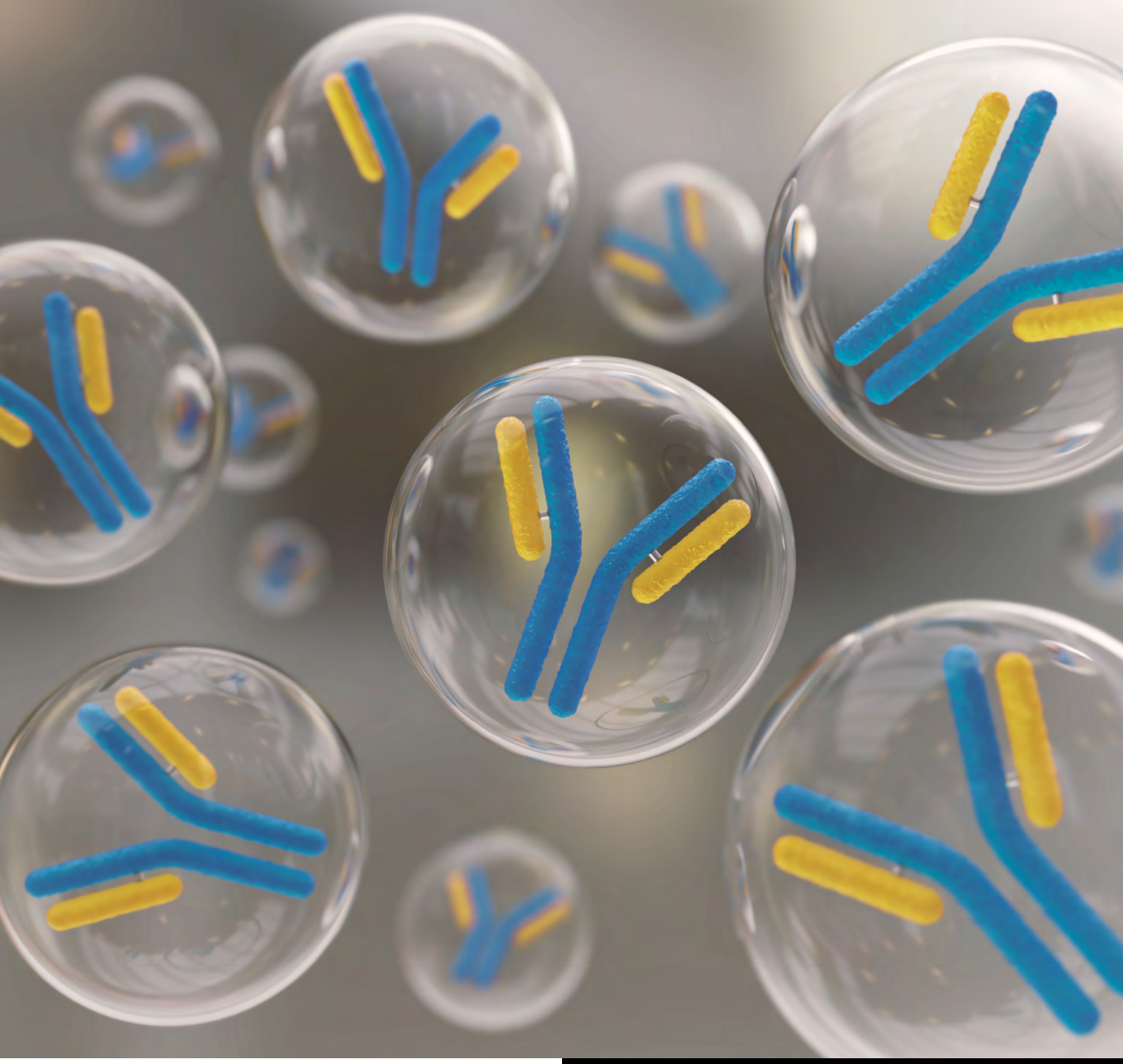

Overcoming Challenges in High Density Cell Culture Harvesting During Antibody Development

Simplifying Progress

SARTORIUS

Sartoclear Dynamics® Lab

Filter hands-free up to 6 × 50 mL cell culture samples in parallel with the Sartolab® Multistation!
Facilitate your filtration of small volumes (≤ 50 mL) using the new innovative stand, Sartolab® Multistation, specially designed to hold 1 to 6 vacuum filtration units, allowing simultaneous filtration of up to 6 samples.

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Simplifying Progress

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Foreword

Monoclonal antibodies (mAbs) have been used successfully for years as therapeutic agents for different pathologies, e.g., various types of cancer and autoimmune diseases, making them one of the top growth drivers of the pharmaceutical industry.

Next generation antibodies are designed to be more specific and are often more potent than traditional monoclonal antibodies. As a result, their commercial potential is significant. Next generation antibody formats include antibody-drug conjugates (ADCs), other bioconjugates, bispecific (multispecific) antibodies, nanobodies, engineered antibodies, antibody fragments, and antibody-like proteins.

While antibody therapy offers the promise of better specificity and safety over other cancer treatment methods, it is significantly more cost-intensive compared to drug therapies with chemically defined small molecules. This is due to both the elaborate development and production processes associated with antibodies. After target identification, the antibody based discovery process begins with cloning different candidates, growth in cell cultures, followed by harvesting, clarification and purification of hundreds to thousands of potential candidates. The specificity and efficacy of the candidates is tested in cell based assays. What then follows is the production of the successful candidate after many years of research and testing.

The reduction of costs per treatment course is becoming increasingly important because of the continuous addition of new therapeutic antibodies while the budgets of national healthcare systems are simultaneously being limited.

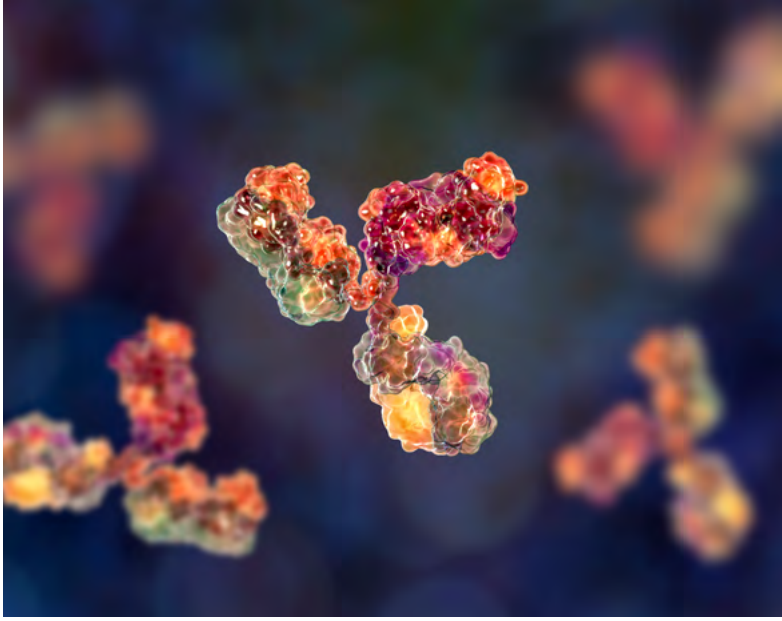
As a result, attempts are being made both to increase the yield of antibodies per production volume and to reduce the development costs by shortening the discovery period. Cost reduction is achieved by continuous optimization of the individual steps.

With over 90 years of expertise in lab filtration, Sartorius offers unique solutions for facilitating and accelerating the cumbersome step of high density cell culture harvest and supernatant clarification. The Sartoclear Dynamics® Lab portfolio allows researchers to harvest and purify drug candidates faster, allowing a higher throughput, enabling candidates to pass on to the production phase more rapidly. As a result, finding the right antibody treatment will be faster and more cost-efficient.

This eBook explores everything from the developmental stages of mammalian cell development to the innovative solutions that are enabling successful antibody therapies.

Content

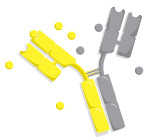
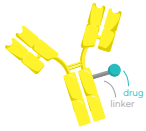
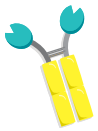
Facilitating Mammalian Cell Culture Harvesting



By harnessing antibody specificity, indispensable tools have been created for basic research (e.g. in radioimmunoassays and cell imaging), *in vitro* diagnostics (e.g. lateral flow assays, microarrays) and therapeutics. More than 80 therapeutic monoclonal antibodies have been approved to treat a range of conditions including autoimmune diseases, cancer and infectious diseases.[1] Monoclonal antibodies (mAbs) can be generated indefinitely by immortal antibody-producing cells in culture, and significant efforts are being directed at optimizing their production and use in next generation therapies. Some therapeutic antibodies are conjugated to another chemotherapy drug, radioactive isotope or toxin, while others are "naked", without any drug or radioactive material attached.[2]

Antibodies and other biotherapeutics are being explored and developed as potential advanced targeted therapies (Table 1). mAbs and other therapeutic proteins represent a constant focus of the biopharmaceutical industry, and the technology behind their production continues to advance.

Table 1. Antibodies are being harnessed to produce advanced targeted therapies, including naked mAbs, bispecific and multispecific antibodies, antibody-drug conjugates, nanobodies, and Fc-fusion proteins.

Image	Type of Ab/ biotherapeutic	Description
	Bispecific and multispecific	Recognize two or more different epitopes or antigens.[3] There are two types: 1. Combinatorial: where the effect could also be achieved by combining separate antibodies with the same specificities, usually at a higher cost of development 2. Obligate bi- or multispecific antibodies: produce sequential or simultaneous binding events which depend on the physical linkage of the bound antibodies
	Antibody-drug conjugates	Cytotoxic small molecule drugs are conjugated to a mAb via a chemical linker. Designed to target potent anti-cancer agents to tumor cells
	Nanobodies	Highly specific and stable antigen-binding domains derived from unique, heavy-chain-only antibodies that are produced by species of the Camelidae family (camels and llamas)[4]
	Fc-fusion protein	Binding proteins (Fc domain of an IgG) that can be linked to a therapeutic peptide or protein to extend its half-life[5]

SARTORIUS

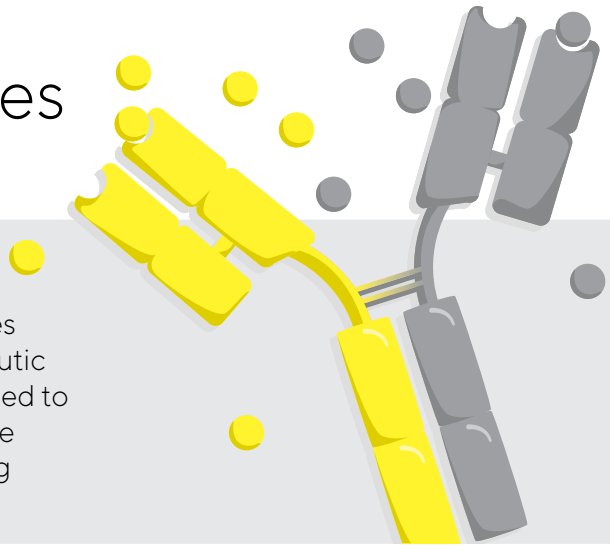
Simplifying Progress

Developing Next generation Therapeutic Antibodies

Therapeutic antibodies are one of most successful immunotherapy strategies for the treatment of both hematologic cancers and solid tumors. The success of this class of drugs stems from the promise of better specificity and safety due to innovative technical advances in antibody engineering and development.

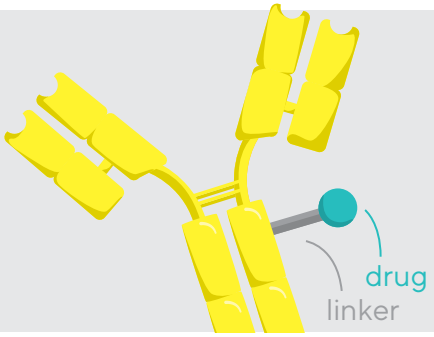
With over 100 approved antibody drugs, antibodies are the fastest growing class of drugs

Next Generation Antibodies



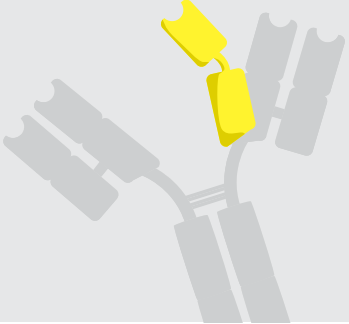
Bi- & Multi-Specific Antibodies

The development or progression of a disease typically involves more than one signaling pathway, therefore multiple therapeutic targets may exist. Bi- and multi-specific antibodies are designed to recognize two or more different targets by combining multiple antigen-recognizing elements into a single molecule, showing promise for cancer immunotherapy.



Antibody-Drug Conjugates

Antibody-drug conjugates (ADCs) combine monoclonal antibodies with biologically active or cytotoxic compounds to produce highly targeted chemotherapeutic agents. After selective binding of the antibody to tumor cells, the conjugated compound is released into the cell causing tumor cell death.



Nanobodies

Nanobodies are modified single antigen-binding variable domain (VHH). They are highly stable, soluble and bind targets that are out of reach of full-length antibodies. Bi-specific nanobodies are currently in development.

Traditional Methods for Antibody Target Production

Traditional Technologies

Most antibody targets are extracellular; found on the surface of cells and tissues or circulating in blood. Traditionally, enzyme-linked immunosorbent assays (ELISAs) have been used to screen for these targets, however, several limitations exist.

Innovative Technologies

Innovative technologies for antibody-based discovery and development, such as advanced flow cytometry, live-cell analysis and Sartoclear Dynamics® Lab kits, have been developed to overcome these challenges.

Challenges	Solutions
Labor intensive workflow with only endpoint measures	Reduced workflow with intuitive, kinetic analysis for every sample
Multiple screens to confirm biological ELISA results such as cell health, specificity and cross-reactivity	Integrated multi-parametric analysis to fully characterize therapeutic antibodies
Biochemical approach (antibody extraction) can disrupt the biology being evaluated	Non-invasive, live-cell analysis to detect true biological changes
Multi-step mammalian cell culture harvest is tedious and time consuming	Single step clarification and sterile filtration collapses cell harvest workflow saving significant time and resources

Click here to learn more about the solutions

Introduction to cell line development

By introducing a gene that deregulates the cell cycle, or by regulating telomerase activity, cells can be manipulated to proliferate indefinitely. The use of immortal cell lines is common in cell culture-based research, and tens of thousands of cell lines have been developed from a variety of donor tissues. Cancer cell lines are used to study the biology of cancer and drug responses,[6] while others have been established to model specific physiological processes, e.g. intestinal absorption.[7] Meanwhile, the development of cells that produce large, complex proteins has revolutionized the biopharmaceutical industry; protein therapeutics represent the largest group of new products in development.[8] While alternative host systems exist (e.g. plants, insect cells, yeasts and transgenic animals),[8] mammalian cells remain the dominant vehicle for biotherapeutic production.[9]

Most reagent antibodies are produced from hybridomas – fusions of an antibody-producing B cell from an immunized animal with a myeloma cell. While hybridomas can produce antibodies indefinitely, they can suffer from genetic drift, compromising the quality and consistency of antibody production.[10] To improve antibody purity, recombinant technology has been pursued as an alternative, and it is an increasingly popular approach. Recombinant antibodies are produced by cloning desired antibody genes in an expression vector that is introduced into the host cell for protein synthesis. Common mammalian cell hosts include Chinese hamster ovary (CHO) and human embryonic kidney (HEK 293) cell lines, which produce highly reproducible antibodies based on the initial defined sequence.[11]

Attaining a production cell line with desired traits is critical to the overall success of the therapeutic protein. Cell line

Stages of Cell Line Development

Vector Design and Transfection Protocol	Optimization of the vector design and protocol for introducing foreign DNA to the host cell
Stable Transfection	"Selection markers" are co-expressed with the target protein to identify cells that stably express transfected DNA
Selection	Selection of cells that stably express transfected DNA and possess desirable manufacturing-related traits
Cell Line Optimization Expansion	Media, feed, and processes are refined to maximize growth and productivity
Single Cell Cloning Clone Selection Characterization	High-producing cells with robust growth characteristics are isolated from the original population. Growth, productivity and stability is assessed, usually in well plates or flasks
Expansion Process Development	The manufacturing process is simulated on a smaller scale to refine process performance and further assess final production clones
Production	Cells are cultivated to achieve high cell densities and antibody concentrations. Can be achieved using a range of formats, e.g. in a bioreactor or shaker flask

Figure 1: Typical stages of cell line development

development involves the production of cell line candidates, and the eventual selection of an antibody-producing clone that will be grown in large volumes. Typically, cell line development will follow a common workflow (Figure 1).[9]

Current standard practice for cell culture harvest

To obtain the antibody or protein of interest following production, cells and cell debris must be removed, leaving behind the antibody-containing supernatant. This separation process, known as "clarification", is carried out before the supernatant is subject to further purification and quality control measures downstream (e.g. endotoxin testing). Whether cells are harvested in a large-scale bioprocessing unit (where bioreactor volumes are typically above 50 L) or on a smaller scale in a research laboratory, clarification is typically achieved using centrifugation, followed by filtration.

After one or two rounds of centrifugation, where cells and cell debris settle to the bottom of the centrifuge tubes, supernatant can be removed using a hand-held syringe or a vacuum-driven bottle top filter. Filters are designed to trap remaining cells and debris while allowing mAbs and proteins to pass through; however they are prone to clogging. Additional filtration steps may be required if a filter becomes clogged, or if the harvested supernatant needs to be sterile.

Optimizing your cell culture harvest: nature holds the key

Despite mAbs retaining their position as important therapeutic agents and growth drivers of the pharmaceutical industry,[12] their development and production remains elaborate and cost-intensive, compared with small molecule drugs. Consequently, there is a strong incentive to optimize cell culture processes to shorten development periods and reduce the cost per treatment course.

The typical two-step clarification process creates inefficiencies for antibody production. Limited centrifuge space leads to multiple runs, and increases the time spent clarifying the supernatant. Overall, clarification is time-consuming and reliant on manual labor, with each additional step providing an opportunity for error and contamination. Before each centrifugation round, samples must be balanced manually, while filtration can take minutes to hours, depending on the volume. Furthermore, the clogging of filter membranes increases spending on expensive consumables.

Fortunately, there is now a much faster way to recover antibodies and other proteins from cell culture. By harnessing the properties of a natural, pharmaceutical grade filtration aid, antibodies can be recovered without the need for a tedious two-step clarification process. Diatomaceous Earth (DE) is the highly porous, insoluble

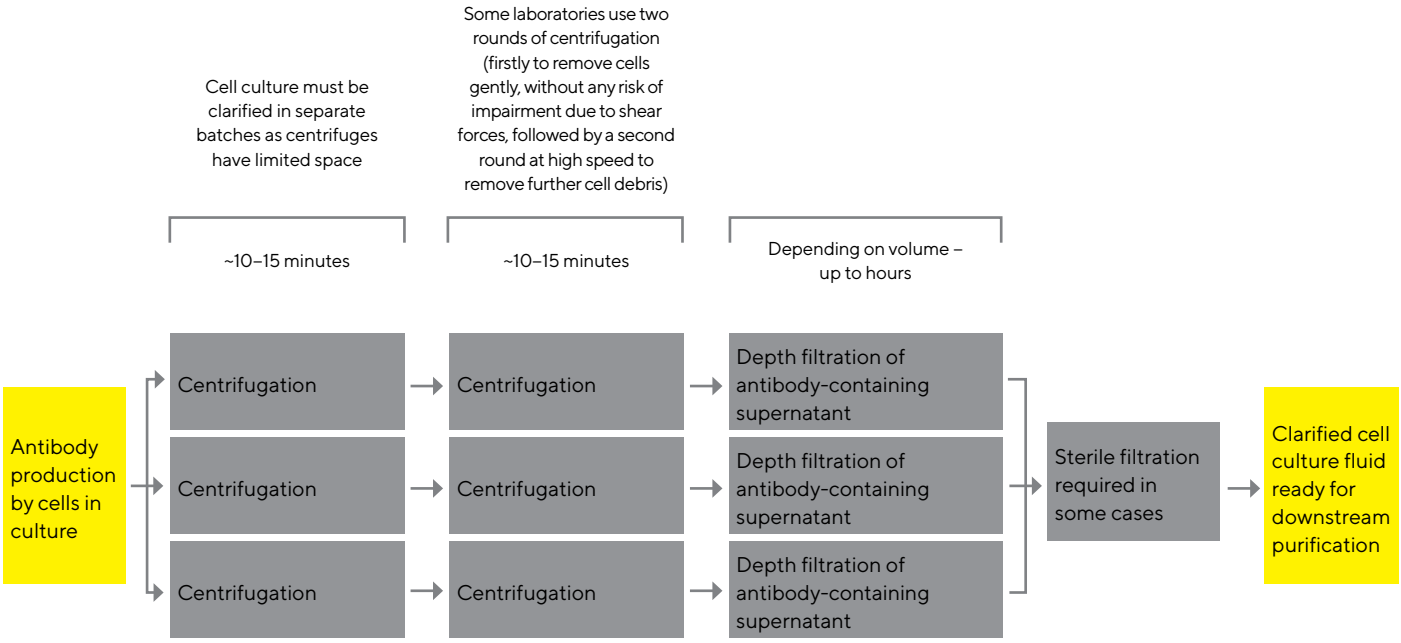


Figure 2: Standard practice for cell culture clarification

material that removes the need for centrifugation. Comprising single-cell algae shells, DE is used widely across industries as a filter aid. In this eBook, we show you how the DE filter aid can transform your cell culture harvest workflow. Using a simple, one-step purification process you can significantly reduce cell removal time – without compromising on protein yield or product quality.

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Optimizing Your Cell Culture Workflow with Diatomaceous Earth

Despite their success as therapeutic agents against a range of pathologies, the production of monoclonal antibodies (mAbs) is limited by cost-intensive and time-consuming workflows that are required to separate cells from antibody-containing cell culture fluid.[1]

Most typical harvesting methods involve a 2-step process:

Centrifugation pellets the cells and the debris in a cell culture. The remaining clear supernatant can then be used for the following filtration/clarification step.

STEP 1
Centrifugation

Centrifugation takes 10–15 mins and is therefore a time-consuming part of the workflow.

Depth filtration*
Captures contaminants within the structure rather than on the surface of the membrane

STEP 2
Filtration

Sterile filtration*
Traps contaminants larger than the pore size on the surface of the membrane

*Depending on the sample volume these filtration methods can take hours.

What is Diatomaceous Earth?

Diatomaceous earth (DE) is a naturally occurring, soft sedimentary rock formed by the fossilized remains of microalgae, or diatoms. 80–90% of DE is made up of silica, making it a popular material used in a variety of applications including, cosmetics, filtration and as an insecticide.[2]

There are two types of DE:

FOOD GRADE[3,4]

Food grade DE is purified and is therefore safe for consumption and commonly used:

- In anticaking materials for food production
- As clarifiers for beer and wine
- In cosmetics
- As an insecticide

FILTER GRADE[3,4]

Filter grade DE is toxic to humans and is therefore used commonly for:

- Swimming pool filtration
- Industrial filtration

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3. https://www.azom.com/article.aspx?ArticleID=16463
4. http://npic.orst.edu/factsheets/degen.html

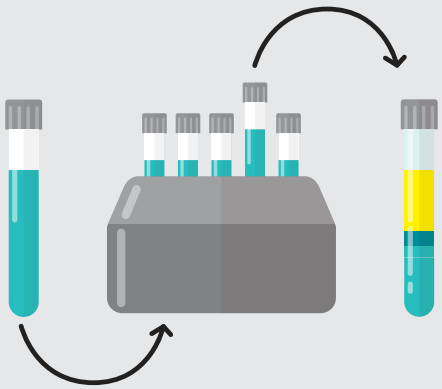
Diatomaceous Earth Filtration

DE crumbles easily to form a white powder. This coupled with the fact that it is highly porous, insoluble and inert, makes it a useful filter aid. This technique, known as body feed filtration, is used in cell culture harvesting. It is facilitated by DE which forms a permeable filter cake due to its porous structure. This avoids the need for a centrifugation step, circumventing issues around centrifuge capacity and availability as well as preventing filters from clogging even after centrifugation.

Conventional Harvesting

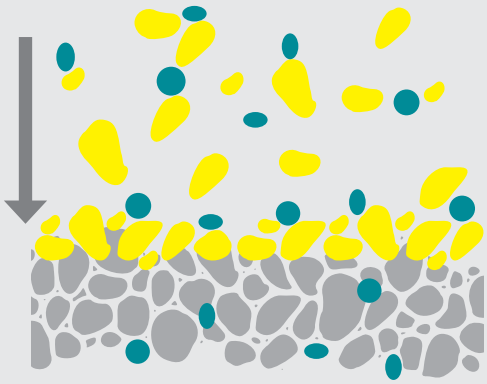
15 mins for centrifugation

Step 1: Centrifugation



~60 mins for filtration*

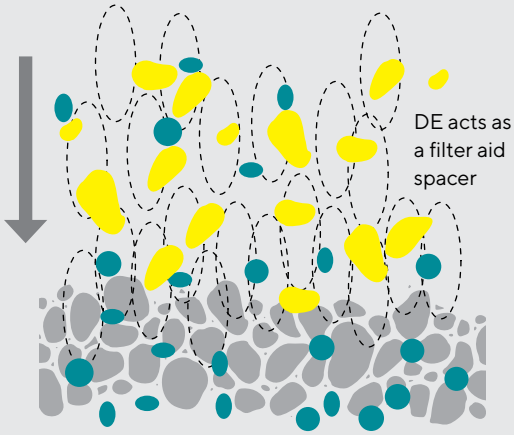
Step 2: Conventional Filtration



DE Filtration

8 min/ 500 ml

Skip Centrifugation



- mAb / proteins
- Cells and cells debris
- Diatomaceous earth

**volume dependent*

Advantages of One-step DE Filtration

- **70% reduction in process time**
- **Removes unnecessary manual steps**
- **Single step lowers the risk of contamination**

To learn more click here

Exploring the Value of Antibodies

Isotypes, Applications and Production

Image provided by Absolute Antibody

In 1890, Emil von Behring and Shibasabura Kitasato published a landmark study showing that the serum taken from animals immunized against diphtheria could be transferred to other infected animals and cure them – this was in fact the ***earliest*** reference to antibodies. Today, therapeutic antibodies have evolved to become valuable tools in numerous fields.

Absolute Antibody Ltd was founded in 2012, with the vision that all antibody users should be able to use recombinant antibodies that are *absolutely* defined by their amino acid sequence. Rather than creating entirely new antibodies, they manufacture defined versions based on an antibody's DNA sequence. They offer antibody sequencing, engineering and recombinant production as services, as well as a catalog of recombinant antibodies engineered into new and useful formats.

Here, experts from Absolute Antibody share insight into the value of antibodies, the evolution of antibody production over the years, and the value of using diatomaceous earth (DE) for filtration.

Q: How can antibodies be harnessed as tools for research, diagnostics and therapeutics?

A: Antibodies have always had the potential to be significant biochemical tools for a range of applications, due to their high specificity and selectivity and have truly revolutionized the field of biological science over the past 20 years. As a tool for research, antibodies are key to a variety of the laboratory techniques used to answer basic research queries. Antibodies allow investigators to identify molecules that cannot be seen by the naked eye and thus enable conclusions to be drawn about target molecules and pathways. Assays such as western blots, flow cytometry, enzyme-linked immunosorbent assays (ELISA), immunohistochemistry (IHC) and a multitude of others rely on antibodies.

In terms of diagnostic assays, antibodies are a critical component of many kits that are used extensively in the clinic for both humans and animals. Diagnostic assays that utilize antibodies include those that detect infection, measure hormones and other blood markers, and identify allergies.

Antibodies bind to a nearly infinite number of targeted proteins in a highly specific manner, making them ideal for therapeutics. The power of antibodies has been harnessed for treatment of cancer, transplant rejection, autoimmune disease and many other disorders. This class of therapies regarded as biologics make up a significant number of the clinical trials currently underway for the treatment of a variety of diseases. Nearly 100 antibody-based drugs are currently on the market, including the top-selling pharmaceutical, Abbvie's Humira® (adalimumab), which treats a variety of autoimmune diseases.

Q: Can you briefly touch on the different types of antibody?

A: Antibodies are available in three main forms:

Polyclonal antibodies are collected from the serum/blood of the animal that has been injected with an immunogen. This heterogeneous mix of antibodies results from multiple B cells' immune responses. The mix includes antibodies that recognize a different epitope on the same antigen.

Monoclonal antibodies are created by fusing spleen cells that secrete the antibody with an immortalized cell line to

generate a hybridoma. These hybridoma cell lines then continue to express the antibody which can be recovered from the cell culture supernatant. These clones produced from a single B cell recognize a single antigen.

Recombinant antibodies are the third form. Antibodies produced recombinantly from a sequence are identical in subsequent preparations.

Structurally, antibodies are composed of light chains and heavy chains. Antibodies in mammals are classified into five main categories, referred to as subtypes: IgA, IgD, IgE, IgG, and IgM (Figure 1). Classification is based on the type of heavy chain they are composed of – alpha, delta, epsilon, gamma or mu, respectively.

IgG is by far the most abundant antibody in normal human serum and is the major antibody of the secondary immune response. IgG consists of four human subclasses (IgG1, IgG2, IgG3 and IgG4), each containing a different heavy chain. These subclasses differ mainly in the hinge region and the extent to which they activate the host immune system. Therapeutic antibodies are all IgGs.

IgM accounts for 5–10% of the antibodies in serum and is involved in the body's primary immune response. IgM does not contain a hinge region like IgG but does contain an additional constant domain and an 18 amino acid tailpiece at the carboxy terminus, which contains a cysteine that enables multimers of the molecule. Soluble IgM is quite large and is thus largely located intravascularly.

IgA represents approximately 5–15% of the body's antibodies and can exist as a monomer or a dimer. IgA is located in mucous secretions such as saliva, tears, milk and intestinal juice.

IgD accounts for less than 1% of the total plasma immunoglobulin but is present in significant quantities on the membrane of B cells. IgD has the same basic structure as IgG but with an extended hinge region which is very susceptible to proteolytic digestion. The purpose of IgD is not completely understood.

IgE is rare in the serum but is found on the basophils and mast cells. This class may play a role in immunity to parasites but is more commonly associated with type I immediate hypersensitivity, where an IgE immune response occurs to generally non-reactive environmental antigens such as pollen and peanuts.

Q: What are the key steps involved in the process of antibody production and how has this evolved over the years?

A: The traditional means of production of an antibody involves the identification and preparation of the antigen – for example, a peptide that the antibody should recognize and bind to. The antigen is conjugated to a carrier protein that is immunogenic. This antigen preparation is then injected into animals, causing the animal to undergo an immune response and produce antibodies in their serum specific to that antigen. The resulting antibody preparations are then screened for their

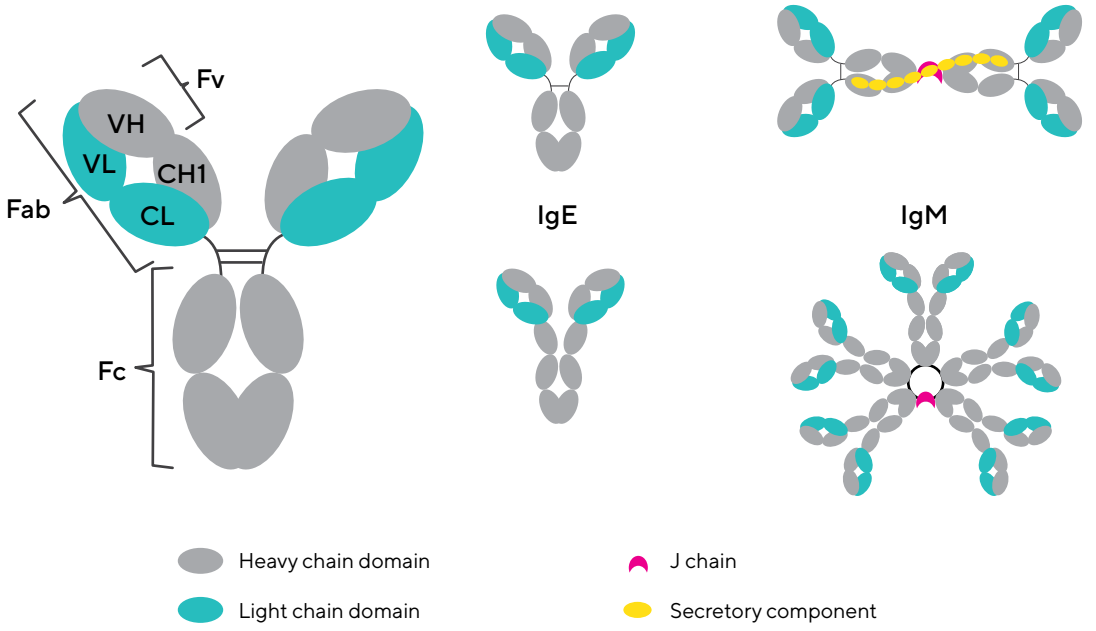


Figure 1. Immunoglobulin isotypes. Schematic representation of the five immunoglobulin classes or isotypes in mammals. Credit: Absolute Antibody

ability to bind to the antigen of interest and characterized.

Manufacturing of polyclonal antibodies involves purifying the serum of animals that have been immunized. Most monoclonal antibodies are manufactured by growing hybridoma cell lines in bioreactors. Generally, therapeutic antibody manufacture involves the production of stable cell lines, most often in Chinese Hamster Ovary (CHO) cells in very large bioreactors.

Absolute Antibody utilizes a recombinant method of antibody production from milligram-to-gram scale. The recombinant production platform we use at Absolute Antibody is completely animal and animal-component free. The platform is based on transient, chemical transfection of a HEK293 cell line cultured in a precisely defined, serum-free medium. We do not use viral transfection approaches.

Other than the benefit to animal welfare, recombinant antibodies offer additional advantages. They are biologically defined, with a known DNA and protein sequence, and as such it is ensured that the identical antibody is produced in each manufacturing batch. Unlike hybridoma-derived antibodies, recombinant antibodies can be reformatted to a different species, isotype or subtype. This opens up the possibility of switching antibodies into a more preferable format for *in vitro* or *in vivo* use. Recombinant antibodies can be engineered at the genetic level enabling production of recombinant antibody fragments, e.g. Fab and Fab2, bispecific antibodies, site-specific conjugation of antibodies and a myriad of other possibilities that are not available with conventional antibody technologies. Additionally, recombinant antibodies are expressed in a chemically defined serum-free mammalian expression system. This eliminates contamination from serum components, such as bovine albumin and IgG, leading to a product with very high purity (>98%).

Q: What challenges have you faced whilst using traditional methods for cell culture harvesting?

A: Traditional cell culture harvesting methods have the potential to introduce errors. These include contamination from manual manipulation of cell lines, poor producing hybridomas that result in low antibody concentrations and the potential for inconsistencies in antibody composition due to genetic drift. While companies producing at very large scale using bioreactors use continuous centrifugation and depth filtration, this method is impractical and expensive at research scale. Research-level work generally utilizes either a manual syringe-based process or a vacuum-driven bottle top filter. Although easy and inexpensive, these can be prone to clogging.

Q: How is diatomaceous earth used for filtration and what are some of the advantages to using this approach?

A: Over the past seven years we've tried various different approaches to improve the efficiency of the clarification step including wine filters, which were very cheap but very slow, and chitosan flocculation, which was very inefficient and high in endotoxin. Eventually we found a solution for research-grade filtration based around diatomaceous earth or DE. The DE comes as a powder in sachets which is mixed with your cells, with no centrifugation required. This mix is then added directly to a vacuum-driven filter. This DE solution (commercially available from Sartorius) has completely removed the need for centrifuges at this stage of the process and allows us to go directly from shake flasks into a sterile filtered supernatant. It really has revolutionized our process and turned clarifying 100 liters plus of cell culture from a real chore into quite a simple activity.

Overcoming Challenges in High Density Cell Culture Harvesting

Therapeutic monoclonal antibodies (mAb) are a type of targeted drug therapy that can be used to treat many diseases, such as cancer.[1] However, the intricate process of mAb development and production can make these treatments intensive in cost. National healthcare systems need cost-effective treatments; therefore, for mAb treatments to become commonplace, researchers must establish robust workflows that increase the yield and shorten the developmental period.[2,3]

The mAb production workflow is schematically described in Figure 1. However, in brief, there are three stages: inoculum, cell culture and primary recovery. Primary recovery is an important step in the workflow, and a step which can be optimized to reduce cost. This step involves the removal of cells, cell debris and impurities (such as adventitious and endogenous viruses, endotoxin, host cell protein, DNA) by filtration.[4]

In a conventional primary recovery process, the cell culture is first centrifuged before it is filtered (this process will be referred to as conventional filtration). Centrifugation separates a mixture into different sized components and is necessary for effective filtration; without it, large and high-density particles can form an impermeable layer, clogging up the filter. However, this extra centrifugation step adds time to the process and increases the cost.[5]

Body-feed filtration is an alternative primary recovery process method that relies on a filter aid instead of the centrifugation step. Once added to the cell culture, the porous filter aid prevents an impermeable layer clogging the filter.[5] Diatomaceous earth (DE) is a good example of a well-established filter aid, and is made up of pure amorphous silica and comprising of different species of fossilized diatom algae.[6,7,8] The Sartoclear Dynamics® Lab Products are filtration kits that have a Diatomaceous Earth (DE) filter aid.

This body filtration step (also referred to as DE filtration) produces a cost-effective workflow, minimizes clogging,

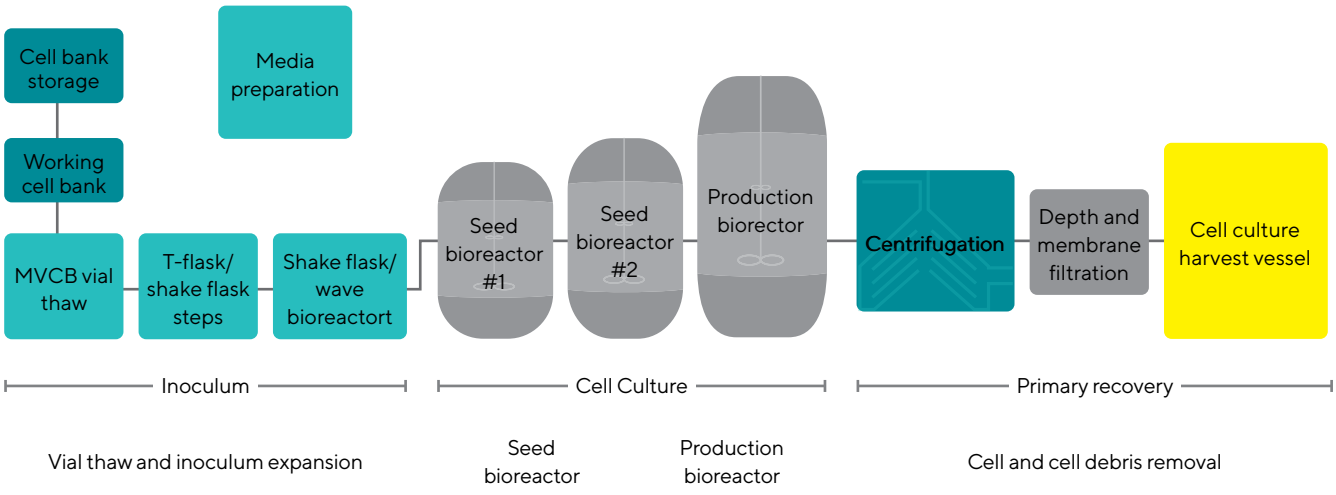


Figure 1: The industrial production of monoclonal antibodies

separates complete cells, and creates a one-step filtration process. This article will summarize the key points from *Grauf et al. (2018)* and compare DE filtration against the conventional method – testing for turbidity, antibody recovery, charge heterogeneity and glycosylation.

Conventional versus DE filtration

Turbidity

Turbidity is the "cloudiness" of a fluid sample; the value is calculated by measuring the presence of suspended matter within the sample.[9] The turbidity values were calculated using a turbidimeter. Pre- and post-filtration cell culture samples from both methods were measured and a reduction ratio calculated. The reduction ratio specifies how well the filtration method removes cell components from the cell culture fluid.

The results are presented in Figure 2A; there is no statistical difference in turbidity reduction between both methods. Therefore, DE and centrifugation methods are both equally effective at removing coarse and fine particles from the cell culture.[5]

Antibody recovery

High antibody recovery rates are essential to minimize the sample loss for the following cell based assays after purification. The mAb titer was calculated on pre- and post-filtration cell culture samples from both methods, using a label-free biomolecular interaction analysis instrument, Octect from ForteBio. The recovery ratio was calculated from these values (Figure 3A on page 18). There is no statistical difference of the antibody recovery between either filtration methods. Therefore, DE and centrifugation methods are both equally effective at maintaining a high antibody level in post-filtration samples.

A

Cultivation system & mAb product	Reduction Centrifuge	Reduction DE
A1 (IgG1) 5 L UniVessel	97.9 %	97.8 %
A2 (IgG1) 5 L UniVessel	98.7 %	97.9 %
A3 (IgG1) 5 L UniVessel	97.9 %	98.3 %
B (fc fusion protein) 25 mL in 125 mL SF	98.0 %	97.3 %
C (IgG1) 25 mL in 125 mL SF	n.a.	94.2 %
D1 (fc fusion protein) 300 mL in 1 L SF	92.5 %	92.7 %
D2 (fc fusion protein) 300 mL in 1 L SF	96.1 %	95.9 %
E (IgG2) 300 mL in 1 L SF	98.0 %	98.2 %
F (IgG1) 300 mL in 1 L SF	98.6 %	98.6 %
G (IgG1) 300 mL in 1 L SF	96.3 %	97.4 %
H (bispecific antibody) 300 mL in 1 L SF	97.9 %	98.7 %

B

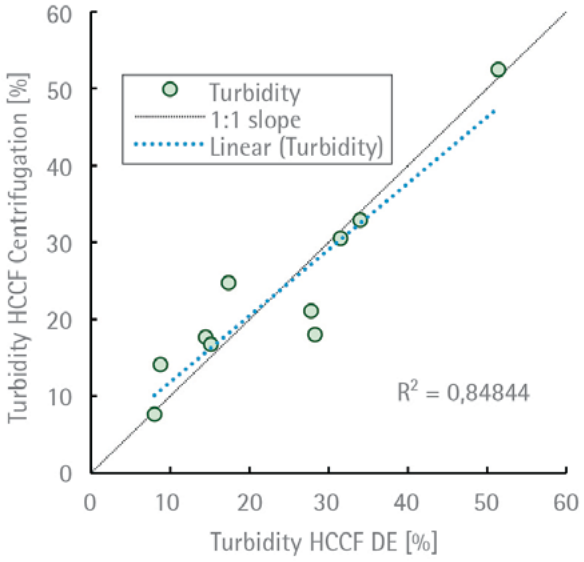


Figure 2: The turbidity values of DE and conventional filtration method. A. A table representing the turbidity reduction values of centrifugation and DE filtration method in different mAb samples. B. A graph representing the turbidity of the post filtered samples by both the centrifugation method and diatomaceous earth (DE). Adapted from From Grauf et al. (2018).

A

Cultivation system & mAb product	Recovery Centrifuge	Recovery DE
A1 (IgG1) 5 L UniVessel	95 %	99 %
A2 (IgG1) 5 L UniVessel	82 %	96 %
A3 (IgG1) 5 L UniVessel	96 %	91 %
B (fc fusion protein) 25 mL in 125 mL SF	93 %	120 %
C (IgG1) 25 mL in 125 mL SF	n.a.	97 %
D1 (fc fusion protein) 300 mL in 1 L SF	103 %	101 %
D2 (fc fusion protein) 300 mL in 1 L SF	94 %	94 %
E (IgG2) 300 mL in 1 L SF	98 %	99 %
F (IgG1) 300 mL in 1 L SF	98 %	102 %
G (IgG1) 300 mL in 1 L SF	100 %	102 %
H (bispecific antibody) 300 mL in 1 L SF	108 %	109 %

B

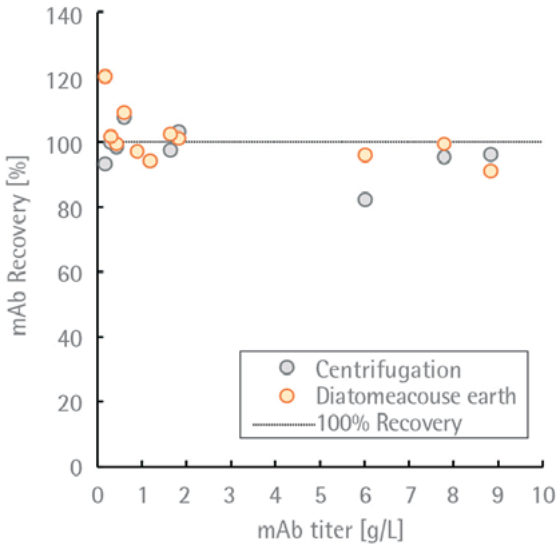


Figure 3: The antibody recovery results of the DE and conventional filtration method. A. A table representing the antibody recovery values of centrifugation and DE filtration method in different mAb samples. B. mAb titer and recovery of the post filtered samples by both the centrifugation method and diatomaceous earth (DE). Adapted from Grauf et al (2018).

Charge heterogeneity

Post-translational modifications can lead to heterogenous mAb isoforms. The presence of different mAb isoforms in a cell culture can create unpredictable net charges. Any changes in charge can modify the biophysical nature of mAbs (such as binding affinity, chemical properties, and stability), and lead to altered adsorption, creating an unwanted depletion of a certain antibody isoform.[10]

The charge variants of the post-filtration DE and centrifugation samples were calculated using a microfluidic assay used to measure relative percentages of variant in recombinant monoclonal antibody samples. The coefficient of variation (CV) was calculated for the acidic, neutral, and basic isoforms in a sample. The CV was used to determine whether the charge variants change depending on the clarification method. Analysis of the charge heterogeneity revealed that there was no significant difference in the charge distribution of the charge variants, and therefore the relative composition of isoforms did not change (Figure 4).[5]

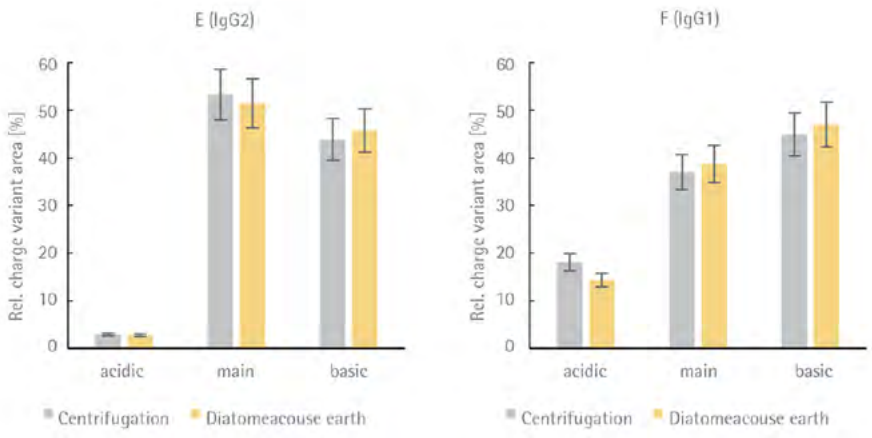


Figure 4: Relative area [%] of acidic, neutral (main) and basic charge variants of IgG2 (E) and IgG1 (F). Error bars show the standard deviation (2s) based on a CV < 5% of the microfluidic assay. Figure from Grauf et al (2018).[5]

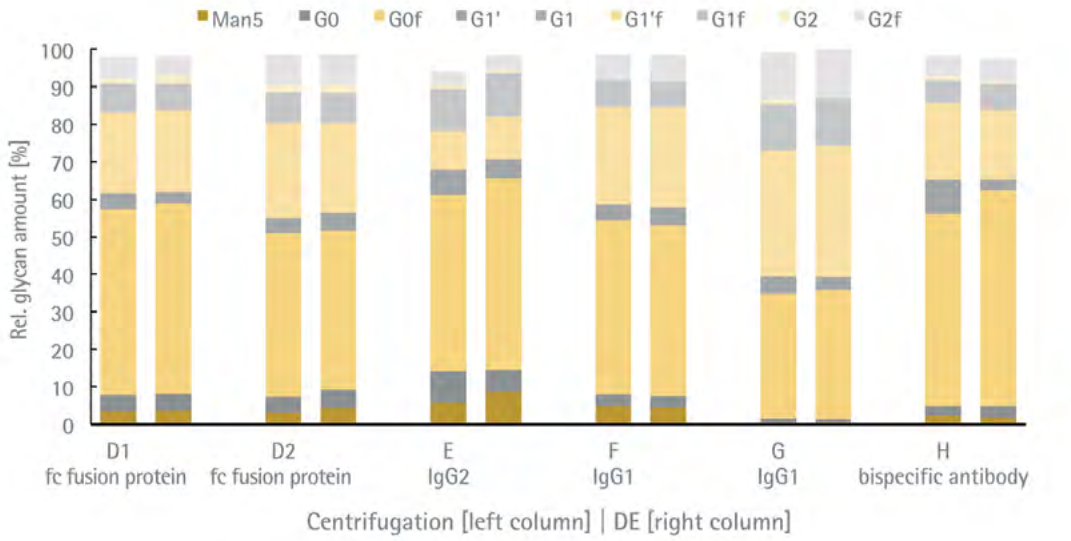


Figure 5: Relative amount [%] of nine main glycan patterns (Man5, G0, G0f, G1', G1, G1'f, G1f, G2, and G2f) determined for the mAb's fc fusion protein, IgG1 (two products), IgG2, and a bispecific antibody. The antibody samples were taken from the filtrate obtained by centrifugation or DE method. Figure from Grauf et al (2018).

Glycosylation

Glycosylation is a post-translational modification that influences mAb efficiency. Nine glycosylation species were examined in post-filtration DE and centrifugation samples. The N-glycans were removed enzymatically using a glycan screening reagent kit. The DE and centrifugation samples were fluorescently labeled and analyzed by capillary electrophoreses with laser-induced fluorescence. There was no significant difference in antibody glycoforms in either DE or centrifugation filtration method samples (Figure 5).

Conclusion

This study compared the conventional centrifugation filtration method with the DE filtration method. Overall, there was no significant difference in turbidity, mAb recovery, relative composition of charge heterogeneity and glycosylation isoform patterning. Therefore, DE filtration is as effective to use as conventional filtration. Additionally, DE filtration is a safe and simple method that greatly reduces protocol time. With the simple addition of Sartoclear Dynamics Lab Products to your workflow, the developmental costs for mAb production will be reduced!

You can learn more about DE filtration from this webinar.

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Body Feed Filtration – The Novel Method for Rapid Harvesting of Mammalian Cell Cultures

Monoclonal antibodies (mAbs) have revolutionized cancer therapy, however their purification can lead to a production line bottleneck. One time-consuming hurdle is the clarification and sterile filtration of a high-density culture before further processing.

This webinar presents body feed filtration as an innovative new approach to tackle the first step of mAb purification. This method uses diatomaceous earth (DE) as a filter aid. DE has been used for plasma fractionation and by the food and beverage industry for years.

From this webinar you will learn:

- How to harvest high-density cell cultures from 15 mL–1,000 mL in minutes
- The application of body feed filtration for fast, clean and reliable filtration of high-density mammalian cell cultures
- How body feed filtration compares to traditional centrifugation and subsequent filtration step in terms of mAb recovery, turbidity of cell culture and charge heterogeneity of the clarified molecules

Dr Noushin Delmdahl
Head of Product Management Lab Consumables, Sartorius

Dr Delmdahl received her PhD in 2001 from the University of Frankfurt, Germany in Protein Biochemistry. She started her career as application specialist for Vivascience, and later moved to the position of product management in Sartorius, for lab ultrafiltration products.

Currently, Dr Delmdahl leads the Lab Consumables Product Management team at Sartorius, and is involved in bringing new products to market for facilitating sample prep solutions, especially for the monoclonal antibody development market.

Compendium

From Mammalian Cell Cultures to Pure Proteins: Sartoclear Dynamics® Lab Significantly Reduces Cell Harvest Time

In this application note, IgG expressing mammalian MEXi-293E (HEK293) cells were removed from cell cultures using the novel Sartoclear Dynamics® Lab V Kit. This method was directly compared to the present standard method, which requires a two-step two centrifugation process.

Rapid Mammalian Cell Harvest without Centrifugation for Antibody Purification Using the Sartoclear Dynamics® Lab Filtration System

Sartoclear Dynamics® Lab V is a novel system for rapid clarification of cell culture media without the need for centrifugation or any other costly equipment. This application note compares the experiments conducted using this system against the traditional filtration process.

Application Note: Lab-Scale Clarification of Mammalian Suspension Cultures Using Sartoclear Dynamics® Lab V Kits

This application note evaluates a novel method for lab-scale clarification of mammalian cell culture supernatants. The Sartoclear Dynamics® Lab V Kit is not only a significant time-saving method compared with the conventional clarification process, but it also yields an improved clearance rate that was observed in combination with high flow rate filtration.

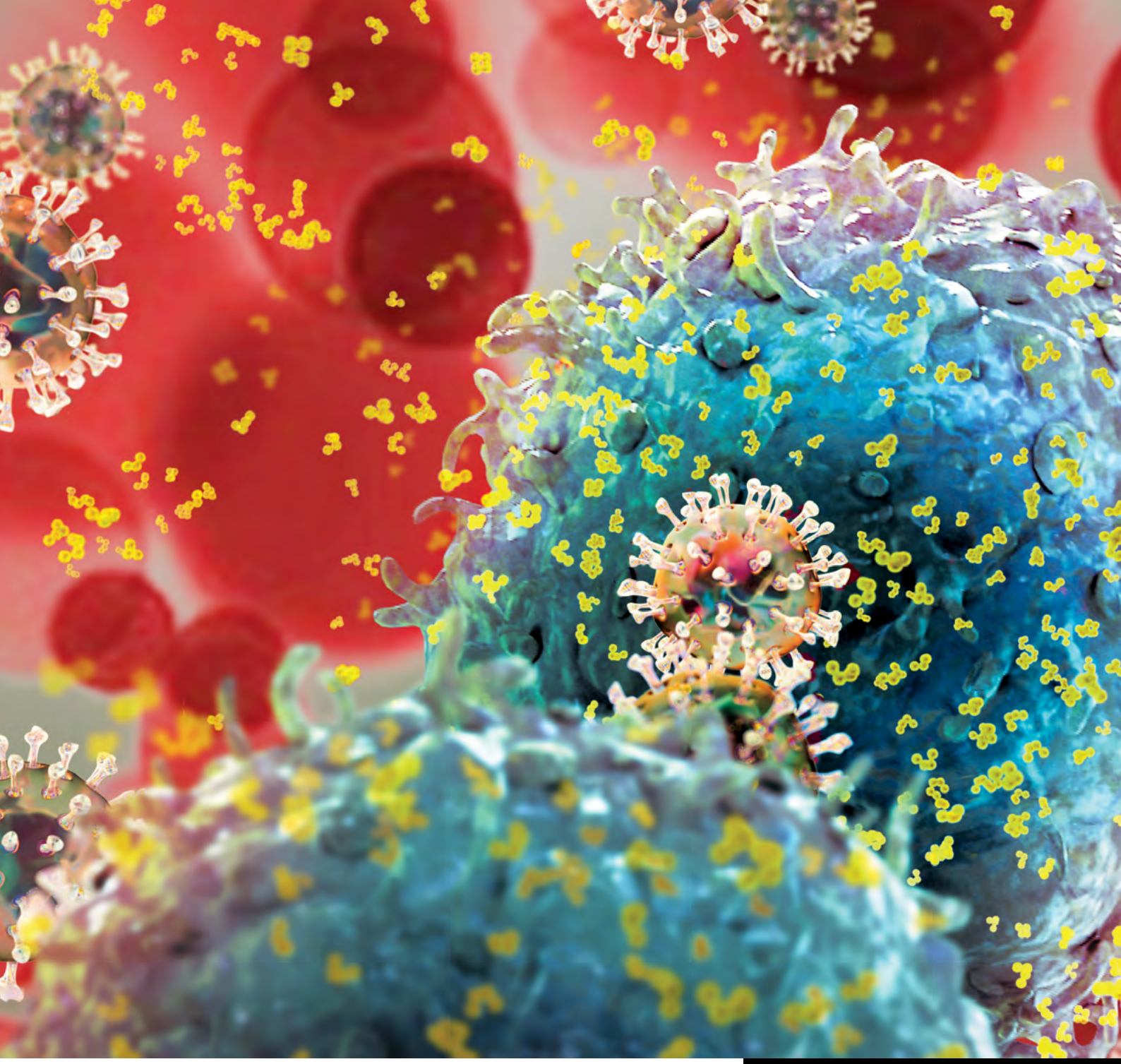
Compendium

Simplified Small-Scale Harvest of CHO Cells for mAb Analytics

In this study, the body-feed filtration method was tested to see if it produced different monoclonal antibody (mAb) and filtrate characteristics compared to the well-established centrifugation method. The parameters investigated were the reduction of turbidity, recovery of mAbs, mAb molecular weight, charge heterogeneity, glycosylation pattern, and overall work efficiency.

Sartolab® Multistation - Hands-Free Cell Culture Filtration for Small Volumes

Sartoclear Dynamics® Lab kits are designed for rapid harvesting 15 mL to 1,000 mL volumes of cell cultures in the lab, enabling clarification and sterile filtration to be performed in one step. These kits simplify the process by fully eliminating the centrifugation step otherwise needed for clarification. Click the link to find out more about the different lab kits available.



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