authors, who disclosed that they were able to rapidly progress ten different fully human antibodies into human clinical trials (Murphy, 2014).

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Another approach used to develop human mAbs in mice has been to use immunodeficient mice engrafted with human cells that have the ability to ultimately mount a human immune response, such as human peripheral blood lymphocytes and hematopoietic stem cells (HSCs) (e.g., Laffleur, 2012; Becker, 2010; Nguyen, 1997). For example, inoculating newborn immunodeficient mice with human fetal or umbilical cord HSCs can result in a robust engraftment of a number of immune cells, including T, B, NK, and dendritic cells (Becker, 2010). As an illustration, Becker et al. used vaccinated human immune system mice to generate human mAbs against a number of different antigens. The investigators used mice deficient of recombinase activating gene-2 (Rag2) and the common gamma chain of the IL-2 receptor (Il2rg) [i.e. $Rag2^{-/-}IL2R\gamma c^{-/-}$], which are permissive for human HSC xenografts. Using this approach, the authors successfully generated fully human mAbs based on the immunization of these mice engrafted with human CD34+CD38- HSCs (Becker, 2010).

While many researchers have used humanized immune system models to generate mAbs, the results have generally been less than stellar (e.g., Villaudy, 2014). In an analysis by Seung et al. (2013), the authors reviewed multiple studies that measured circulating human antibody levels in various humanized immune system mice. The authors found that the data were highly variable, particularly the total IgG and IgM blood serum levels in naive humanized HSC and BLT mice; the levels were also substantially lower than those found in humans (Seung, 2013).

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# Key steps of optimal antigen specific mouse mAb production

**As discussed in the previous articles in this series, each step in the production of a mAb requires careful attention, including selection of antigen and adjuvant, choice of host species and the immunization procedure for inducing a B-cell antibody response. Additional considerations specific to mAb production include protocols for hybridoma generation, cryogenic preservation and liquid nitrogen storage, as well as methods for scaling up the production of the antibody to obtain sufficient amounts of reagent.**

One of the major differences between the production of mAbs and that of pAbs is the animal species most frequently used for immunization. Whereas rabbits or larger animals are often used for pAb production, mice are most commonly used for the generation of mAbs. The BALB/c mouse strain has traditionally been used because it is syngeneic with the myeloma cell lines required for the cell fusion process and hybridoma generation (Leenaars *et al.*, 2005).

Initial attempts to use other species were not successful due to poor hybridoma stability (Greenfield, 2013) and non-specific secretion of antibodies. However, over the last decade, several companies have developed technologies that enable the production of hybridomas and mAbs in alternative species, notably hamsters, and rabbits (Feng *et al.*, 2011).

The fusion of B-cells with myeloma cells requires tissue culture facilities and technical expertise. Cell culture considerations, such as choice of media, media supplements, myeloma cell line selection and plating method, are just as important as other aspects of antibody production like antigen selection and host species. Due to alterations of the lipid membranes, hybridoma cells are very fragile immediately after the fusion process. Great care must be taken during this period, because no backup stock of cells yet exists and a long immunization period is required even to reach this phase of production. Fortunately, once hybridomas have been maintained in tissue culture for several hours, they are relatively stable and easier to maintain.

The routine culture of myeloma and hybridoma often includes the use of fetal bovine serum (FBS) to provide necessary cellular factors that are required for growth. FBS batches with low immunoglobulin concentration are often used, because high immunoglobulin (Ig) content may interfere with some downstream assays (Greenfield, 2013).

The use of FBS also has major drawbacks, for example, the considerable lot-to-lot variation in biological properties and the potential for contamination with infectious agents (Bovine spongiform encephalopathy (BSE), mycoplasma, etc.). To overcome these challenges, a number of commercial serum-free media (SFM) products have been developed to support the growth and maintenance of cells.

When using a SFM product, it is important to consider the intended use of the antibody product, potential regulatory issues, as well as the increased costs associated with these more defined media formulations. It is also vital to determine whether the cells can adapt to serum-free conditions and whether antibody secretion or downstream processing will be affected by some of the media components. For instance, antibodies produced in SFM can exhibit different glycosylation profiles (Serrato *et al.*, 2007).

Depending on the intended use of the mAb, it may also be worthwhile to ensure that a drug master file (DMF) is on record with the FDA for the particular media formulation.

A DMF provides detailed and confidential information regarding the facilities, processes or components used in the manufacturing, processing, packaging and storage of the media formulation for use in human drugs or biological diagnostics.

The use of an SFM product to generate a mAb for a licensed antibody-based diagnostic or therapeutic drug will lead the FDA to consult the DMF in its assessment of any regulatory filing. Furthermore, because SFM products are proprietary, it is important to assess the financial stability of the vendor, how easy they are to work with and whether there are any licensing issues to address.

In the event that the product is discontinued or the relationship deteriorates, the impact of potentially having to switch growth media during the manufacturing process could be significant, especially if the change arises without warning. For instance, with regard to diagnostic antibodies, if a change in the manufacturing process occurs, the FDA notes that sponsors should "develop a plan for demonstrating that the products made by the old and new schemes are comparable" (FDA, 1997). As such, appropriate testing often needs to be conducted.

Although a full discussion of the types of analytical, functional and animal tests that one might need to conduct is beyond the scope of this article, the FDA has issued guidance that discusses appropriate comparability testing for mAb reagents (FDA, 1997). Therefore, the decision to use a serum-free or more chemically-defined medium is not always simple. All aspects associated with its use should be considered.

The production of mAbs is technically demanding. Difficulties can usually be traced back to improper selection or subcloning of the hybridomas. Myeloma cells should be screened for deficiency of the hypoxanthine-guanine-phosphoribosyl transferase gene to ensure that they are suitable for cell fusion, are non-secreting and are amenable to drug-susceptible selection.

If cells are no longer killed in the presence of aminopterin, they will eventually take over the culture. Any myeloma lines that lose their sensitivity to drug selection should be discarded.

Additionally, because more than one hybridoma clone is often present in the tissue culture well or plate following drug selection, it is absolutely vital to carry out single-cell cloning, which involves multiple rounds of cloning by limiting dilution analysis until every cell in the well secretes the antibody of interest in a stable fashion (Greenfield, 2013).

One of the main advantages of mAbs is consistency. The use of a single immortal hybridoma clone provides a stable source of a particular mAb. For antibodies that perform well in downstream applications, these clones are an invaluable resource for the research, diagnostic and therapeutic communities.

Hybridoma clones that are acceptable for production are frozen in cryopreservation media and stored in the vapor phase of liquid nitrogen. Clones designated for good manufacturing practices (GMP) production are frozen in accordance with the FDA's *Points to Consider in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use* (1997). This includes creating a master cell bank and a working cell bank that provide an adequate supply of the cell lines that are free of cross-contaminants and adventitious agents.

One of the main advantages of mAbs is consistency

# Scaling up production of monoclonal antibodies

There are many different options for scaling up the production of mAbs. This subject could form a lengthy article in its own right. For the purposes of this article we will focus upon hollow fiber production as Inotiv has invested extensively in this technology over many years.

Hollow fiber reactors are vessels used to collect tissue culture supernatants, a popular method of producing large amounts of mAbs (Figure 3). Although these reactors can play host to a variety of cell types (e.g., CHO, 293, etc.), in the case of mAb production, they allow the hybridoma cells to expand on their porous surface instead of on plastic (Dewar et al., 2005). Thousands of semi-permeable hollow fibers (5–30 kDa) are arranged in parallel within a cartridge that provides a 3D environment for cellular growth and is fitted with inlet as well as outlet ports on both sides of the fibers (Dewar et al., 2005).

Briefly, cells are seeded within the cartridge, but outside the hollow fibers. Freshly oxygenated culture medium is continuously pumped through the hollow fibers. This flow generates a relatively consistent environment for the cells, permitting nutrients and waste products to diffuse across fiber walls.

Because the cells are isolated from the rapidly flowing fresh media, shear forces are virtually eliminated. Together, these features allow for high-density cellular growth (estimated at >109/mL) and the removal of waste products, like lactate and $CO_2$. The semi-permeable fiber retains high molecular weight proteins, which results in a high concentration of the secreted antibody. Hollow fiber bioreactors can be used for several months at a time, with little loss of production (Greenfield, 2013; Dewar *et al.*, 2005; Evans *et al.*, 1988; NRC, 1999).

The continuous removal of waste products is a significant feature that distinguishes hollow fiber reactors from systems like the WAVE-rocking bioreactor and most stirred tank reactors. In essence, these other systems permit the buildup of toxic waste products, which when not removed, leads to the premature death of the cells. In addition, because semi-permeable hollow fibers separate the cells from the fresh media supply, the use of costly media reagents, such as FBS, growth factors, hormones, etc. can be significantly reduced.

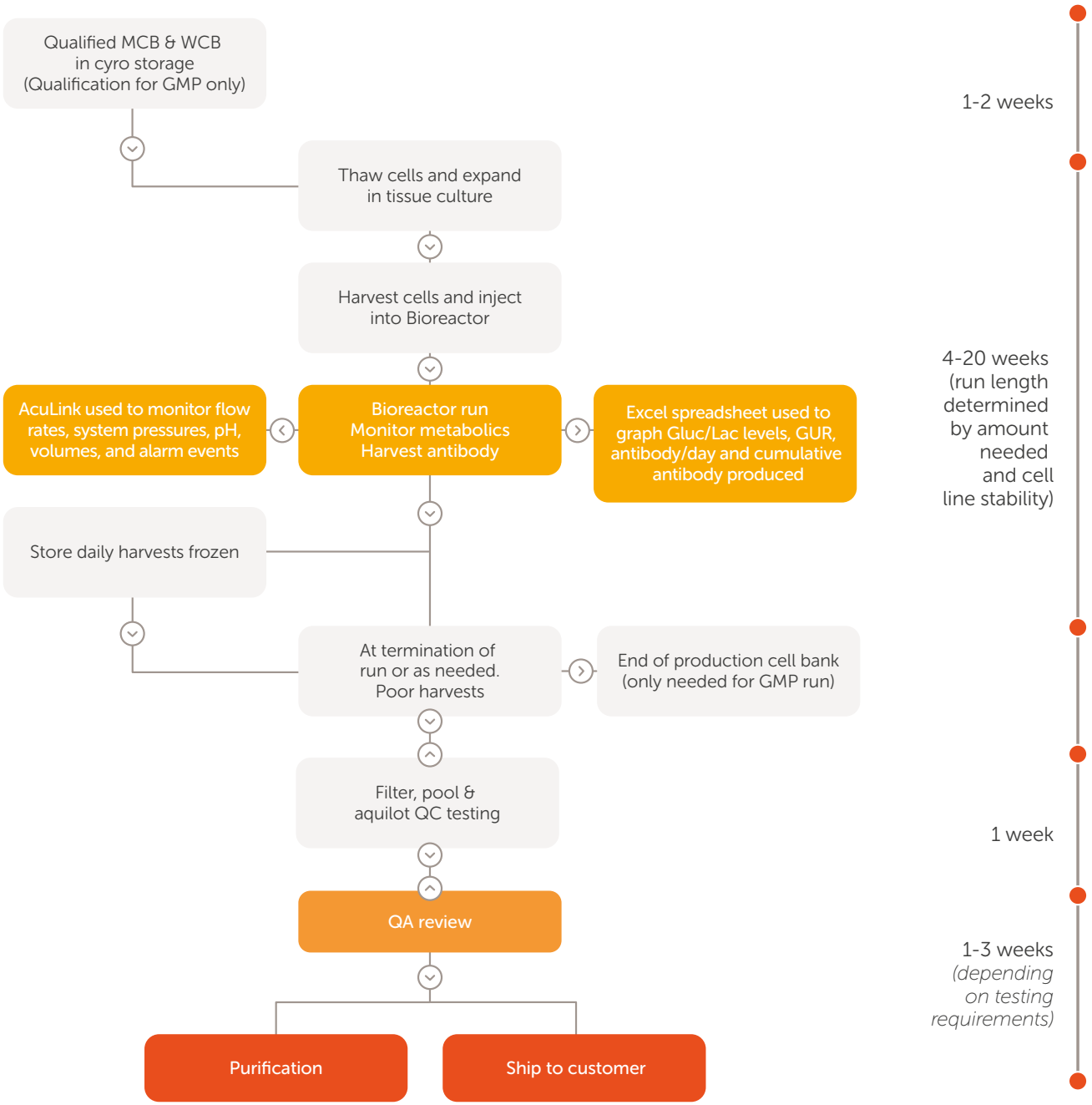
When using hollow fiber reactors to scale up antibody production, cartridge size, media formulations, feed strategies, hollow fiber composition and pore size should be considered. Also to be considered are the capacity of circulation pumps, oxygenator needed to provide adequate oxygenation, pH control, antibody harvesting strategies (batch, perfusion or timed batch), process control (e.g., temperature, pH control, nutrients) and analytics (e.g., nutrients, waste products, antibody concentration, endotoxins, virus, end product integrity). Once a hollow fiber reactor has been optimized for mAb production, one can expect to reliably and consistently generate superior, high-titer antibodies with a well-defined quality over a period of several months.

Hollow fiber production processes allow for scale-up, production according to either good laboratory practices (GLP) or GMP standards, and the generation of a highly concentrated product. An advantage over *in vivo* production methods are the lack of extraneous animal proteins, the absence of contaminating host antibodies that must be purified, and the flexibility to use a variety of cell lines (Peterson et al., 1998; Marx *et al.*, 1995). Additionally, antibodies can be harvested at any point in time from the hollow fiber production for use in experiments, downstream processing, and/or inventory while maintaining production to generate additional antibody.

Hollow fiber reactors are vessels used to collect tissue culture supernatants, a popular method of producing large amounts of mAbs.

# Scaling up production of monoclonal antibodies

## Figure 3: Typical processes and timelines associated with the generation of purified mAbs using hollow fiber bioreactor production.



# Purification

**As discussed in the previous pAb article in this series, the purification of antibody products will eliminate other host cell material, microorganisms and immunoglobulins, all of which can increase background and negatively affect antibody performance. Refer to Inotiv's pAb article and its citations for more on protein A/G-based affinity chromatography for antibody purification (Grodzki et al., 2010; Roque et al., 2007; Liu et al., 2010).**

Purification strategies can also be used to eliminate antibody aggregates that may affect the performance of the mAb as well as to separate the whole antibody molecule into different fragments following selective cleavage using papain or pepsin. To eliminate antibody aggregates, various chromatography techniques can be used, including ion-exchange chromatography and hydrophobic interaction chromatography (Evans *et al.*, 2015; Vázquez-Rey *et al.*, 2011).

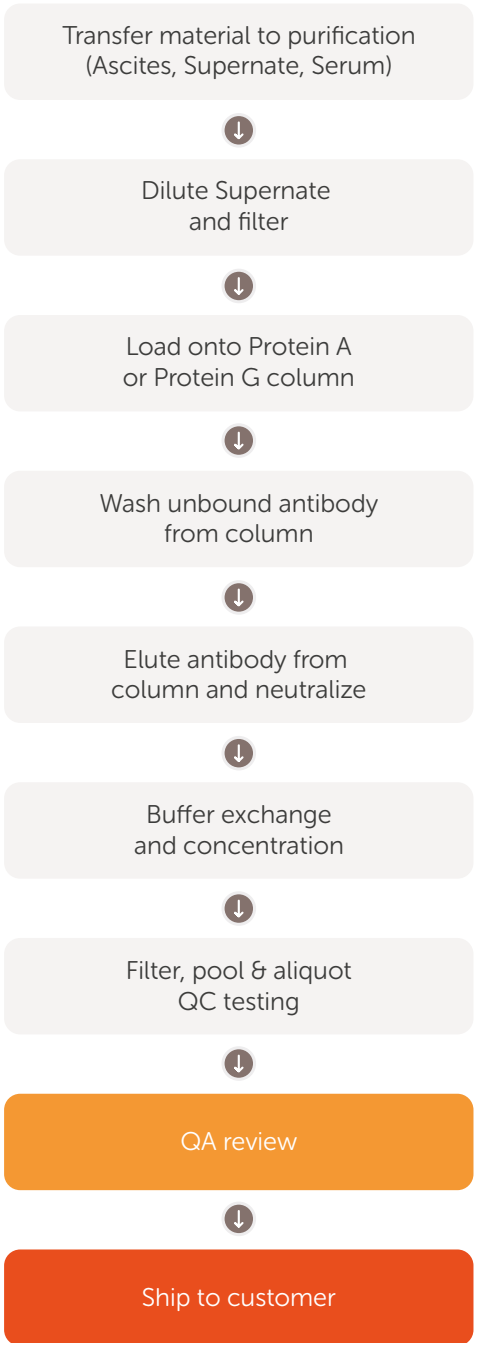
With respect to employing antibody fragments, some assays, such as immunohistochemical approaches, exhibit better results when antibody fragments are used due to a reduction in nonspecific Fc interactions. In addition, the use of antibody fragments for *in vivo* experiments often results in lower immunogenicity (Low et al., 2007; Roque et al., 2007).

Overall, the selection of a particular purification technique is not always straightforward, and multiple factors should be taken into consideration in order to strike an appropriate balance between antibody yield, quality and practicality. The flowchart in Figure 4 depicts typical processes and timelines in the purification of an antibody.

Purification strategies can also be used to eliminate antibody aggregates that may affect the performance of the mAb.

Overall, the selection of a particular purification technique is not always straightforward, and multiple factors should be taken into consideration.

Figure 4: Typical processes and timelines associated with the production of a purified antibody.



# Quality testing

**Although quality testing does not impact the quality of a mAb antibody per se, many of the considerations given to pAb production should be given to the generation of mAbs, including, at minimum, specificity testing against the target antigen. In addition, adequate characterization at the hybridoma-development stage (i.e., stage 2 in Figure 1) removes the need for repeated testing procedures for each cycle of production, because the master and working cell banks allow the production process to start from the same point each time (FDA, 1997).**

Lastly, mAbs generated for use as reagents should be sterilized to maintain the integrity of the product, and antibody purity should be assessed using SDS-PAGE. Refer to Inotiv's pAb article for additional details on the quality testing of antibody products.

# Conclusions

**The production of a custom antigen specific mouse mAb is a time-consuming and technically demanding process. However, once stable hybridomas have been established, they can be expanded and frozen to create a reliable source of the mAb. Scaling up the production of mAbs can be done using a wide range of various methodologies. Additional technologies, such as APD and humanized mice, have provided some great successes in generating human or humanized mAbs.**

However, it is important to carefully evaluate the potential benefits and risks relative to other available technologies, and the downstream use of your custom antibody.

Careful planning and preparation are required to produce high-quality hybridomas and mAbs. Working with a partner with deep practical experience can both ensure the optimization of each step along the way and save time and money by eliminating the most common pitfalls.

## REFERENCES


1. **Barbas, C.F.,3rd, A.S. Kang, R.A. Lerner, and S.J. Benkovic. 1991.** Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. *Proc.Natl.Acad.Sci.U.S.A.* 88:7978-7982.
2. **Becker, P.D., N. Legrand, C.M. van Geelen, M. Noerder, N.D. Huntington, A. Lim, E. Yasuda, S.A. Diehl, F.A. Scheeren, M. Ott, K. Weijer, H. Wedemeyer, J.P. Di Santo, T. Beaumont, C.A. Guzman, and H. Spits. 2010.** Generation of human antigen-specific monoclonal IgM antibodies using vaccinated "human immune system" mice. *PLoS One.* 5:10.1371/journal.pone.0013137.
3. **Bruggemann, M., M.J. Osborn, B. Ma, J. Hayre, S. Avis, B. Lundstrom, and R. Buelow. 2015. Human antibody production in transgenic animals. Arch.Immunol.Ther.Exp. (Warsz). 63:101-108.**
4. **Dewar V., et al. (2005).** Industrial implementation of *in vitro* production of monoclonal antibodies. *ILAR J.* 46(3):307-13.
5. **Evans, W. (2015).** Removing Aggregates in Monoclonal Antibody Purification. *Pharmaceutical Technology.* 39 (3).
6. **Evans T.L., Miller R.A. (1988).** Large-scale production of murine monoclonal antibodies using hollow fiber bioreactors. *Biotechniques.* Sep;6(8):762-7.
7. **FDA (US Food and Drug Administration).(1997).** *Points to Consider in the Manufacturing and Testing of Monoclonal Antibody Products for Human Use.* Available at: http://www.fda.gov/downloads/ BiologicsBloodVaccines/Guidance ComplianceRegulatoryInformation/ OtherRecommendationsforManufacturers/UCM153182.pdf
8. **FDA (US Food and Drug Administration). (1996).** *Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products.* Available at: http://www.fda.gov/Drugs/Guidance ComplianceRegulatoryInformation/Guidances/ucm122879.htm
9. **Feng, L., et al. (2011).** Rabbit monoclonal antibody: Potential application in cancer therapy. *Am. J Transl Res* 3(3), 269-274.
10. **Goding, J.S. (1986).** Monoclonal Antibodies: *Principles and Practice.* Academic Press, London.
11. **Green, L.L., M.C. Hardy, C.E. Maynard-Currie, H. Tsuda, D.M. Louie, M.J. Mendez, H. Abderrahim, M. Noguchi, D.H. Smith, Y. Zeng, N.E. David, H. Sasai, D. Garza, D.G. Brenner, J.F. Hales, R.P. McGuinness, D.J. Capon, S. Klapholz, and A. Jakobovits. 1994.** Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nat.Genet.* 7:13-21.
12. **Greenfield, E.A. (2013).** Antibodies: A Laboratory Manual. 2nd Ed. Cold Spring Harbor, NY: CSHL Press.
13. **Grodzki, A.C., Berenstein, E. (2010).** Antibody purification: affinity chromatography: Protein A and protein G Sepharose. In C. Oliver & M.C. Jamur (eds.), Immunocytochemical Methods and Protocols (pp. 33-41). New York, NY: Springer.
14. **Hendriksen, C.F., de Leeuw, W. (1998).** Production of monoclonal antibodies by the ascites method in laboratory animals. Res. Immunol 149(6), 535-42.
15. **Hoogenboom, H.R. 2005.** Selecting and screening recombinant antibody libraries. *Nat.Biotechnol.* 23:1105-1116.
16. **Jakobovits, A., R.G. Amado, X. Yang, L. Roskos, and G. Schwab. 2007.** From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. *Nat.Biotechnol.* 25:1134-1143.
17. **Köhler, G., Milstein, C. (2005)** Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256(5517), 495-497.
18. **Laffleur, B., V. Pascal, C. Sirac, and M. Cogne. 2012.** Production of human or humanized antibodies in mice. *Methods Mol.Biol.* 901:149-159.
19. **Lee, C.M., N. Iorno, F. Sierro, and D. Christ. 2007.** Selection of human antibody fragments by phage display. *Nat.Protoc.* 2:3001-3008.
20. **Leenaars, M., Hendriksen, C.F.M. (2005).** Critical steps in the production of polyclonal and monoclonal antibodies: evaluation and recommendations. ILAR J 46(3), 269-279.
21. **Lerner, R.A. 2016.** Combinatorial antibody libraries: new advances, new immunological insights. *Nat.Rev.Immunol.* 16:498-508.
22. **Liu, H.F. et al. (2010).** Recovery and purification process development for monoclonal antibody production. *MAbs* 2(5), 480-499.
23. **Lonberg, N., L.D. Taylor, F.A. Harding, M. Trounstine, K.M. Higgins, S.R. Schramm, C.C. Kuo, R. Mashayekh, K. Wymore, and J.G. McCabe. (1994).** Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature.* 368:856-859.
24. **Lonberg, N. 2008.** Fully human antibodies from transgenic mouse and phage display platforms. Curr.Opin.*Immunol.* 20:450-459.
25. **Lorenz, H.M. 2002.** Technology evaluation: adalimumab, Abbott laboratories. Curr.Opin.Mol.Ther. 4:185-190.
26. **Low D., O'Leary R., Pujar N.S. (2007).** Future of antibody purification. J Chromatogr B 848(1), 48-63.
27. **Marx, U., Merz, W. (1995).** *In Vivo* and *In Vitro* Production of Monoclonal Antibodies. In Davis W.C. (ed.), Monoclonal Antibody Protocols (pp. 169-176).
28. **McCafferty, J., A.D. Griffiths, G. Winter, and D.J. Chiswell. 1990.** Phage antibodies: filamentous phage displaying antibody variable domains. *Nature.* 348:552-554.
29. **Moore, J.M., Rajan, T.V. (1994).** Pristane retards clearance of particulate materials from the peritoneal cavity of laboratory mice. J Immunol Methods 173(2), 273-278.
30. **Murphy, A.J., L.E. Macdonald, S. Stevens, M. Karow, A.T. Dore, K. Pobursky, T.T. Huang, W.T. Poueymirou, L. Esau, M. Meola, W. Mikulka, P. Krueger, J. Fairhurst, D.M. Valenzuela, N. Papadopoulos, and G.D. Yancopoulos. 2014.** Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normal mice. *Proc.Natl.Acad.Sci.*U.S.A. 111:5153-5158.
31. **Nguyen, H., J. Sandhu, and N. Hozumi. 1997.** Production of human monoclonal antibodies in SCID mouse. *Microbiol.Immunol.* 41:901-907.
32. **Nixon, A.E., D.J. Sexton, and R.C. Ladner. 2014.** Drugs derived from phage display: from candidate identification to clinical practice. *Mabs.* 6:73-85.
33. **NRC (National Research Council). (1999).** Chapter 2: *In Vitro* Production of Monoclonal Antibody. *In Monoclonal Antibody Production* (pp. 12-15). Washington, DC: The National Academies Press.
34. **Peterson N.C., Peavey J.E. (1998).** Comparison of *In Vitro* Monoclonal Antibody Production Methods with an In Vivo Ascites Production Technique. Contemp Top Lab Anim Sci. Sep;37(5):61-66.
35. **Roque A.C., Silva C.S., Taipa M.A. (2007).** Affinity-based methodologies and ligands for antibody purification: advances and perspectives. J Chromatogr A 1160(1), 44-55.
36. **Seung, E., and A.M. Tager. 2013.** Humoral immunity in humanized mice: a work in progress. J.Infect.Dis. 208 Suppl 2:S155-9.
37. **Serrato, J.A., et al. (2007).** Differences in the glycosylation profile of a monoclonal antibody produced by hybridomas cultured in serum-supplemented, serum-free or chemically defined media. *Biotechnol Appl Bioc* 47(2), 113-124.
38. **Shukra, A.M., N.V. Sridevi, C. Dev, and M. Kapil. 2014.** Production of recombinant antibodies using bacteriophages. *Eur.J.Microbiol.Immunol.*(Bp). 4:91-98.
39. **Smith, G.P. 1985.** Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science. 228:1315-1317.
40. **Stewart, S.J. (2001).** Chapter 6: Monoclonal Antibody Production. In G.C. Howard & D.R. Bethel (eds.), Basic methods in antibody production and characterization (pp. 51-68). Boca Raton, FL: CRC Press.
41. **Stohl, W., and D.M. Hilbert. 2012.** The discovery and development of belimumab: the anti-BLyS-lupus connection. Nat.Biotechnol. 30:69-77.
42. **Vázquez-Rey, M., Lang, D.A. (2011).** Aggregates in monoclonal antibody manufacturing processes. Biotechnol Bioeng. Jul; 108(7):1494-508.
43. **Villaudy, J., R. Schotte, N. Legrand, and H. Spits. 2014.** Critical assessment of human antibody generation in humanized mouse models. J.Immunol.Methods. 410:18-27.
44. **Winter, G., A.D. Griffiths, R.E. Hawkins, and H.R. Hoogenboom. 1994.** Making antibodies by phage display technology. Annu.Rev.Immunol. 12:433-455.

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