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Your Practical Guide to Basic Laboratory Techniques

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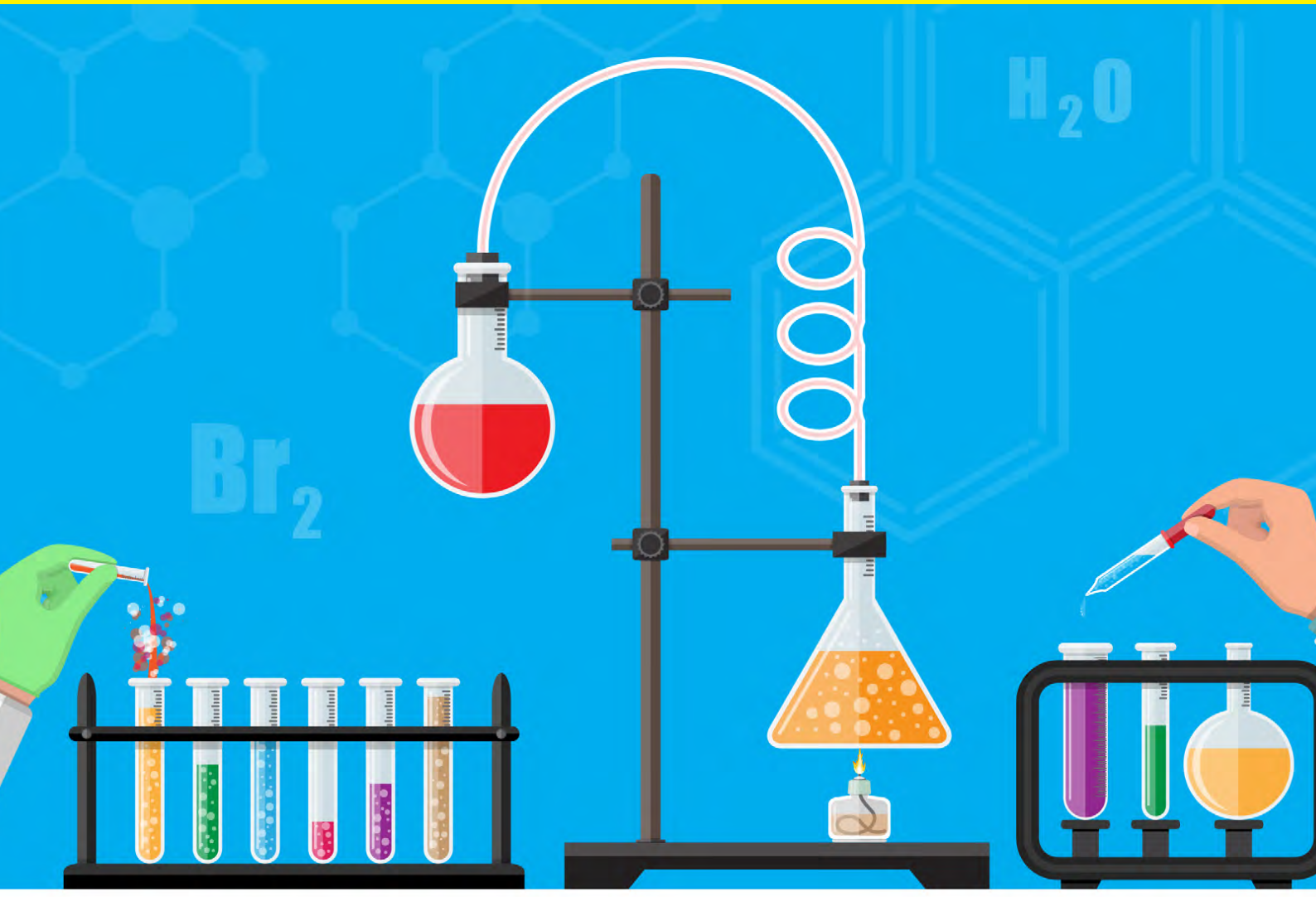

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Editor: Sean Sanders, Ph.D.
Proofreader | Copyeditor: Bob French
Designer: Amy Hardcastle
ROGER GONCALVES, ASSOCIATE SALES DIRECTOR
Custom Publishing: Europe, Middle East, and India
rgoncalves@science-int.co.uk
+41-43-243-1358

Ready... Set... Pipet!

It behooves all researchers to ensure that their core lab skills are solid and up to date.

Sean Sanders, Ph.D.
Senior Editor,
Custom Publishing
Science | AAAS

"*You need to learn to walk before you can run*" is a saying many of us probably heard when we were children. The clear message here is that there are some basic skills we need to master before we can move on to the next level. And there are plenty of good reasons that following this mantra will set one up for success, not the least of which – to continue the metaphor – is to avoid tripping and falling on your face.

In a scientific laboratory, there are also fundamental skills that require mastering before more complex tasks can be undertaken. Building a solid foundation of core lab skills is critical not only to producing accurate, reproducible experimental results, but also to prevent damage to expensive equipment and maintain a safe environment for ourselves and our fellow labmates.

Gaining competence in accurately weighing dry reagents is a critical skill, particularly when making stock solutions that might be used across multiple experiments and by multiple researchers in the lab. When an experiment doesn't work, we often don't know why – but we certainly don't want its failure to be the result of incorrectly prepared solutions due to poor weighing proficiency.

Filtration is a foundational technique used ubiquitously in the biological sciences and is an essential step in many protocols. One of its common applications is the generation of clean water needed in many aspects of lab work, but probably most importantly for making up and diluting reagents. Impurities in improperly filtered water, even at low levels, can negatively impact biological processes or, even worse, generate spurious results. Filtration is also essential for purification and | or concentration of solutions as well as the sterilization of biological reagents for which autoclaving is not an option due to heat sensitivity.

Most, if not all, life science laboratories have at least one set of micropipettes. If they're lucky, some might even have a set for each researcher. Correct pipetting technique for small volumes of reagents is an essential skill for researchers performing almost any type of molecular biology experiment. Knowing how to accurately pipet a range of fluids | from viscous glycerol to highly volatile phenol – can make the difference between a successful experiment and yet another confusing result. And anyone who remembers learning to pipet will recall that it's nowhere near as easy as it looks.

With increasing focus in the scientific community on reproducibility of results, it behooves all researchers to ensure that their core lab skills are solid and up to date. The latest advances in lab techniques need to be studied and absorbed, and basic skills revisited and refreshed. In other words, keep practicing your walking skills so that you're able to sprint when it's really needed!

Sean Sanders, Ph.D.
Senior Editor, Custom Publishing
Science | AAAS

Taking the First Steps

"*A Journey of a Thousand Miles Begins with a Single Step.*"
Laozi

In a scientific world that is more competitive than ever before, it is imperative to gain a deep understanding of biological novelties and phenomena at both a macro and micro scale, and to do this as quickly and accurately as possible. This knowledge will potentially enable scientists to formulate novel hypotheses, make new discoveries, and share their findings with the world. The creation and dissemination of scientific information is the cornerstone of scientific and societal advancement.

With such a strong focus on exciting discoveries – like the next generation of cancer therapies – it is easy to forget that it all starts with the basics. As the Chinese philosopher Laozi once said, "*The journey of a thousand miles begins with a single step.*" Every cell culture medium, and every sample of DNA, RNA, or purified protein, needs at some point during the experimentation process to undergo a variety of different treatments. These materials may need to be dissolved or diluted in purified water, weighed, filtered, pipetted, or generally experience aseptic handling or transfer. All these small, seemingly insignificant steps and minor details tend to be forgotten as a user gains experience and confidence in the daily routines of their laboratory, or even disregarded when it comes to complete beginners.

Since nothing that stands the test of time can have a weak foundation, it is extremely important for today's young scientists entering the lab world for the first time to be able to build a robust foundation in basic lab techniques, starting on day one. This underpinning is crucial to their future success. It is equally important that experienced scientists revisit these basic topics in order to remedy potential misconceptions, and to fill in the gaps in their knowledge that have developed over time.

Sartorius, a global laboratory products and services supplier for the academic and (bio)pharma markets, has been dedicated to providing solutions that strengthen scientific experimentation for more than 140 years. Sartorius engages with its customers over the full spectrum of their work, catering not only to their basic laboratory needs (such as weighing, pipetting, and filtering), but also by offering high-end and high-throughput (live) cell-analysis instrumentation. By offering this booklet in partnership with Science | AAAS, we hope that we can contribute to building a secure and prosperous scientific future for the benefit of all stakeholders involved.

Ferencz Paldy, Ph.D.
Head of Segment Marketing Academia, Sartorius

Fiona Coats, Ph.D.
Head of Marketing, LPS Marketing, Sartorius

Building Skills in Basic Lab Techniques

Useful Tips from the Experts

Safety and competency in a science laboratory depend on a set of basic skills. As science advances, so do some of the capabilities required for it. Nonetheless, some skills are almost as old as science itself, and these remain vital – even though the way of doing these tasks has evolved. With both old and new techniques, beginner and experienced scientists alike need to maintain their competency in the use of numerous standard methods. Even after learning and mastering a technique, a refresher never hurts, and keeping current on changing methods maintains the foundation of a lab and the integrity of its findings. **By Mike May, Ph.D.**

Here, we'll explore familiar, everyday methods along with some newer ones – all aimed at helping scientists build and maintain a skillset. Many of these skills will apply to various applications. For example, Donald Spratt, assistant professor of chemistry and biochemistry at Clark University (Worcester, Massachusetts), says, "*Protein scientists need, for example, to have excellent planning and organizational skills so they can design and successfully execute their experiments.*" He adds, "*These skills are translatable to many different scientific disciplines.*" In fact, most lab skills build on others and help scientists learn new ones.

Weigh it right

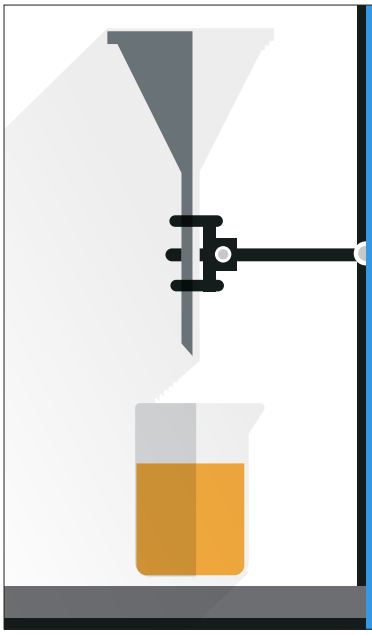
Weighing samples is one of the oldest procedures in all of science. It's one of the first things that scientists learn how to do, and a skill that most of them need throughout their careers. The ubiquity of weighing makes it a top-priority skill for scientists at all levels. The first step to weighing involves picking the right balance. "*People do not need a four-place analytical balance for routine powder dispensing, and conversely they cannot achieve precise weighing on a top-loading balance,*" says Kevin Olsen, instrumentation specialist in the chemistry and biochemistry department at

New Jersey's Montclair State University. "I*t is important to understand the limitations of whatever kind of balance you are using.*" For instance, any balance produces a more accurate weight for larger over smaller samples. "T*his is why we typically weigh out an analytical standard in the grams range and dilute it rather than weighing the same material in the milligram range,*" Olsen explains. "*Different balance models have different features and if they are used incorrectly, the weighing may not be accurate.*" For every balance, keeping it clean and calibrated impacts all weight measurements. So, a little care goes a long way.

Proper pipetting

After weighing samples, the next most common technique, at least in the biological sciences, might be pipetting. For some scientists, pipetting could even be the most important skill to master. To get it right, scientists need to pay attention, and not just to the proper technique. In fact, becoming distracted is a common mistake in pipetting, according to Tamara Mandell, associate director of education and training at the University of Florida's Biotility, a center that prepares people for the biotech industry.

Others agree on the value of the right attitude with this process. "*The most important thing to keep in mind while pipetting is slowing down and taking my time,*" says VJ Tocco, lecturer in the department of chemical engineering at the University of Florida, Gainesville. "*Sometimes, I get tempted to rush, which can lead to mistakes.*"



One critical role for filtration in these industries is sterilization, since the use of heat to sterilize would cause undesirable product degradation.

Tocco suggests other things to remember as well, including picking the right pipette. "*You should use the pipette that dispenses the smallest volume,*" he says. "*For example, to pipet 18 microliters of fluid, use the 20-microliter pipette, not the 100-microliter pipette.*" And the pipette tip should be wet before using it. As Tocco says, "*It's best to aspirate liquid and dispense it at least once before actually pipetting your liquid.*" Lastly, Tocco reminds scientists to take their time and not to "*aspirate so quickly that bubbles form in the solution.*" Those bubbles cause errors in volume measurement.

Purifying the processes

Many protocols in a lab require a variety of solutions, including culture media, buffers, and more. And these solutions usually require water. In most cases, not just any water will do. Instead, water for lab processes must be filtered and purified, and the application determines the level of purity required. According to ASTM International, water can be categorized as Type I–IV, with Type I being the purest. One metric that distinguishes these categories is resistivity (Ω-cm); water with fewer impurities shows higher resistivity. For example, the resistivity of Type I and IV water is 18 and 0.2 megaΩ-cm, respectively. The less-pure Type IV water can be used as a source for a lab distiller, for example, and ultrapure Type I water is used for cell culture, gas chromatography, high-performance liquid chromatography (HPLC), and other applications that are very sensitive to impurities.

The secret is matching the right water to an application, and not overspending to make water that is more purified than necessary. The volume of water necessary will also determine how to make it. In some situations, a water system for a lab is enough, while other applications require building-wide purification systems. In the latter case, a building-wide system might make reasonably pure water, for example, Type III; and then lab systems can further treat that water as needed.

Filtering fluids

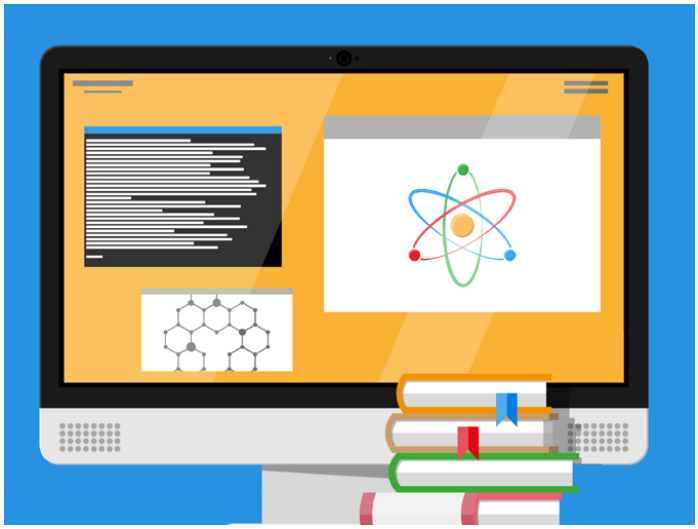
To remove unwanted solids from a sample and increase purity, scientists often use various forms of filtration, which extend from a simple piece of filter paper in a funnel to advanced membrane-based devices. Many molecular methods include filtration to concentrate a sample. Filtration is used extensively to concentrate and purify proteins or DNA, for example, for crystallography studies or for use in the polymerase chain reaction (PCR).

Filtration processes can also be distinguished by general application. One of the most common applications for analytical filtration is sample preparation for HPLC. Filtering out particles is essential to prevent blocking of the column, which can lead to failure of the analysis; it also reduces background in the chromatogram and improves sensitivity and accuracy.

The biotechnology and pharmaceutical industries also require filtration in many processes, using a variety of membranes and devices that often have the added requirement of meeting specific criteria, such as ASTM International standards or good manufacturing practices (GMP) regulations. One critical role for filtration in these industries is sterilization, since the use of heat to sterilize would cause undesirable product degradation.

Keeping cultures healthy

From basic science to biotechnology and pharmaceutical sciences, many labs include cell or tissue culture as a standard method. The basic idea of keeping cells alive in culture is over 130 years old – starting in 1885 with work by German zoologist Wilhelm Roux, who cultured chicken embryonic cells in a saline solution. Today, scientists culture cells in two and three dimensions, even mimicking complete organs in some cases. Despite its increasing complexity, some of the steps for cell culture are easier than ever.



Scientists who have been thinking about writing all along can get a head start by using an electronic lab notebook to keep track of protocols and results.

In the early 1980s, for example, I worked in a cell-culture lab, and we made most of what we needed, including materials like rat-tail collagen to coat the coverslips on which the cells grew. Today, scientists can purchase a wide variety of media and reagents as well as labware designed for specific culture techniques, such as 3D culture.

Still, some of the key skills remain the same. "*The most important aspect of tissue culture is good sterile technique,*" says Katy Phelan, director of the cytogenetics laboratory at Florida Cancer Specialists & Research Institute (Fort Myers, Florida). "*This applies to initial setup of cultures as well as feeding, subculturing, and cryopreservation.*" This means that everything – culture media and additives, pipettes, culture vessels, and other equipment – must be kept sterile and tested to confirm sterility. "*Practicing good sterile technique will reduce the chance that cultures will become contaminated,*" Phelan explains. "*Valuable cell lines can be lost or compromised due to failure to practice good sterile technique.*" In fact, keeping cultures contamination-free is one of the biggest challenges of this general method.

Plus, it's crucial to ensure that a culture includes only what is intended. "*A common mistake in cell culture is sample mix-up or cross-contamination of samples,*" Phelan explains. "*Various techniques can be employed in an attempt to prevent this error, such as working with only one sample at a time in the tissue culture hood, avoiding the use of prelabeled flasks or petri dishes, and double-checking two unique identifiers on all paperwork and culture vessels.*"

Increasingly, scientists must ensure the integrity of cultures. "*In a research lab, a sample mix-up can lead to false and unreliable results,*" Phelan notes. In a diagnostics lab, however, such an error could be deadly for a patient. Many journals require that researchers authenticate cell lines used, and this can be done using DNA fingerprinting.

The American Type Culture Collection (ATCC), says Phelan, "*actually provides a service for human cell authentication and has an online course called Cell Line Authentication Training.*"

Processing proteins

Many protocols in life science and clinical labs involve proteins. When asked about the top skill required for working with these molecules, Daniel J. Kosman, SUNY Distinguished Professor in biochemistry at the University of Buffalo's Jacobs School of Medicine and Biomedical Sciences, picks the ability to use fast protein liquid chromatography (FPLC), which can isolate proteins in a mixture. He also notes that protein scientists must be able to perform heterologous expression, in which DNA or RNA from one species is expressed in another to create a specific protein. With this technique, though, Kosman notes that the key challenges are ensuring the "*correct folding and posttranslational modification of heterologously expressed proteins.*"

Spratt also points out the need for protein-expression capabilities. When asked about the most common technique for obtaining proteins for further research, he selects bacterial expression in Escherichia coli using recombinant DNA technology, calling it "*the most common and cheapest way to make a protein.*" With this technique, the overexpressed protein "*can then be purified using chromatography, based on its unique physicochemical properties, such as size, charge, affinity, solubility, and | or oligomeric state,*" Spratt explains. "*Once the protein is pure, it needs to be quantified prior to further biochemical examination.*"

In fact, getting adequately pure protein for downstream techniques can be challenging. "*Many protein biochemists have to contend with frustrating obstacles, including protein yield, solubility, and degradation issues,*" Spratt says. "*Speaking from personal experience, it can take many attempts to overcome these challenges.*"

That brings up perhaps the most crucial lab skills of all: patience and persistence.

Writing up the results

Once those skills pay off, it's time to write. Scientists who have been thinking about writing all along can get a head start by using an electronic lab notebook to keep track of protocols and results. At the very least, they can cut and paste methods and results to get started on an article.

Beyond collecting all the information, more challenges arise in knowing how to describe the work. For even seasoned writers, it's worth reading "The Science of Scientific Writing" by writing consultant George Gopen and Judith Swan, associate director for writing in science and engineering at Princeton University (American Scientist, November–December 1990). As they concluded, "*In real and important ways, the structure of the prose becomes the structure of the scientific argument.*"

To build the best structure, make an outline or develop some organization before writing begins. It doesn't need to be a formal system of Roman numerals or capital letters, but just something that works for the writer. A research article comes with an overall organization, including introduction, methods, discussion, and conclusion. So it's worth making time to organize topics within each section. In short, know what you want to write before you write it. Writing and the other techniques described here take time and practice. Also, these scientific skills should be refreshed as needed. Only then can scientists produce their best work.

Mike May is a publishing consultant for science and technology.

The Paperless Lab

Some scientists keep experimental records on sticky notes. Some groups maintain ordering information in the head of a single technician. But for researchers looking for more stable, searchable, and sharable records, digital options such as electronic laboratory notebooks (ELNs) and laboratory information management systems (LIMS) are readily available. Scientists can start with a simple online notebook or choose a complete lab management package to track the entire lifecycle of their projects. **By Chris Tachibana**

A paper notebook seems like it should last forever. After all, Gutenberg Bibles have survived since the 1400s. Still, paper is not perfect. Consider these true stories: an Australian university, 30 years of notebooks became a of loose pages after the bindings crumbled during relocation. In the United States, a postdoc spent days combing through three-ring binders for experimental details requested reviewers. In a positive example of going paperless, a Swiss contract manufacturing organization wowed clients with realtime, online chromatography runs of their samples. Electronic laboratory tools have definite advantages, but scientists have been reluctant adopters. The major barriers for going digital are cost, the activation energy required to change work habits, and the daunting number of options.

Where to Start

LIMSwiki is an excellent starting point for laboratory informatics newbies. The online resource is a community service from the Laboratory Informatics Institute, a trade organization founded in 2006 by **LabLynx**, a vendor of browser-based research management software. LabLynx emphasizes transparency, for example in pricing, and LIMSwiki provides prices when possible in its up-to-date vendor descriptions.

"*We've tried to maintain neutrality throughout,*" says Shawn Douglas, LIMSwiki curator, "*avoiding marketing and self-promotion. The wiki is an evolving tool, and we're always looking for quality contributors.*"

LIMSwiki provides definitions for terms such as ELN (electronic laboratory notebook, generally used to document experiments) and LIMS (laboratory information management systems traditionally used for tracking standardized processes such as production). But the distinction between informatics products is blurring, says Markus Dathe, good manufacturing practice and computer system validation coordinator at **Roche**, because "*convergence is happening.*" ELNs, LIMS, and equipment software are expanding functions, interconnecting, and overlapping. Informatics packages increasingly aim to cover the entire lifecycle of an R&D project including reagent inventories, regulatory forms, and work requests in addition to experimental details. Most researchers start small, though, with a homegrown ELN with protocols in text documents and electronic data files.

"*Everyone sees the value of ELNs, from scientists to principal investigators to lab managers,*" says Erik Alsmyr, senior director of software development for the **Accelrys** Notebook (previously Contur's iLabber) for small-to-medium-sized research groups. Alsmyr says most labs start with all-purpose organizing and sharing software such as Evernote or SharePoint, then realize they need more storage capacity or intellectual property (IP) protection. Electronic systems provide 24/7 global access to your records, says Alsmyr, and most commercial ELNs are compliant with regulatory requirements for electronic records, for example Part 11 of the Code of Federal Regulations Title 21, which covers the U.S. Food and Drug Administration, and European Union Annex 11 for the European market. Researchers are still slow adopters, though, particularly at universities. That's why **LabArchives** offers a free ELN in addition to a subscription-based version with more storage and features. "*Our research says that in academia, about 95% of scientists still use a paper notebook,*" says Earl Beutler, LabArchives' chief executive officer.

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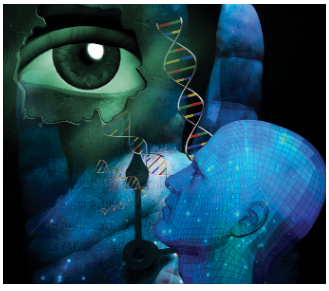
Beutler, whose entire family are scientists (including a Nobel Prize winner), thinks it's time for labs to go digital. "*I've worked around smart, technologically proficient scientists my entire life,*" he says, "*and I'm amazed that their state-of-the-art is still taking a photo of a gel, printing it out, and gluing it into a paper notebook.*"

Realizing that adhesives disintegrate and notes on laptops don't have the strongest IP protection, universities are buying informatics site licenses that cover entire departments, says Beutler. This removes the cost barrier for scientists and ensures proper archiving of potentially patentable results. LabArchives also targets an audience that doesn't have paper nostalgia: students. "*Many of our users are academic researchers who teach, so we created our classroom ELN at their request,*" says Beutler. "*It lets instructors provide background information and give and grade assignments electronically. The largest class it's been used in was more than 2,000 students.*"

Tammy Morrish is an academic researcher who went digital from day one, setting up her laboratory with **Labguru**, a webbased research management system. As a postdoc, Morrish kept a homemade database of project resources but wanted an advanced, sharable system when she started as an assistant professor at the **University of Toledo** Biochemistry and Cancer Biology Department. That's a great time to set up a new system, she says, because you know all the mice, cell lines, and plasmids you have available for projects.

Morrish praises Labguru's customer service and says the system is a huge timesaver. It streamlines ordering by putting product numbers, vendors, and current orders in one place, she says. Labguru holds her laboratory's mouse records with full genotypes, and plasmid information including maps. Morrish says the system is particularly helpful for locating items. "*Think how much time we waste looking for things,*" she says. "*Now when I need something, even if other people aren't around to ask, I can type it into the database and find it. Of course,*" she adds, "*people have to put things back where they found them.*" Her lab has a technician who checks inventories against the database weekly.



In this (nearly sci-fi) vision of the future laboratory, scientists simply do their work while an automated tracking system simultaneously keeps records.

At a higher level, the system facilitates group interactions, for example by making data sharing easy. It also teaches best practices. "*It helps students learn that with any database,*" says Morrish, "*you have to enter information correctly and consistently or you won't be able to find it.*"

Going Digital But Maintaining Control

Science-based businesses also appreciate the efficiency of digital research management, but long-term stability is a high priority, too. "*The challenge is assuring the accessibility and usability of data 20 years from now,*" says Dathe. Choosing a major informatics supplier such as IDBS, PerkinElmer, or Accelrys might give some assurance of permanence, but the market is so dynamic that any vendor will likely undergo changes. In the past decades, Thermo Fisher Scientific acquired InnaPhase; PerkinElmer purchased Labtronics, CambridgeSoft and ArtusLabs; Accelrys, which has its own lengthy merger and acquisition history, was recently acquired by the French software company Dassault. Still, after consolidating, companies strive to retain users. "*We still carry software developed in the 1990s and we've always shown customers a path forward,*" says Leif Pedersen, senior vice president at Accelrys. Nonetheless, industries are not uniformly adopting laboratory informatics. Although agencies such as the Food and Drug Administration encourage electronic documentation, Dathe says, "*The pharmaceutical industry is generally conservative, and it's often easier and cheaper to stay with a paper system that is known to be accepted by regulatory agencies.*"

At **LEO Pharma** in Denmark, head of discovery informatics and data management Ulrik Nicolai de Lichtenberg developed a model for committing to a commercial informatics system. Start with in-depth stakeholder analyses, he says. Define your needs and goals and "*how much pain you can put up with,*" meaning the money, time, and effort available for implementing a new system. Realize that your ELN or LIMS is just a part of an information ecosystem. LEO Pharma chose the Accelrys ELN for its Medicinal Chemistry R&D Department, but the ELN is just one element in a comprehensive infrastructure designed by de Lichtenberg's team. Their system will capture, validate, and permanently store records so they are accessible, searchable, and legally defensible in case of IP disputes. It's a complex project and de Lichtenberg recommends seeking advice from independent consultants who understand the ever-changing informatics market.

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Looking to the Cloud And Beyond

Michael Elliott, chief executive officer of **Atrium Research & Consulting**, advised de Lichtenberg and endorses his approach. "*Don't get enamored with a demo,*" he says. "*Look under the hood and check out the capabilities of an informatics system.*" Clients dream of a single system that streamlines process management and securely and permanently stores data while rapidly retrieving needed information. An ideal system would even find "dark data" – previous work that could answer current research questions but is buried in disorganized files. Clients want scalability, a user-friendly interface, and outstanding global support. However, products vary in these capabilities, says Elliott. "D*on't choose based on a presentation or brand name. Think carefully about your needs now and in the future.*"

If expandability and ease of use are priorities, a cloudbased system, for example from Core Informatics, might be the answer. In principle, the cloud can house unlimited amounts of data and has a familiar interface since accessed is through a web browser. Brower-based systems don't require specialized software, so they're easy to upgrade. Informatics vendors are also creating user-friendly modular packages. Similar to choosing mobile phone apps, users select only the components they need.

Also on the horizon is greater mobility and compatibility. Researchers are taking smartphones and tablets into the laboratory so informatics developers are making products compatible with handheld devices. Increasingly, data needs to be compiled across different instruments and informatic platforms, so Pedersen says he is personally pushing for increased standardization to facilitate information sharing. Ever the realist, though, Elliott says progress in standardization is slow because even within a single department, users might employ different terminology and definitions. The force that could drive both standardization of scientific informatics and better data integration, says Elliott, "*is the move toward more collaborative work.*" To the wish list of informatics improvements, Dathe adds features that give data context: when and where they were collected and for what project. Data should be linked to relevant molecular and clinical information and the entire data-generating process, including the type and status of equipment used. "*Without context,*" says Dathe, "*the mountain of data we can collect is meaningless.*"

Being Open-Minded

Scaling the data mountain is Britt Piehler's job. Piehler is president of **LabKey Software**, which develops tools for data management and integration. The trend toward globalization and multisite collaboration, he says, means project managers must coordinate data collected at farflung sites under diverse conditions with a variety of instruments.

"*That's where LabKey comes in,*" says Piehler. "*We build tools for specific tasks, usually data integration for multisite collaborative projects that need to standardize heterogeneous data.*" An unusual feature of LabKey Software is that its product is open source.

"*We grew out of the academic community,*" says LabKey's Science Outreach Director Elizabeth Nelson, "*so we believe it's an advantage for the software platform to be freely available.*" Open source code allows researchers to tailor their systems, says Piehler, and building and sharing LabKey tools creates a community.

If the code is free, what does LabKey offer? "*Customization,*" says Piehler. LabKey Software experts can create tools that directly address Dathe's call for giving context to data, for example by adding demographic information. And in August 2013, open source and open access came together via LabKey to promote scientific transparency and reproducibility. For a clinical trial of a vasculitis therapy published in the New England Journal of Medicine, the LabKey open source platform was used to create a web portal with free public access to participant-level data, stripped of identifying information.

Researchers who are committed to transparency and are also do-it-yourselfers have a choice of open source workflow management tools. Carl Boettiger, an ecology and evolution postdoctoral researcher at the **University of California**, **Santa Cruz** has traveled the entire DIY lab notebook journey. Boettiger started keeping publicly accessible lab records in the Open-WetWare platform. "*It's a bit radical,*" says Boettiger. "*Anyone can go in and edit other peoples' notes, although that rarely happens.*" After OpenWetWare, Boettiger moved to platforms that give him increasing control over his research records, starting with WordPress, which is usually used for blogging. Boettiger now uses the online software development site GitHub as his note-book and Jekyll website-generating software to publish his notebook online.

A blog-type ELN creates a robust, cached history of your research, says Boettiger. It discourages fraud because any changes leave records. You choose what is public, private, and password protected. And think of the advantages when talking to people at conferences or answering reviewer requests, he says. You can just pull up records on a handheld device to see what you tried and when, and how it worked out.

What's Next

"*The trends in laboratory records,*" says Boettiger, "*are toward more open and collaborative, more secure, and more automated.*" Although Boettiger and Dathe should have different perspectives as an ecology researcher in Santa Cruz and a pharma development and information

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technology specialist in Basel, respectively, they share a nearly sci-fi vision of the future laboratory. In this vision, scientists simply do their work while an automated tracking system simultaneously keeps records. Barcoding will note reagents, samples, and instruments used, providing context to the data for subsequent analysis. The entire process will be recorded, showing the provenance of every byte and definitively establishing IP claims. "*It will give a much more extensive record that can be transparent or shared if you want,*" says Boettiger. A fully automated system would simplify research by capturing experimental details with no manual data entry. Then, all we'd need is a robot to return reagents to the right shelves.

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Atrium Research & Consulting
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LabArchives
www.labarchives.com

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LabKey Software
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LIMSwiki
www.limswiki.org

LEO Pharma
www.leo-pharma.com

Roche
www.roche.com

University of California, Santa Cruz
www.ucsc.edu

University of Toledo
www.utoledo.edu

Additional Resources

Core Informatics
www.corelims.com

Evernote
www.evernote.com

GitHub
www.github.com

IDBS
www.idbs.com

Jekyll
www.jekyllrb.com

OpenWetWare
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PerkinElmer
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Chris Tachibana is a science writer based in Seattle, USA, and Copenhagen, Denmark
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Pipetting, contamination, cross-contamination, sample preparation, filter tips, sterile tips

How to Avoid Contamination in Pipetting

Joni Ake[1]*

1. Sartorius Biohit Liquid Handling Oy, Laippatie 1, 00880 Helsinki

* Correspondence
E-Mail: Joni.Ake@sartorius.com

Introduction

Preventing contamination in pipetting is paramount to achieving reliable results. It requires identification of the potential contamination mechanisms in order that they can all be addressed.

Aerosols, suspensions of solid or liquid particles in a gas, are formed in many laboratory activities such as pipetting with air displacement pipettes, and aerosols are the major contamination source in pipetting. They may transfer into the pipette body when unfiltered pipette tips are used and consequently contaminate subsequent samples. A slow and careful pipetting rhythm helps minimize aerosol formation.

This paper addresses the three contamination types that originate from pipetting: pipette-to-sample contamination, sample-topipette contamination, and sample-to-sample contamination.

Pipette-to-Sample Contamination
This type of contamination occurs when a contaminated pipette or pipette tip contaminates the sample.

Pipette tips are available in multiple purity grades from most manufacturers. Purity grades can be divided into three categories:
- no purity certification
- certified free of contaminants like DNase, RNase, and endotoxins
- sterilized to be free of microbial life

Contaminants such as DNase, RNase, and endotoxins are difficult to remove by any sterilization method, so it is very important to prevent contamination during manufacturing. The absence of these contaminants is separately tested, usually by a third-party laboratory. Sterilization after manufacturing ensures that the tips do not contain any microbial life (bacteria, viruses etc.) when delivered to customers.

Pipette tips can also be a potential source of leachables – trace amounts of chemicals originating from materials or process equipment that can contaminate the samples. Examples of potential leachables are heavy metals, UV stabilizers, antioxidants, pigments, release agents, biocides, and surfactants. High quality tips manufactured from 100% virgin polypropylene in a high quality manufacturing facility do not contain leachables. It is recommended that you confirm this with the tip manufacturer. In daily laboratory work, pipette-to-sample contamination can be avoided by following these simple guidelines:
- Select a tip with the relevant purity class for your application.
- Use (sterilized) filter tips.
  Alternatively, you may be able to use tip-cone filters with some manufacturers' pipettes. The filters prevent aerosols from reaching the pipette body and potentially contaminating subsequent samples.
- Always change the pipette tip after each sample.
- Regularly autoclave, or disinfect, the pipette or the components that may come into contact with the sample.

Sample-to-Pipette Contamination

This type of contamination takes place when the pipetted liquid or aerosol particles from it enter the pipette body. To minimize the risk of sample-to-pipette contamination, the following precautions are recommended:

- Always release the pipette's push button slowly to prevent aerosol formation and uncontrolled liquid splashing within the pipette tip.
- Hold the pipette in a vertical position during pipetting and store the pipette in an upright position. This prevents liquids from running into the pipette body.
- Use filter tips to prevent aerosol transfer from the sample into the pipette body. Alternatively, filters can be used on pipette tip cones.

Sample-to-Sample Contamination

Sample-to-sample contamination (or carry-over contamination) occurs when aerosol or liquid residue from one sample is carried over to the next sample. This may take place, for example, when the same pipette tips are used multiple times. To avoid carry-over contamination:

- Use filter tips to prevent aerosol transfer from the sample into the pipette body, and again to the next sample. Alternatively, filters can be used on pipette tip cones.
- Always change the pipette tip after each sample.
- If you suspect pipette contamination, autoclave or disinfect the pipette according to the manufacturer's instructions.

Definitions:

Decontamination	Any activity that reduces microbial load to prevent contamination. Includes methods for sterilization, disinfection, and antisepsis.
Sterilization	The destruction of all microbial life, including bacterial endospores. Can be accomplished e.g. using steam, heating, chemicals, or radiation.
Autoclaving	Autoclaving (moist heat) is an efficient sterilization method for laboratories. A hot, pressurized, and saturated steam is applied to destroy microorganisms and decontaminate e.g. laboratory plastic and glassware. Exposure time and temperature are critical. Moreover, the steam needs to penetrate through the entire load to be efficient.
Disinfection	The elimination of virtually all pathogenic microorganisms (excluding bacterial endospores) and reduction of the microbial contamination to an acceptable level. A practical method for surface decontamination. The disinfectant (e.g. alcohols, phenolic compounds, halogens), concentration, and exposure time should be selected according to the assumed contamination type.
Antisepsis	The application of an antimicrobial chemical to living tissue to destroy microorganisms.
DNase	Powerful enzymes (nucleases) that degrade DNA by hydrolyzing it into short fragments. Even trace amounts of DNases can lead to low or no yields in DNA techniques such as PCR, or to degradation during DNA purification. Contamination sources: human contact, saliva, bacteria.
RNase	Powerful enzymes (nucleases) that catalyze the degradation of RNA into short fragments. Very stable enzymes that are difficult to remove. Contamination sources: oils from skin, as well as hair, tears, bacteria.
Endotoxins	Lipopolysaccharides, large molecules that are part of the outer membrane of Gram-negative bacteria such as E. coli, Salmonella, Shigella, Pseudomonas, and Haemophilus. Cause fever in humans and impair the growth of cell cultures. Are released into the environment when bacteria die and the cell wall is destroyed. Contamination sources: endotoxins are present wherever bacteria are able to grow, i.e. air, water, soil, skin, raw materials, any non-sterile environment.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

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Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories

Rik McRae[1], Hannes Landmann[2,*]

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Goettingen, Germany

* Correspondence

E-Mail: adam.green@sartorius.com

Abstract

Concentration and Purification of Proteins in Cell Culture Supernatant Using Sartorius Vivaflow®, Vivaspin® and Vivapure® Products

This protocol demonstrates how the Vivaflow® cassettes, Vivapure® Ion Exchange spin columns and Vivaspin® devices can be used in order to perform a complete protein purification workflow, from concentration and diafiltration of the original protein source, a cell culture supernatant, to final concentration | desalting of the purified protein. This protocol shows in detail the recoveries after each step along with the time needed for every purification and concentration step.

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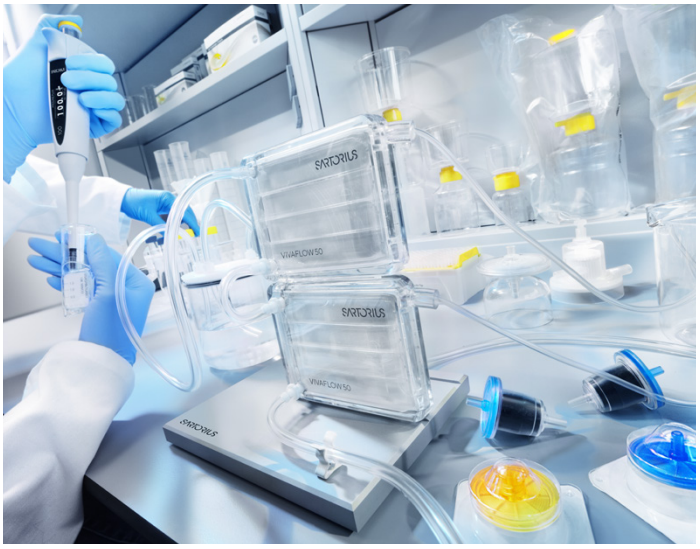
Introduction

Efficiency and efficacy of a multiple cycle experimental procedure was performed using Vivaflow® tangential flow cassettes for initial concentration and diafiltration of a cell culture supernatant, followed by Vivapure® Ion Exchange spin columns for the protein purification step and finally Vivaspin® 20 ultrafiltration devices for the final sample concentration and desalting. An artificial mixture of proteins in a RPMI-1640 culture medium was created to mimic the type of product that many researchers culture using e.g. the UniVessel device. This procedure further reflects a method that can be adapted to a large number of protein purification protocols, adapting MWCOs and device sizes where necessary.



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Methods

Part 1 – Creating and concentrating the culture medium
2 bottles (4 g) of RPMI-1640 were dissolved into 1.8 L dd-H_2O and 4 g of Sodium Acetate was added.

The pH was adjusted to 7.2 using 1M HCl. 2 g of BSA and 1 g of Lysozyme was added as protein samples, meant to be separated by chromatography. The volume of the cell culture supernatant sample was brought up to 2 L using dd-H_2O. After every preparation, concentration and purification step, 1 mL sample was set aside for SDS gel analysis at the end of the preparation.

Ion Exchange chromatography was chosen as the method of choice for purifying lysozyme from the cell culture supernatant, especially from the "contaminant" BSA. For this, the 2 L cell culture supernatant needed to be concentrated and then diafiltered to adjust the sample to the starting conditions needed for the ion exchange chromatography binding step.

For concentration and diafiltration, the Vivaflow® 200 was used with a 5 kDa PES membrane. Vivaflow® 200 is a ready-touse laboratory crossflow cassette in an acrylic housing, which allows caustic cleaning and 4-5 re-uses. Two cassettes can be run in parallel for the concentration of up to 5 L sample volumes. For the 2 l sample to be concentrated in this experiment, one cassette was sufficient. A Masterflex pump with an Easy Load, size 16 pump head was used to run the Vivaflow® 200 cassette. Figure 1a. and 1b. show the Vivaflow® 50R set up with a single module and with two modules.

The Vivaflow® 200 system was set up and run at 3 bar. Once 1.8 L of filtrate had been collected, the pump was stopped, the tubes removed from the cell culture medium concentrate and filtrate and the Vivaflow® system was purged with dd-H_2O. This solution now contained a 10 fold concentration of the constituent proteins from the original culture-medium.

A BCA protein detection test conveyed a 100% recovery of protein after this first concentration step. Table 1 indicates the time needed for the sample concentration.

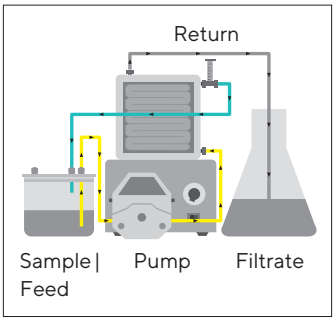

Fig. 1a. Vivaflow® 50R – single module

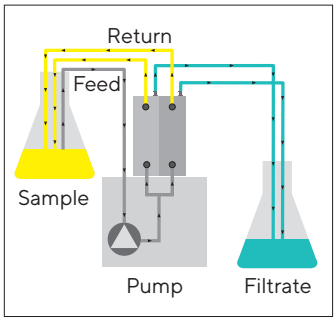

Fig. 1b. Operation – two modules

Table 1: Vivaflow® 200, PES, 5 kDa MWCO concentration speed.

Vivaflow® 200 (5kDa MWCO)

Filtrate Volume (mL)	Time taken (hr:min:secs)
0	0:00:00
100	0:03:16
200	0:06:50
300	0:10:45
400	0:14:38
500	0:18:36
600	0:22:43
700	0:26:57
800	0:31:14
900	0:36:01
1000	0:40:50
1100	0:45:46
1200	0:50:36
1300	0:55:32
1400	1:00:24
1500	1:05:26
1600	1:10:28
1700	1:15:52
1800	1:21:50

Part 2 – Buffer exchange of culture medium concentrate

The Vivaflow® 200 system was used for fast and easy diafiltration. To this end, the diafiltration cup, a Vivaflow® accessory, was filled with the 200 mL concentrated sample. Figure 2 shows the diafiltration set up. The Vivaflow® 200 system was set up as before, however attaching an additional tube to the diafiltration lid and placing this new inlet tube into a 25 mM Sodium Acetate (pH 5.5) buffer (needed to re-adjust the sample concentrate for the ionic starting conditions of the ion exchange chromatography step which was to follow). This leads to the concentration of the sample in the reservoir and to the extent in which the original buffer is removed and collected as waste (filtrate), new buffer (25 mM Sodium Acetate) is sucked into the closed system, gradually leading to a buffer exchange while keeping the sample volume constant at 200 mL. The system was run at 3 bar. Once 1 L of buffer had been exchanged, the filtration was stopped.

The 200 mL solution now contained the correct buffer to maintain the stability of the proteins of interest for the next part of the protocol and had the correct pH and salt concentration for the ion exchange binding step. BCA protein quantification again showed a 100% protein recovery.

Table 2 shows the time needed for diafiltration of 200 mL sample against 1000 mL exchange buffer, again using Vivaflow® 200 with a 5 kDa PES membrane.

Fig. 2: Diafiltration system set up for buffer exchange. Culture medium concentrate can be seen in the center of the image. 1 L 25 mM Sodium Acetate (exchange buffer) can be seen connected to the system on the left of the image.

Filtrate Volume (mL)	Time taken (hr:min:secs)
0	0:00:00
100	0:06:58
200	0:14:16
300	0:22:39
400	0:29:40
500	0:37:02
600	0:44:15
700	0:51:34
800	0:58:54
900	1:06:03
1000	1:13:02

Table 2: Diafiltration of 200 mL concentrated cell culture supernatant containing the proteins lysozyme and BSA against 1000 mL 25 mM Sodium Acetate.

Part 3 – Purification of Lysozyme, the protein of interest

The purification of lysozyme was performed using a Vivapure® cation exchange membrane adsorber devices (Vivapure® Maxi H S). The membrane adsorber matrix holds the active ligands and performs like a traditional cation exchanger. Membrane adsorbers represent a special form of chromatography matrix. Unlike traditional chromatography resins, they make use of convective transport to bring proteins to the ion exchange surface; hence, binding, washing and elution is performed quickly and high binding capacities are even achieved at high flow rates. This allows the use of the chromatography matrix in fast and convenient centrifugal spin columns (Fig. 3).

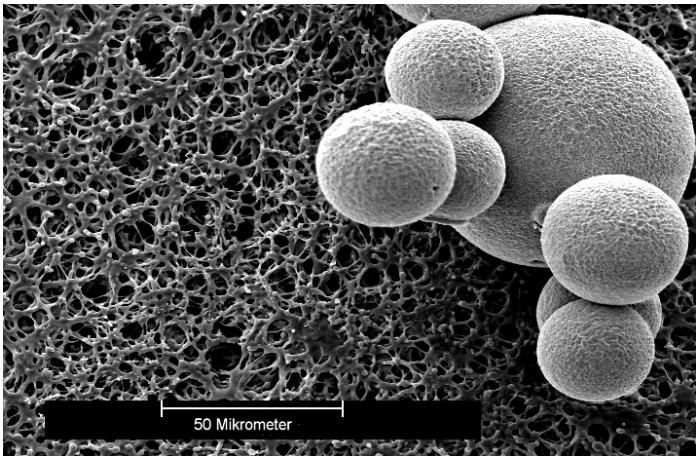

Fig. 3: The electron microscopic image of chromatography gel beads (upper right) in comparison to a Q ion exchange membrane adsorber (background) reveals 100 fold larger pore sizes of the membrane adsorber.

Two Vivapure® Maxi H S type devices (Fig. 4) were equilibrated with 10 mL of 25 mM Sodium Acetate, pH 5.5 each, by filling with 10 mL of this buffer and centrifuging for 5 min. in a swing bucket centrifuge at 500 × g and discarding the flow through. Using the concentrated and buffer exchanged sample from Part 2, 10 mL sample were pipetted into each of these two equilibrated Vivapure® devices and centrifuged again for 5 min. in a swing bucket centrifuge at 500 × g. The Vivapure® devices were washed with further 10 mL of 25 mM Sodium Acetate, discarding the flow through, followed by an elution step with 5 mL of 1 M NaCl in 25 mM Sodium. A BCA test revealed a 95% lysozyme recovery.

Fig. 4: Vivapure® Maxi spin columns can be used in a centrifuge for fast and easy protein purification.

The eluate was then concentrated in a Vivaspin® 20 (PES, 5 kDa MWCO), Figure 5., and centrifuged at 5000 × g for 10 min. or until approximately 2 mL of concentrate had been collected. The device was then re-filled with 18 mL 50mM Potassium Phosphate buffer, pH 7.2 to 20 mL for a final buffer exchange and desalting of the purified sample. The sample was again centrifuged until a final sample volume of 2 mL had been attained. A BCA test revealed a 97% lysozyme recovery.

Fig. 5: Vivaspin® 20 ultrafiltration device, on the right with a pressure cap which allows pressurization of the device as well and the regular utilization in a centrifuge.

Part 4 – Analyzing the samples

The samples of the individual steps were analyzed by SDS gel, using reducing sample buffer (prepared by adding 50 µL 2-mercaptoethanol to 950 µL Laemmli sample buffer). For all steps, 5 µL of the 1 mL sample taken during the experiment were diluted with 95 µL reducing sample buffer, of which 20 µL were loaded onto a 12% tris-HCl SDS gel (Fig. 6)

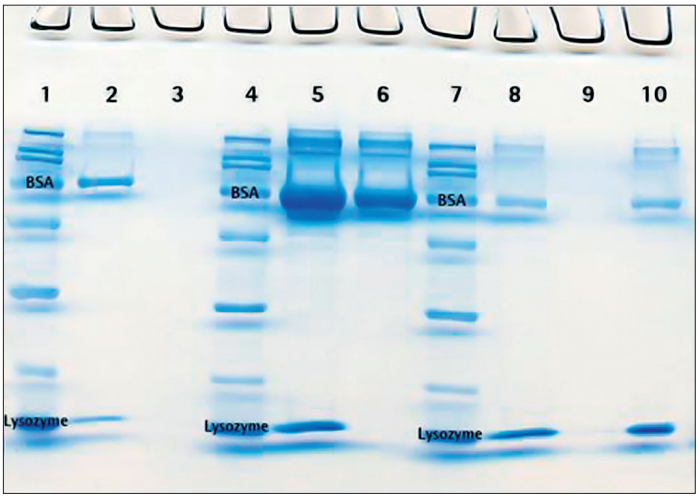

Fig. 6: Coomassie stained 12% tris-HCl SDS gel loaded with 20 µL sample preparations. Lane 1: Marker (SDS Broad range marker); Lane 2: Original sample; Lane 3: Original sample filtrate (Part 1); Lane 4: Marker; Lane 5: Buffer exchange concentrate (Part 2); Lane 6: Filtrate after binding (Part 3); Lane 7: Marker; Lane 8: Filtrate after eluting (Part 3); Lane 9: Filtrate after concentrating and desalting (Part 3); Lane 10: Concentrate after concentrating and desalting.

3. Conclusion

The overall result shows that a standard and straightforward procedure can be followed to concentrate, purify, isolate and analyze a protein of interest from a cell culturing device, using Vivaflow® 200 tangential flow units for cell culture supernatant concentration and diafiltration, Vivapure® for ion exchange chromatography followed by Vivaspin® 20 for final sample concentration and desalting.

In many cases dialysis, which is an overnight procedure would be performed instead of the much quicker alternative ultrafiltration. Here, we show how time saving and efficient ultrafiltration is for diafiltration and desalting applications, as well as for protein concentration.

The complete set up and completion of protein purification takes approx. 3.45 h using this method, starting form a culture supernatant, with high protein recoveries in each step (see Table 3) The total protein purification procedure can be completed within 1 working day, including SDS gel analysis, utilizing this time saving strategy, when adapted to individual needs.

Task	Time	Recovery
Vivaflow® 200 set up and run through	1 hour 25 min.	100%
Vivaflow® 200 Diafiltration set up and run through	1 hour 20 min.	100%
Vivapure® purification	45 min.	95%
Vivaspin® Lysozyme desalting | concentration	30 min.	97%
Total	3 hours 45 min.	92%

Table 3

Products used in this experiment	Order No.
Vivaflow® 200, PES, 5kDa	VF20P1
500 mL Diafiltration cup	VFA006
Vivapure® S H Maxi	VS-IX20SH08
Vivaspin® 20, 5 kDa	VS2011

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

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Scouting Protein Purification Conditions Using Vivapure Centrifugal Ion Exchange Membrane Absorbers

C. Naumann, N. Kashani-Poor

Correspondence
E-Mail: adam.green@sartorius.com

Introduction

For separation and purification of proteins from biological samples, different characteristics of the target protein, e.g., its size, charge, hydrophobicity, or specifically engineered tags, are exploited.

With ion exchange chromatography, separation is achieved on the basis of different charges of biomolecules. This makes it a versatile method often used for prefractionation or purification of a target protein from crude protein mixtures. To optimize the purification procedure for an individual target, several binding and elution conditions have to be tested on cation and anion exchange matrices.

Materials and Methods

In contrast to traditional column chromatography methods, Vivapure IEX centrifugal columns allow scouting of several chromatography conditions in parallel, leading quickly to different fractions which can be further analyzed for enriched or even already purified target protein.

Here, we demonstrate the performance of Vivapure® IEX Mini spin columns for evaluation of optimal purification conditions of cloned SH2 domains from an *E. coli* lysate in a two-step procedure. This protocol can generally be employed for finding a purification method based on ion exchange chromatography for a given target protein, as it is fast and only uses up small amounts of the sample.

In the first step of this protocol, binding conditions are evaluated by loading the sample on Vivapure® Q and S columns at various pH values, eluting bound proteins with a high salt concentration buffer and analyzing all fractions for the target protein. This step results in the optimal binding pH and the best ion exchange chemistry for the purification.

In the second step, the best elution method is evaluated by applying increasing salt concentrations to columns which were shown to bind the target protein in step one, leading to a complete purification protocol in less than one hour.

Experiment

Using the described scouting procedure, a purification method for a SH2 domain expressed in *E. coli* was developed. In Step One, proteins were bound to the Vivapure® IEX membranes at different pH values, then eluted with high-salt buffer. In Step Two, a fresh sample was adjusted to the respective pH elucidated previously as the best choice for binding the protein and was loaded onto a new column for refining optimal elution conditions.

Materials

- Vivapure® Mini Q H spin columns
- Vivapure® Mini S H spin columns
- Minisart® syringe filter (0.45 µm CA, Sartorius AG)
- Centrifuge, 45°-fixed-angle rotor; 2000 × g

Buffers used

Buffer A	25 mM citrate, pH 4
Buffer B	25 mM potassium phosphate, pH 6
Buffer C	25 mM HEPES, pH 8
Buffer D	25 mM sodium bicarbonate, pH 10
Buffer E	25 mM citrate, pH 4, supplemented with 1 M NaCl.
Buffer F	25 mM potassium phosphate, pH 6, supplemented with 0.2 M, 0.4 mM, 0.6 mM, 0.8 mM, & 1 M NaCl, respectively.
Buffer G	25 mM HEPES, pH 8, supplemented with 1 M NaCl
Buffer H	25 mM sodium bicarbonate, pH 10, supplemented with 1 M NaCl

Step One: Scouting for Binding Conditions to the Appropriate Ion Exchange Chemistry

Expression of Target Protein
300 mL LB media were inoculated with 4 mL of an overnight culture and incubated at 37°C, shaking at 150 rpm until an OD600 of 1.0 was r eached. IPTG was added to a final concentration of 1 mM and incubated for further 4 h with shaking at 150 rpm. Cells wer e harvested by centrifugation at 4000 × g for 30 min at 4°C. The pellet was resuspended in 35 mL PBS (150 mM KPi, pH 7.3) and cells were lysed by addition of lysozyme to a final concentration of 0.1 mg/mL and incubation for 1 h at 37°C. Insoluble particles as cell debris wer e removed by centrifugation at 10000 × g for 30 min at 4°C.

Sample Preparation

4 × 200 µL of the cell lysate wer e diluted with 1.8 mL binding buffer A to D each, to adjust the sample to the respective pH conditions. In order to avoid clogging of the membranes in the Vivapure® Mini spin columns, samples were clarified by passage through Minisart® syringe filters.

Column Equilibration

4 × Q and 4 × S Vivapure® Mini spin columns were labeled 4, 6, 8, and 10, corresponding to the pH of the buffer to be used. To each spin column, 400 µL of the corresponding binding buffer were added and spun for 5 min at 2000 × g.

Binding and Washing

400 µL of the clarified samples adjusted to pH values 4, 6, 8, and 10 were applied each to the correspondingly equilibrated Vivapure® Q and S spin columns. Columns were spun for 5 min at 2000 × g.

Afterwards, Vivapure® Mini spin columns were reloaded with 400 µL sample and spun again for 5 min at 2000 × g. Loosely bound proteins were washed away with the application of 400 µL of the respective binding buffer to each of the columns and spun for 5 min at 2000 × g. Flow-through and wash fractions were collected for subsequent detection of the target protein.

Complete Elution of Bound Proteins

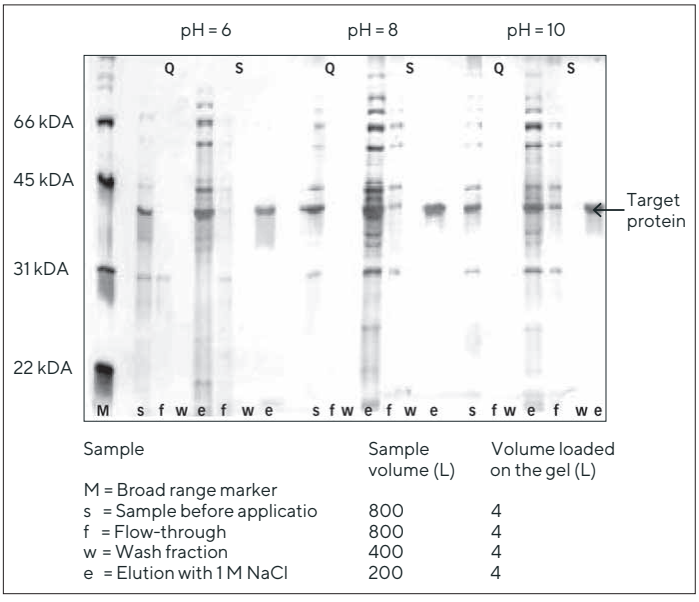
200 µL of elution buffer E, F, G, and H were applied to the washed columns and spun for 3 min at 2000 × g. Eluates were saved for subsequent analysis.

Analysis

4 µL of flow-through, wash, and elution fractions from each column were analyzed on reducing SDS-PAGE, followed by silver staining.

Result of Step One

Dilution of the *E. coli* lysate with binding buffer A (25 mM citrate, pH 4) led to complete precipitation of sample proteins. Thus, pH 4 could not be tested in this experiment. As can be seen on the SDS gel in Figure 1, the target protein was present in the eluate of the Vivapure® Q Mini spin column at all pH values tested together with most of the *E. coli* proteins (Lanes Q "e"). In contrast, using the Vivapure® S Mini spin column, at all pH-values tested, most *E. coli* proteins did not bind to the membrane and were found in the flow-through (Lane S " f"), thus resulting in pure target protein in all elution fractions (Lane S "e").



Sample	Sample volume (L)	Volume loaded on the gel (L)
M = Broad range marker		
s = Sample before applicatio	800	4
f = Flow-through	800	4
w = Wash fraction	400	4
e = Elution with 1 M NaCl	200	4

Figure 1: Scouting for optimal binding conditions of a SH2 domain expressed in E. coli. SDS gel (reducing, 12%), silver stained. Shown are sample before loading, flow-through, wash, and elution fractions (1 M NaCl) from Vivapure Q and S Mini spin columns, at the various pH values tested.

Differences could be detected in the binding efficiency of the target protein, as at pH 8, traces of the target protein were already found in the flowthrough, with slightly higher amounts at pH 10 (Lane S "e"). At pH 6, the most efficient binding of the target protein to the S membrane was observed. Now that the binding conditions, i.e., the binding pH and the best suited ion exchange chemistry, were found, the elution protocol of the target protein was optimized in a second step.

Step Two: Optimizing Elution Conditions

Sample Preparation
Taking account of the results of Step One, 200 µL cell lysate were diluted with 1.8 mL binding buffer B (25 mM KPi, pH 6). In order to avoid clogging of the membrane in the Vivapure® Mini spin column, the pH adjusted sample was clarified by passage through a Minisart® syringe filter.

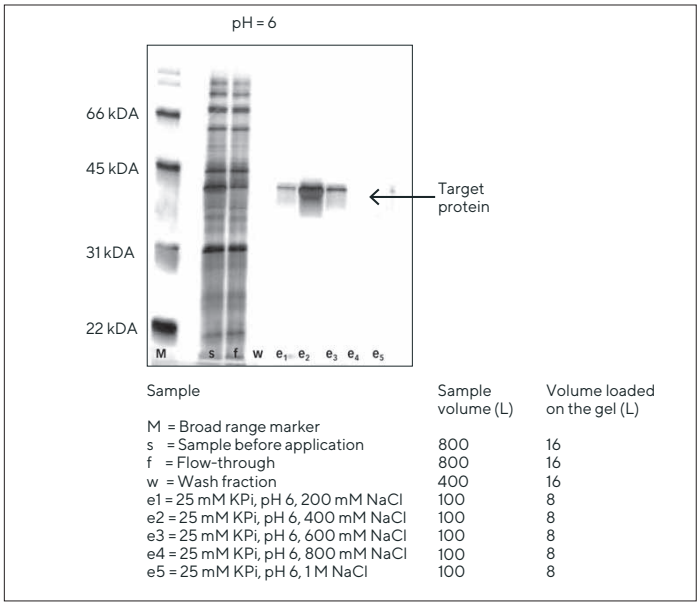
Column Equilibration
400 µL binding buffer B were applied to one Vivapure® S Mini spin column and spun for 5 min at 2000 × g.

Binding and Washing
400 µL of the clarified sample were applied to the equilibrated Vivapure® S column and spun for 5 min at 2000 × g. Afterwards, the Vivapure® S Mini spin column was reloaded with 400 µL sample and spun again for 5 min at 2000 × g. Loosely bound proteins were washed away by application of 400 µL binding buffer to the column and spun for 5 min at 2000 × g. Flow-through and wash fraction were saved for analysis.

Stepwise Elution
100 µL elution buffer F, supplemented with 0.2 M NaCl, were applied to the Vivapure® S Mini spin column and spun for 3 min at 2000 × g. The eluate was collected. In the next step, 100 µL of elution buffer F, supplemented with 0.4 M salt, were applied and again spun for 3 min at 2000 × g. Elution was continued until the entire gradient had been tested, saving the eluates from each step.

Analysis
4 µL of flow-through, wash, and elution fractions from each column were analyzed on reducing SDS-PAGE, followed by silver staining.

Result of Step Two
The target protein started to elute with 200 mM NaCl, however the main fraction eluted with 400 mM NaCl. Traces of the target protein were also found in the next elution step with 600 mM NaCl, but this might be due to the low elution volume.



Sample	Sample volume (L)	Volume loaded on the gel (L)
M = Broad range marker		
s = Sample before application	800	16
f = Flow-through	800	16
w = Wash fraction	400	16
e1 = 25 mM KPi, pH 6, 200 mM NaCl	100	8
e2 = 25 mM KPi, pH 6, 400 mM NaCl	100	8
e3 = 25 mM KPi, pH 6, 600 mM NaCl	100	8
e4 = 25 mM KPi, pH 6, 800 mM NaCl	100	8
e5 = 25 mM KPi, pH 6, 1 M NaCl	100	8

Figure 2: Scouting for optimal elution conditions of a SH2 domain expressed in *E. coli*. SDS gel (reducing, 12%), silver stained. Sample before loading, flow-through, wash, and elution fractions from Vivapure® S Mini spin column at pH 6 are shown.

Results

A two-step procedure was used to rapidly scout optimal purification conditions for a target protein (a SH2 domain from *E. coli* lysate) with ion exchange chromatography. In the first step, the most suited buffer pH for binding the target protein to the most adequate ion exchanger was verified. In the second step, the elution condition was optimized, building on the results gained in Step One of this protocol (elution optimization after optimal binding of the target to the proper ion exchanger). With the scouting procedure described here, it was possible to quickly and conveniently purify the target protein to homogeneity. The results obtained in this experiment can be used for various ends, e.g.:

- polishing a specific protein after a first chromatography step with another chemistry
- establishing quickly a FPLC method for a new protein
- finding a purification method for a new protein for upscaling with Vivapure® Maxi or Mega.

For these purposes, Vivawell 96-well plates, Vivapure® Maxi, and Sartobind® membrane adsorber units with FPLC connectors are available.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

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Lab Ultrafiltration

Tips and Tricks

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The use of ultrafiltration membranes for concentration and purification of proteins and DNA is ubiquitous in biological laboratories. Filter devices with ultrafiltration membranes can also be used for concentration of other macromolecules such as inorganic polymers, nanoparticles or even viruses. Although performing sample concentration and buffer exchange using an ultrafiltration device is relatively simple, some tricks of the trade can improve your recovery or speed up your work flow considerably.

The following Application Notes will give you an overview of how to:

Desault Samples	Concentrate Samples	Recovery
Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators	Concentration to a defined final volume with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES	Treatment of Vivaspin® concentrators for improved recovery of low-concentrated protein samples

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Application Note

April 03, 2019

Keywords or phrases:
Diafiltration, buffer, exchange, de-salting

Desalting and Buffer Exchange With Vivaspin® Centrifugal Concentrators

Pieter Eyckermann[2], Rik McRae[1], Andreas Kocourek[2], Robert Zeidler[2] and Adam Green[1,*]

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Göttingen, Germany

* Correspondence

E-Mail: adam.green@sartorius.com

Abstract

This short application note highlights the ability to reduce protein sample salt concentrations by up to 99%, or to exchange the buffer sample entirely, using Vivaspin® 20 and Vivaspin® 6 centrifugal ultrafiltration devices. This process is known as 'diafiltration' and prevents the over concentration of proteins with a tendancy to precipitate at higher salt concentration.

Find out more: www.sartorius.com

Introduction

Vivaspin® centrifugal concentrators, with patented vertical membrane technology, combine fast filtration with high recovery of target proteins. This makes Vivaspin® the technology of choice for desalting or buffer exchange, avoiding lengthy dialysis steps.

While proteins are retained by an appropriate ultrafiltration membrane, salts can pass freely through, independent of protein concentration or membrane MWCO. In consequence, the composition of the buffer in the flow-through and retentate is unchanged after protein concentration. By diluting the concentrate back to the original volume, the salt concentration is lowered. The concentrate can be diluted with water or salt-free buffer if simple desalting is required; however, it is also possible to dilute the concentrate with a new buffer, thereby exchanging the buffering substance entirely. For example, a 10 ml protein sample containing 500 mM salt, if concentrated 100× still contains 500 mM salt. If this concentrate is then diluted 100× with water or saltfree buffer, the protein concentration returns to normal, while the salt concentration is reduced 100× to only 5 mM, (I.E. a 99% reduction in salt).

The protein sample can then be concentrated again to the desired level, or the buffer exchange can be repeated to reduce the salt concentration even further before a final concentration of the protein. This process is called "diafiltration". For proteins with a tendency to precipitate at higher concentrations, it is possible to perform several diafiltration steps in sequence, with the protein concentrated each time to only 5 or 10x. For example, if a precipitous protein sample is concentrated to 5x then diluted back to the original volume, and this process is repeated a further two times, this still results in a >99% reduction in salt concentration, without over concentrating the protein.

Desalting and Buffer Exchange Procedure

(See Figure 1.)

1. Select the most appropriate MWCO for your sample. For maximum recovery, select a MWCO ½ to 2 the molecular size of the species of interest.
2. Fill concentrator with up to the maximum volume stated in the device operating instructions*, (e.g. 20 ml if Vivaspin® 20 is used).
3. If the sample is smaller than the maximum device volume*, it can be diluted up to the maximum volume before the first centrifugation step. This will help increase the salt removal rate.
4. Centrifuge for the recommended amount of time at an appropriate spin speed for your Vivaspin® model*.
5. Empty filtrate container†.
6. Refill concentrator with an appropriate solvent.
7. Centrifuge again as before.
8. Empty filtrate container†.
9. Recover the concentrated, de-salted sample from the bottom of the concentrate pocket with a pipette.

Notes

* For guidance on maximum fill volumes, spin speeds and suggested spin times, please refer to the Operating Instructions that accompany your Vivaspin® products.

† Filtrate volumes should be retained until the concentrated sample has been analyzed.

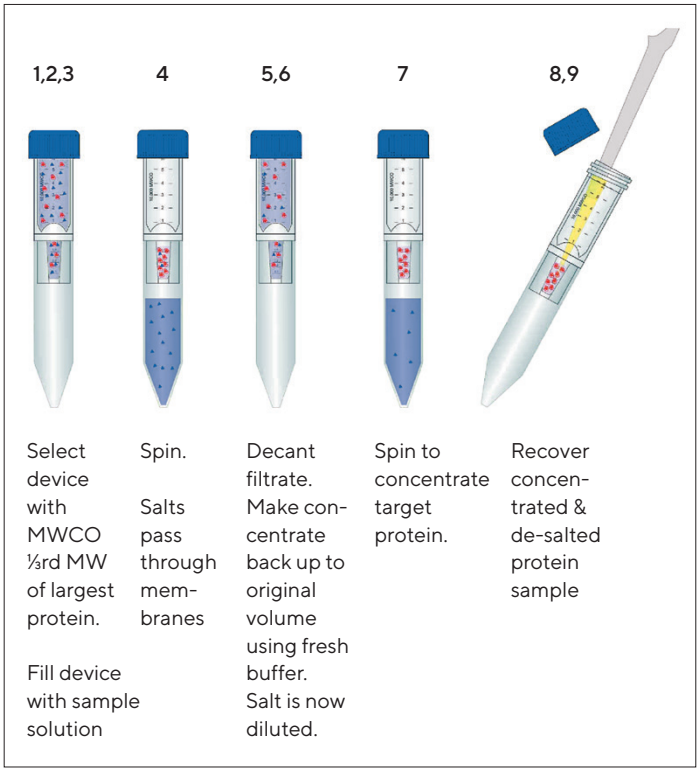

Figure 1: Step-by-step method for desalting and concentration

Test Results

As the results below show, the efficient design of Vivaspin® devices allowed >95% of the salt to be removed during the first centrifugation step. Only one subsequent centrifugation step was needed to increase the typical salt removal to 99% with >92% recovery of the sample.

Vivaspin® 20

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal
Spin 1	100%	99%	97%	99%	97%	99%	90%	98%
Spin 2	96%	100%	92%	100%	93%	100%	87%	100%

Four Vivaspin® 20 devices of each cut-off were tested with 20 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g.
The devices > 5kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and second spin, the retentate was brought up to 20 ml with ultra pure water from the Arium system (Sartorius). OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

Vivaspin® 6

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal	Protein Recovery	NaCL Removal
Spin 1	98%	99%	92%	99%	93%	99%	92%	98%
Spin 2	85%	100%	86%	100%	83%	100%	89%	100%

Four Vivaspin® 6 devices of each cut- off were tested with 6 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g.
The devices > 5 kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and the second spin the retentate was brought up to 6 ml with ultra pure water from the Arium system (Sartorius) OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

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Application Note

January 15, 2018

Keywords or phrases:
Concentration ratio, final volume adjustment

Concentration to a Defined Final Volume with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES

Rik McRae[1], **Hannes Landmann**[2,*]

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Goettingen, Germany

* Correspondence

E-Mail: adam.green@sartorius.com

Abstract

This short Application Note describes how you can use Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES concentrators to concentrate to defined final volumes. By adding a particular volume to the filtrate vessel prior to the concentration, the final volume of the concentrate can be adjusted accurately.

Introduction

It is sometimes desirable to be able to preselect a defined final volume for a concentration step, especially when parallel concentrations are being performed. Vivaspin® centrifugal concentrators have a built-in deadstop feature, which prevents overconcentration to dryness. Due to the fast concentration rates possible with the patented vertical membrane design in the Vivaspin®, the drying out of the sample would otherwise be a possibility.

This note describes a method for achieving reproducible defined final volumes using Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES centrifugal concentrators. The method does not rely on the deadstop pocket but is increasing the retained volume by adding liquid to the filtrate vessel prior to centrifugation.

Equipment

- Vivaspin® Turbo 15 PES 10kDa MWCO
- Vivaspin® Turbo 4 PES 10kDa MWCO
- Vivaspin® 500 PES 10kDa MWCO
- Tacta 5 mL mechanical pipette and Optifit pipette tips
- Tacta 1000 µL mechanical pipette and Optifit pipette tips
- Tacta 200 µL mechanical pipette and Optifit pipette tips
- Arium® pro ultrapure water system
- Sartorius Precision Lab Balance
- Centrisart® D-16C Centrifuge with swing out rotor for 50 mL and 15 mL falcon tubes
- Centrisart A-14C Centrifuge with fixed angle rotor for 24 1.5 | 2.2 mL tubes

Reagents

1 mg/mL Bovine Serum Albumin labelled with Bromophenol blue

Methods

1. Add defined amount of water to the filtrate tube (see table).
2. Put the concentrator insert into the filtrate tube and add sample solution.
3. Close the concentrator screw cap (for Vivaspin® Turbo 15 PES or Vivaspin® Turbo 4 PES) or close the cap (Vivaspin® 500 PES) and place in the centrifuge.
4. Concentrate the sample.
5. Remove the concentrator insert and recover the concentrate with a pipette.

Results

Results for Vivaspin® Turbo 15 PES

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
11.5 mL	15 mL	20 min @ 4,000 × g	1.50 ± 0.02 mL
9.5 mL	15 mL	20 min @ 4,000 × g	0.96 ± 0.01 mL
7.5 mL	15 mL	20 min @ 4,000 × g	0.53 ± 0.02 mL

Results for Vivaspin® Turbo 4 PES

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
2.0 mL	4 mL	20 min @ 4,000 × g	0.34 ± 0.03 mL
1.5 mL	4 mL	20 min @ 4,000 × g	0.15 ± 0.02 mL
1.2 mL	4 mL	20 min @ 4,000 × g	80 ± 10 µL

Results for Vivaspin® 500 PES in 40° fixed angle rotor

Volume of water added to the filtrate tube	Volume of sample solution added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
500 µL	500 µL	15 min @ 15,000 × g	103 µL ± 13 µL
380 µL	500 µL	15 min @ 15,000 × g	51 µL ± 11 µL
250 µL	500 µL	15 min @ 15,000 × g	30 µL ± 5 µL
200 µL	500 µL	15 min @ 15,000 × g	23 µL ± 7 µL

Conclusion

Reproducible defined final concentrate volumes can be quickly and easily achieved with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES, and Vivaspin® 500 PES.

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Application Note

November 05, 2020

Keywords or phrases:
Ultrafiltration, passivation, protein concentration, low-concentration protein samples, protein yield, non-specific binding, adsorption

Treatment of Vivaspin® Concentrators

For Improved Recovery of Low-Concentrated Protein Samples

Rik McRae[1,*], Claudia Naumann[2,*], Kristin Hoehne, Robert Zeidler

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Göttingen, Germany

* Correspondence

E-Mail: adam.green@sartorius.com

Abstract

Ultrafiltration is a core technology for the concentration of molecules of interest in the laboratory and research setting. As molecule recovery is a key criteria for high performance; optimal membrane, MWCO and device handling must be in place. Further to this, certain techniques can be employed to minimize loss through non-specific adsorption to device housing material and membrane material. This is most important for samples with low starting concentrations in the nanogram to microgram range, where loss through adsorption can have a significant impact on end recoveries. Here we describe these "passivation" techniques that demonstrate increased recovery when used with low starting concentration samples.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

Introduction

With appropriate device size and membrane cut-off selected, Vivaspin® products will typically yield recoveries for the concentrated sample > 90% when the starting sample contains over 0.1 mg/mL protein of interest. Depending on sample characteristics relative to the membrane type used, solute (protein) adsorption on the membrane surface is typically very low (2 – 10 µg/cm²) and in practice not detectable.

This can increase to 20 – 100 µg/cm² when the filtrate is of interest and the sample must pass through the whole internal structure of the membrane. Whilst the relative adsorption to the plastic of the sample container will be proportionately less important than on the membrane, due to the higher total surface area, this can be also be a source of yield loss. Typically, a higher cut-off membrane will bind more than a low molecular weight alternative.

Whenever possible, the smallest MWCO and device size applicable should be chosen. Swinging bucket rotors are preferred to fixed angle rotors. This reduces the surface area of the concentrator that will be exposed to the solution during centrifugation.

An important factor not to be neglected is the thorough recovery of the retentate. Make sure to carefully remove all traces of solution from the sample container and, if feasible, rinse the device after recovering the sample with one or more drops of buffer and then recover again.

The intention of the following "passivation" procedure is to improve recovery of protein samples in the nano- to microgram concentration range by pretreating the device (membrane & plastic). For this purpose a range of solutions are suggested in Table 1.

Table 1: Passivation Solutions

Type	Concentration
Powdered milk	1% in Arium® water
BSA	1% in PBS
Tween 20	5% in Arium® water
SDS	5% in Arium® water
Triton X-100	5% in Arium® water
PEG 3000	5% in Arium® water

Passivation Procedure for Vivaspin® Ultrafiltration Concentrators

A) Passivation Procedure

1. Wash the concentrators once by filling with Arium® water and spin the liquid through according to the respective protocol.
2. Remove residual water thoroughly by pipetting.
   Caution: Take care not to damage the membrane with the pipette tip.
3. Fill concentrators with the blocking solution of choice as given in Table 1.
4. Incubate the filled concentrators at room temperature for at least 2 hours (overnight is also possible except for Triton X-100 which is not recommended for overnight incubation).
5. Pour out the blocking solution.
6. Rinse the device 3 – 4 × very thoroughly with Arium® water and finally spin through.
7. The "passivated" devices are now ready for use.
   We recommend comparing different passivation reagents with an untreated device.

Note

It is necessary to rinse the device thoroughly before each washspin to ensure that traces of passivation compound are removed from the deadstop. Use the device immediately for protein concentration or store it at 4°C filled with Arium® water, to prevent the membrane from drying.

B) Evaluation Of Passivation Effects (Exemplary With BSA)

1. Prepare a 10 µg/mL BSA stock solution e.g. by diluting 90 µL of the 4 mg/mL stock solution in 36 ml 0.1 M sodium borate pH 9.3. Mix well.
2. Fill Vivaspin® 2 devices with 2 ml of this 10 µg/mL BSA solution and close with cap provided.
3. Spin the device in a swing-out rotor at 4,000 × g until the volume is to app. 100 µL.
4. Recover the concentrate and make back up to 2 mL with 0.1 M sodium borate pH 9.3
5. Determine recovered protein concentrations e.g. according to Bradford or BCA assays.

Results and Discussion

As an example, the effect of milk powder was analysed. It could be shown (Figure 1) that the protein recovery of a 10 µg/mL BSA solution could be increased from around 70 to 90%. If milk powder is not interfering with sample purity and quality, it is a good starting point to improve recovery of diluted sample solutions.

Protein Recovery (10 µg/ml BSA) with Vivaspin® PES 10 kDa after Passivation

In another example, detergents were analysed with only 250 and 500 ng BSA (Figure 2) BSA recovery declined to 50 – 30% in untreated devices as the protein concentration was reduced. Significant improvement to 60 – 90% recovery could be demonstrated when using the passivation strategy. Often, Triton X-100 seemed to work though the optimal reagent has to be selected for the respective protein and its hydrophilic | -phobic characteristics.

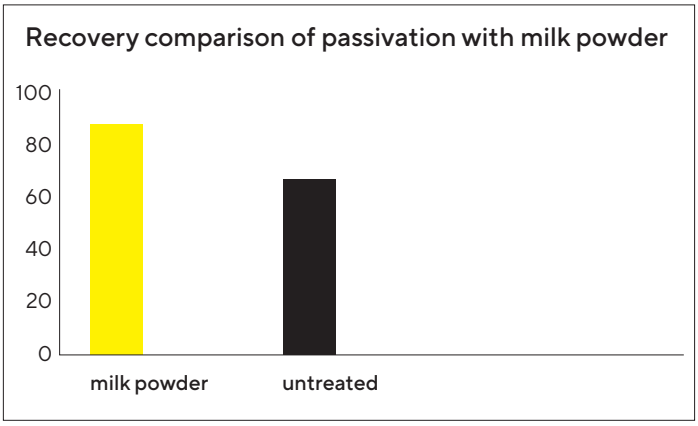

Fig. 1: Protein recovery (10 µg/mL BSA) with Vivaspin® PES 10 kDa after passivation

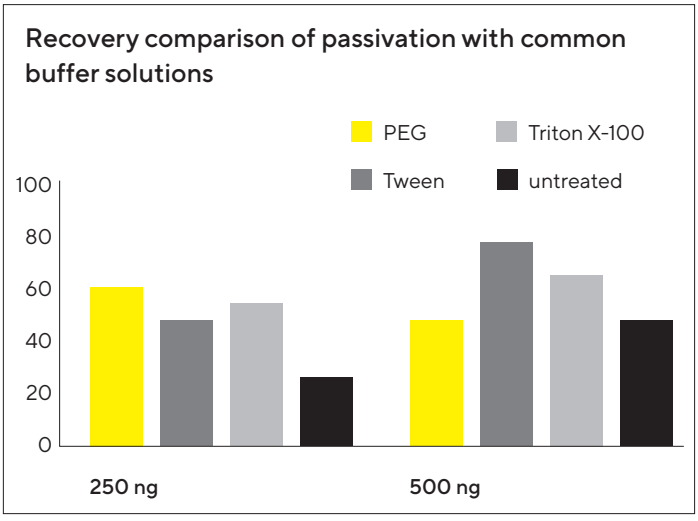

Fig. 2: Protein recovery (250 and 500 ng BSA) with Vivaspin® 2 PES 10 kDa after passivation

Summary

Passivation is an appropriate method to achieve increasing sample recovery when using very dilute samples. In addition to skimmed milk, other proteins (BSA), detergents and compounds are possible. However, it should be noted that this is a general procedure, not specific for any particular application. Depending on the hydrophilic | -phobic character of the protein non-specific binding may be more or less of a problem and the suggested passivation solutions may lead to different results. Even with the Hydrosart membrane, which is recommended for dilute samples, passivation of the device will reduce losses on the plastic surface. One very important thing to remember is that the blocking agent is potentially introduced into the sample. It should be assured that this will not interfere with downstream analysis.

For example, proteins must not be used for passivation if a pure protein is intended to be concentrated for x-ray crystallography, as even the smallest traces would interfere with the diffraction pattern. Other subsequent analyses methods include activity testing, gel electrophoresis or labelling are less problematic.

Additional application notes can be found on:
https://www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
www.sartorius.com

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Application Note

08 May 2018

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mammalian cells, mAbs, clarification, diatomaceous earth, centrifugation, protein purification

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

James Stephenson, Catherine Bladen and Ian Wilkinson*
Absolute Antibody Ltd, Wilton Centre, Redcar, TS10 4RF, United Kingdom
www.absoluteantibody.com

* Correspondence
E-mail: wilkinson@absoluteantibody.com, Tel: +44.1865.920810

Abstract

Monoclonal antibody expression systems typically utilise a signal peptide to ensure secretion of the antibody into the cell culture media. Although this reduces the complexity of purification and avoids the need for cell disruption, it does require the use of expensive and/or time-consuming techniques to separate cells from antibody-containing cell culture fluid. In this study, we describe our tests of Sartoclear Dynamics® Lab V, a novel system for rapid clarification of cell culture media without the need for centrifugation or any other costly equipment.

Find out more: www.sartorius.com/sartoclear-dynamics-lab

Introduction

Monoclonal antibodies are used in a wide range of applications including biopharmaceutical development, basic research and in vitro diagnostics. The production of antibodies from mammalian cells, whether it be standard production using hybridomas or recombinant production in Chinese Hamster Ovary (CHO) or Human Embryonic Kidney (HEK) cell lines, requires a process to separate the cells from the antibody-containing cell culture fluid. At bioreactor scale, typically above 50 litres, this is usually done by processes such as continuous centrifugation and depth filtration.[1,2]

However, at research scale, which is typically less than 10 litres of cell culture per antibody, these processes become both impractical and expensive. The most widely cited method for research scale clarification is an initial centrifugation step followed by filtration of the cleared supernatant through a 0.2, 0.22 or 0.45 µm filter, either using a hand-operated syringe or a vacuum driven bottle-top filter. These are inexpensive and easy to use with standard equipment present in most biotechnology laboratories. However, due to the shallow design of these filters, they are prone to clogging due to the presence of submicron particles remaining in suspension.[2]

At Absolute Antibody, we transiently express recombinant antibodies in HEK293 and CHO-K1 cells, typically working with approximately 50 antibodies per week from a scale of 30 mL up to 20 litres with a total weekly capacity of approximately 100 litres of cells. For the last six years, we have been using centrifugation followed by bottle-top filtration for all our antibodies. As our capacity has grown, so has the need for an increased number of centrifuges. Over this time, we have looked at a number of alternative options, including the use of filters designed for home brewing and flocculants such as Chitosan.[3] These approaches proved to be slow, prone to clogging and low throughput and to be of low quality (e.g. endotoxin contaminated).

The Sartoclear Dynamics® Lab V filtration system was designed to reduce the time and effort involved in clarifying mammalian cell cultures. The addition of diatomaceous earth (DE) to cultures supports the formation of a porous filter cake to prevent blockage of the filter, allowing rapid removal of cells and cell debris from the sample (Figure 1). This avoids the need for a centrifugation step, circumventing issues around centrifuge capacity and availability as well as preventing filters from clogging. We tested the Sartoclear Dynamics® Lab V filtration system and compared it with our standard process.

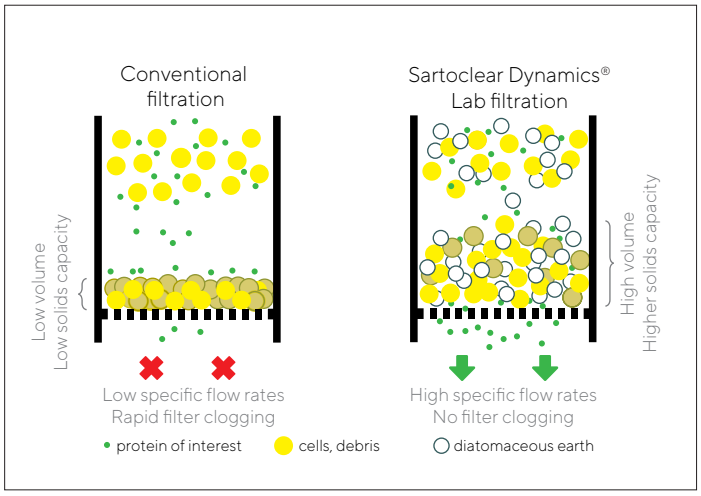

Figure 1: Principles of clarifying cell cultures using conventional filtration and Sartoclear Dynamics® Lab filtration.

Materials and Methods

Suspension adapted HEK293 or CHO cells are grown in serum-free media and transfected with DNA plasmids encoding the heavy chain and light chain of a monoclonal antibody. The cells are harvested for purification 6 to 14 days post-transfection. For a typical expression batch of 1 litre, our original clarification process would involve centrifugation at 3,500 g in a bench top centrifuge (2 × 500 mL) for 45 minutes followed by filtration using one or more vacuum-driven PES 0.45 µm bottle-top filters. Filtered supernatant is then loaded onto an ÄKTA purifier with a 5 mL Protein A column for antibody capture and elution on low pH buffer. Antibody is then neutralised and proceeds either to additional purification steps (e.g. cation exchange or size exclusion chromatography) or directly to quality control depending on the requirements for the particular antibody batch. Quality control is performed by SDS-PAGE under non-reducing and reducing conditions, SEC-HPLC, endotoxin testing and, where required, an ELISA to measure binding activity (Figure 2).

In the modified downstream process, the centrifugation and filtration steps are replaced with the use of Sartoclear Dynamics® Lab V. In the case of a 1-litre culture, 20 g of DE filter aid were added to 1 litre of cells. The cells and DE are mixed vigorously and then added directly to a 1,000 mL PES 0.22 µm Sartolab® RF vacuum-driven filter included in the Sartoclear Dynamics® Lab kit. A vacuum is applied and the clarified cell culture fluid collected in the 1 litre bottle. The filtrate is then taken through the purification and QC process as described above.

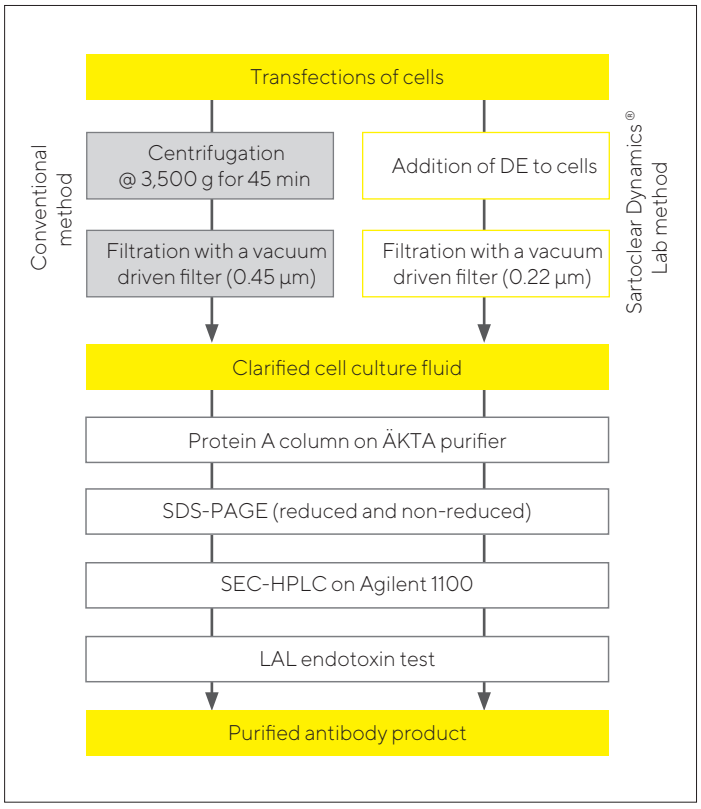

Figure 2: Downstream processing workflow of recombinant antibody production at Absolute Antibody. Grey boxes show the original process of clarification using centrifugation and filtration. The boxes with yellow outlines show the new work flow using Sartoclear Dynamics® Lab V.

Results and Discussion

To benchmark the Sartoclear Dynamics® Lab V against our standard process, a two-litre culture of a human IgG1 anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) was prepared in HEK293 cells. Six days post-transfection the culture was split into two equal volumes. At the point of harvesting, the cell density was 2.4×10^6 cells/mL with a viability of 65%.

The first litre was taken through our conventional process. This involved a 45-minute centrifugation step followed by filtration using three 0.45 µm PES 500 mL bottle-top filters. These filters typically block after about 400 mL of supernatant has been filtered, meaning on average three filters are required per litre of cell culture. Filters with a pore size of 0.45 µm are used rather than 0.22 µm to increase the volume of supernatant that can be filtered prior to blockage. Filtering of 1 litre of cells took 9 minutes, 48 seconds, of hands-on time, which added up to an overallprocess time of approximately 55 minutes.

The second litre was taken through the Sartoclear Dynamics® Lab V process. Two 10-gram pouches of DE were added to the cells, followed by vigorous mixing. The cells were then poured into a 1,000 mL 0.22 µm Sartolab® RF filter and a vacuum was applied. The filtration ran to completion with no blockage and took a total of 8 minutes, 27 seconds, from applying the DE to completion of filtering. This represents approximately 15% of the time taken by our conventional approach, as illustrated in Figure 3.

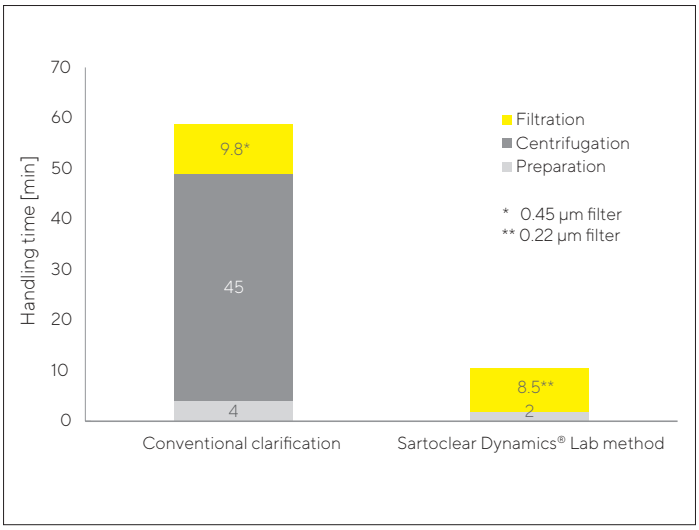

Figure 3: Comparison of clarification methods by handling time. Each 1 litre of cell culture with a density of 2.4×10^6 cells/mL was clarified with the conventional method and with the Sartoclear Dynamics® Lab method. Sartoclear Dynamics® Lab eliminates the need for a centrifugation step and substantially reduces the time required for clarification of mammalian cell cultures.

To confirm that the Sartoclear Dynamics® Lab V process had no effect on the quality of the antibody, the two samples proceeded through purification and quality control separately. The final purified antibodies showed no detectable differences in product quality as determined by SDS-PAGE (Figure 4) or SEC-HPLC (Figure 5). An endotoxin measurement was taken for each sample, with both giving a reading of < 0.05 EU/mg, which is the lower limit of detection of the testing kit we routinely use.

To confirm that the modified process had no effect on the function of the antibody, an indirect ELISA was performed to show binding to human EGFR-Fc (Absolute Antibody catalogue number Pr00117-10.9). As shown in Figure 6, the method of cell clarification had no impact on binding activity. Additionally, the final yields obtained by both processes were almost identical, showing that DE has no impact on both quality and quantity of antibody.

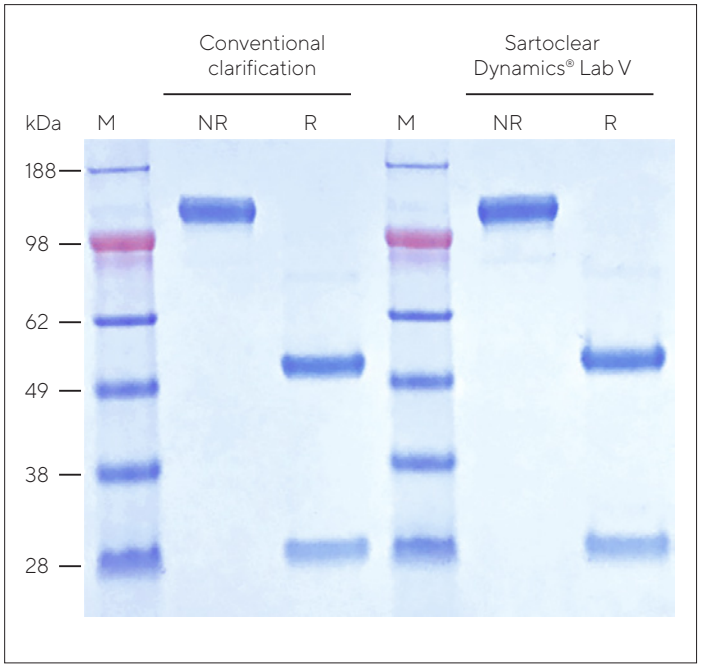

Figure 4: SDS-PAGE gel image (NR – non-reducing, R – reducing, M – markers) of Protein A purified anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) following conventional clarification by centrifugation and filtering and clarification using Sartoclear Dynamics® Lab V.

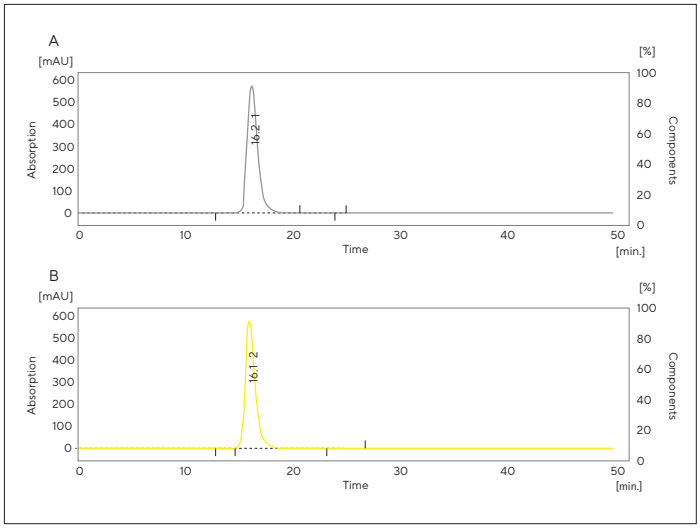

Figure 5: SEC-HPLC of Protein A purified anti-EGFR antibody (Cetuximab; Absolute Antibody catalogue number Ab00279-10.0) following conventional clarification by centrifugation and filtering (A) and following clarification using Sartoclear Dynamics® Lab V (B). Both samples show identical profiles.

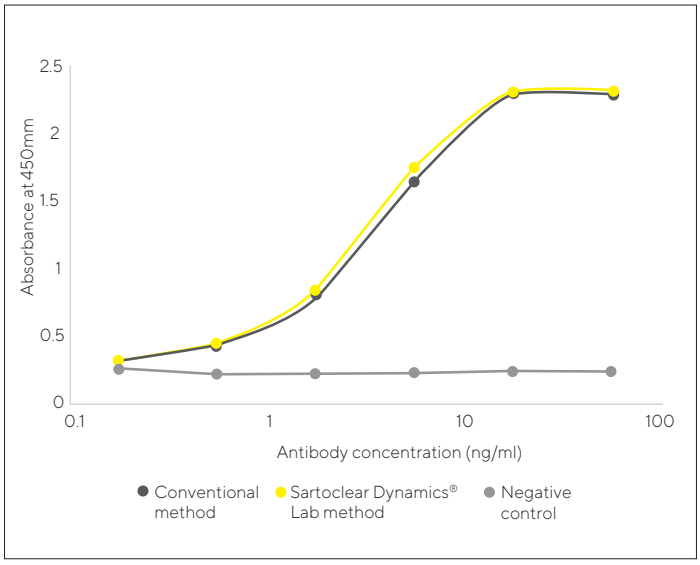

Figure 6: Indirect ELISA showing binding of antibodies to EGFR-Fc. Anti-EGFR antibodies (Cetuximab) were purified by our conventional process and utilising Sartoclear Dynamics® Lab V. There is essentially no difference in binding activity.

Conclusion

We have clearly demonstrated here that Sartoclear Dynamics® Lab V enables rapid clarification of mammalian cell cultures without the need for centrifugation. We have done this by taking two litres of cell culture transiently transfected with an antibody and comparing a standard centrifugation-based clarification process with the diatomaceous earth-based Sartoclear Dynamics® Lab V process. We were unable to detect any meaningful differences in the final product quantity or quality as determined by a host of measurements (SDS-PAGE, SEC-HPLC, ELISA and endotoxin), demonstrating that the diatomaceous earth-based system has no impact on product quality. Importantly, the Sartoclear Dynamics® Lab V process gave an impressive time saving of approximately 85%. This makes Sartoclear Dynamics® Lab V an attractive option to increase productivity and throughput for the clarification step of secreted protein expression and purification systems.

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Abbreviations

DE	Diatomaceous Earth
PES	Polyethersulfone
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SEC-HPLC	Size Exclusion Chromatography-High Performance Liquid Chromatography
ELISA	Enzyme-linked Immunosorbent Assay
EGFR	Epidermal Growth Factor Receptor

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen, Germany
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

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Application Note

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Keywords or phrases:
mammalian cell culture, clarification, diatomaceous earth, affinity chromatography

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

Dennis Karthaus*, Sarah Ludwig, Isabel Schuchardt, Stefanie Krame
IBA GmbH, Rudolf-Wissell-Str. 28, 37079 Göttingen, Germany
www.iba-lifesciences.com

* Correspondence
E-Mail: karthaus@iba-lifesciences.com

Abstract

In this study, the novel Sartoclear Dynamics® Lab V Kit was evaluated for the removal of transiently IgG expressing mammalian MEXi-293E (HEK293) cells from cell cultures. The method was directly compared to the present standard method that required two centrifugation steps. After clarification, recombinant IgG harboring a Twin-Strep-tag® was purified from all samples in parallel by a one-step Strep-Tactin®XT Superflow® high capacity affinity purification process.

Overall, the use of Sartoclear Dynamics® Lab significantly reduced the time for sample clarification by up to 3.6-fold while maintaining total protein yield and quality. Moreover, Sartoclear Dynamics® Lab can be integrated easily into already existing lab processes, substantially decreasing hands-on time and thereby simplifying sample preparation.

Find out more: www.sartorius.com/sartoclear-dynamics-lab

Introduction

The relevance of recombinant proteins produced in mammalian cell lines has increased over the past few years, especially for therapeutic applications. One of the major goals in this field is efficient production of highly pure proteins. Key factors driving this efficiency are the expression system, the purification platform and the purification protocol itself. A transient expression system enables rapid production of proteins in milligram quantities and is the system of choice for obtaining recombinant proteins for research purposes.

Affinity tags are a commonly used and powerful tool for the purification of recombinant proteins. Genetically attached to the protein of interest, they simplify the purification process considerably by using the same strategy simultaneously for many different proteins. The high binding affinity of the Twin-Strep-tag® to Strep-Tactin®XT Superflow® high capacity makes it a powerful tool for nearly all downstream applications, particularly in combination with efficient, one-step purification. This facilitates the overall protein production process and makes the Strep-tag® system a highly attractive platform.

Sample preparation can be a time-consuming procedure. In particular, centrifugation for separating mammalian cells is a tedious step. Therefore, we compared a different method using the novel Sartoclear Dynamics® system with a standard centrifugation procedure as part of preparation of a transiently mammalian expressed Twin-Strep-tagged IgG.

Sartoclear Dynamics® Lab products are based on the principle of dynamic body feed filtration: Diatomaceous earth (DE) used as a filter aid is added to cell culture broth to sieve out cells and cell debris, creating a permeable filter cake that prevents blockage of the final filter. Sartoclear Dynamics® Lab eliminates the need for the individual steps of centrifugation and filtration, thus saving significant time and resources.

Materials and Methods

A Twin-Strep-tag® fused IgG was transiently expressed in a mammalian HEK-293 expression system. Three liters of MEXi-293E suspension cell culture (IBA GmbH, 2-6001-010) were transfected with the IgG encoding plasmid according to the manufacturer's protocol. Cells were cultured at 5% CO_2, 37°C and 125 rpm. Two days after transfection, the temperature was reduced to 32°C. After 16 days, the cell suspension was divided into three identical 1,000 mL aliquots. In order to challenge the clarification and purification process, cells were cultivated until significant cell death occurred.

One aliquot was subjected to the standard method for cell removal in two consecutive centrifugation steps. The first centrifugation was conducted at 300 × g for 10 min. at 4°C to remove cells gently, without any risk of impairment due to shear forces. The supernatant was centrifuged again at 10,000 × g for 20 min. at 4°C to remove cell debris and the remaining cells for subsequent affinity column purification.

The remaining two aliquots were processed using the Sartoclear Dynamics® system (Sartorius, SDLV-1000-40C-E). Diatomaceous earth in a quantity of 40 g or 60 g, respectively, was added, and each solution was mixed to obtain a homogenous suspensionbefore being passed through the 0.22 µm PES sterile filter.

Buffer W (IBA GmbH, 2-1003-100) and BioLock solution (IBA GmbH, 2-0205-050) were added as recommended by the manufacturer in order to prepare samples for protein purification. IgG was purified using a Strep-Tactin®XT Superflow® high capacity via gravity flow (IBA GmbH, 2-4030-010). Samples were loaded on the columns using Wet Fred devices (IBA GmbH, 2-0910-001), which allow semi-automated parallel purification of large sample volumes. After the samples were applied, columns were washed with five column volumes of Buffer W (IBA GmbH, 2-1003-100). The protein was eluted under physiological conditions by adding three column volumes of Buffer BXT (IBA GmbH, 2-1042-025). Protein concentration was measured on a NanoDrop™ photometer at 280 nm.

After purification, the samples (centrifuged supernatant, filtered supernatant, supernatant with Buffer W and BioLock solution, elution fractions) were analyzed under reducing conditions on an SDS-PAGE gel. In addition, the samples were blotted onto a nitrocellulose membrane, and the Twin-Strep-tagged IgG heavy chain was detected with Strep-Tactin®-HRP conjugate (IBA GmbH, 2-1502-001) according to the manufacturer's protocol.

Finally, elution fractions from each cell removal experiment were analyzed by size exclusion chromatography (SEC) on an Äkta™ purifier system using a Superdex 200 Increase 3.2/300 column (GE Healthcare, 28990946).

Results and Discussion

Viable peak cell density was 1.5 × 10^7 cells/mL, and viability was 95% after 13 days of cultivation. Sixteen days after transfection, viable cell density and viability decreased to 6.8 × 10^6 cells/mL and 63%, respectively. Hence, many dead cells and substantial cell debris were in the culture, hampering the cell removal step.

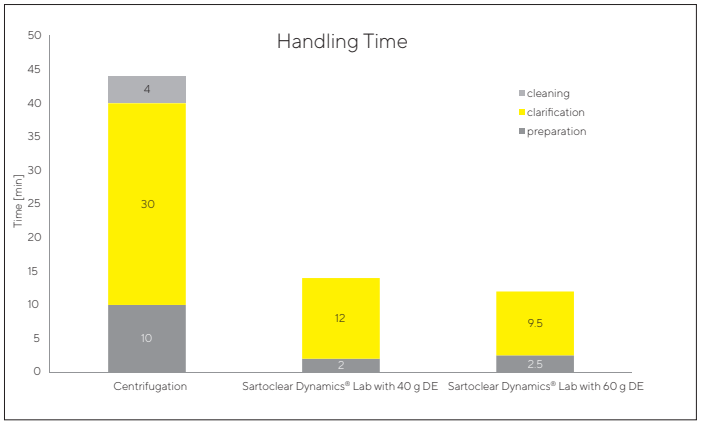
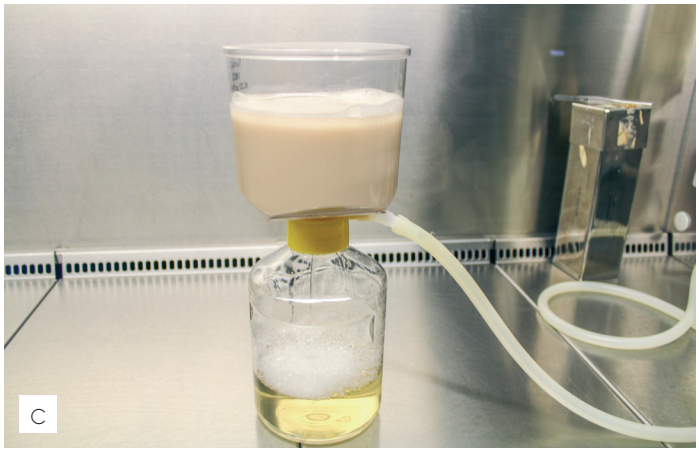


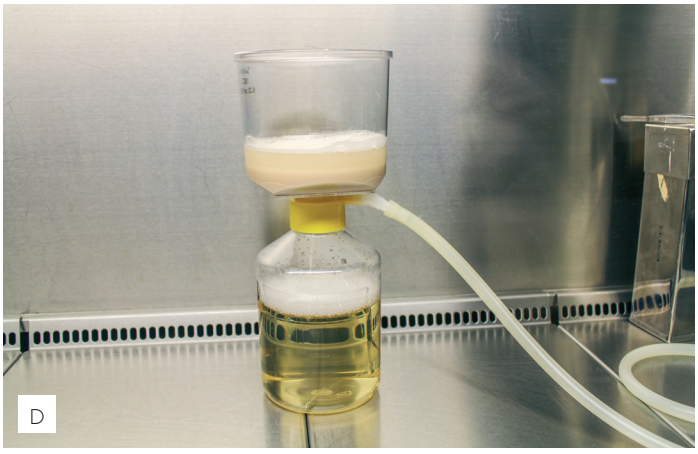
Figure 1: Comparison of clarification methods by handling time. One liter of HEK293 cell culture each was clarified by the standard process (centrifugation) and by Sartoclear Dynamics® Lab with 40 g and 60 g filter aid, resp. Sartoclear Dynamics® Lab significantly reduces the time needed for clarification of HEK293 cell culture media.

Handling times for sample preparation and cell removal for both methods were directly compared (Figure 1). In the case of the standard centrifugation-based method, the sample preparation time included balancing the weight of the centrifuge tubes and removal of the supernatant. The sample preparation time for the Sartoclear Dynamics® system included the addition of DE to the cell suspension.

Hands-on time for cell removal by centrifugation was up to 5-fold higher compared with the sample preparation time needed for the Sartoclear Dynamics® system. In addition, Sartoclear Dynamics® Lab decreased the time required for cell removal by 2.5-fold (with 40 g DE) and 3.1-fold (with 60 g DE). The complete Sartoclear Dynamics® Lab procedure took only 12 min. and 14 min., respectively. In contrast, the full sample preparation and cell removal time for centrifugation culminated in 44 min. of overall processing time. In summary, by using Sartoclear Dynamics® Lab with DE for sample preparation, the total processing time was reduced by up to 3.6-fold.

It is worth mentioning that the centrifuged sample was not sterile. This was not an issue in the present study because column purification was started immediately after clarification under non-sterile conditions. However, if the harvested supernatant needs to be sterile, an additional





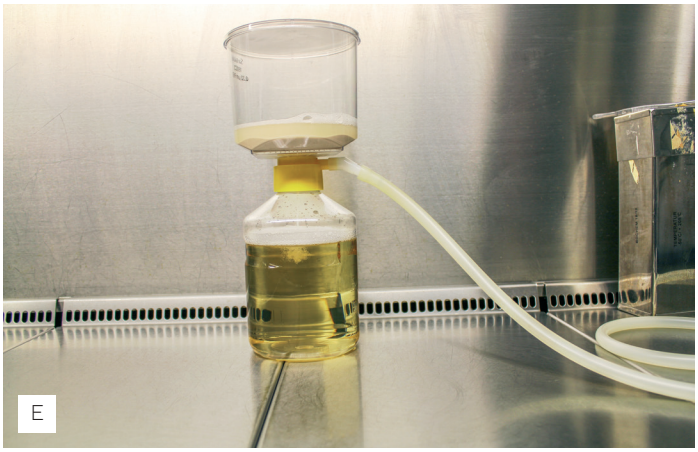


Figure 2: Pouring in and clarification of mammalian cell culture supernatant with 40 g of filter aid using the filter unit Sartolab® RF included in the Sartoclear Dynamics® Lab kit (A – F). A tube is connected to a vacuum pump.

filtration step must be performed after centrifugation. This is not the case for the Sartoclear Dynamics® Lab procedure as all samples are automatically sterile-filtered during clarification.

Besides reducing process time, the Sartoclear Dynamics® system is advantageous with regard to its scalability. There is no need for any instrumentation, except for a vacuum pump, to use the Sartoclear Dynamics® system (Figure 2). In contrast, the conventional centrifugation method requires a centrifuge and a rotor to accommodate tubes with a sufficient volume.

The protein yield was determined after affinity column purification to exclude the possibility that unspecific binding of IgG to the components might occur in the Sartoclear Dynamics® system. The resulting IgG yields of the centrifuged sample and Sartoclear Dynamics® Lab processed samples were comparable (Figure 3), ruling out any negative effect of Sartoclear Dynamics® Lab on the protein yield.

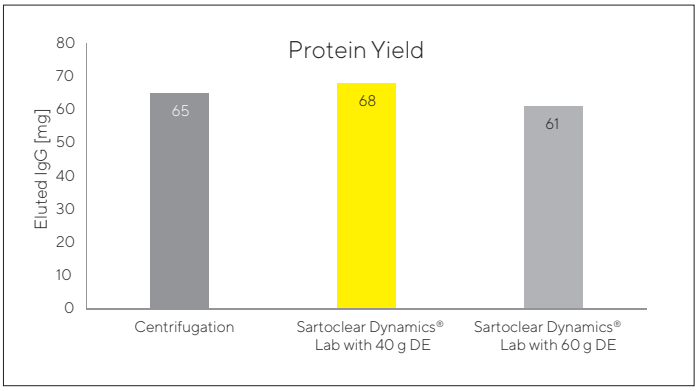


Figure 3: Comparison of clarification methods by protein yield. Comparable mAb yields were purified independently of the clarification method used.

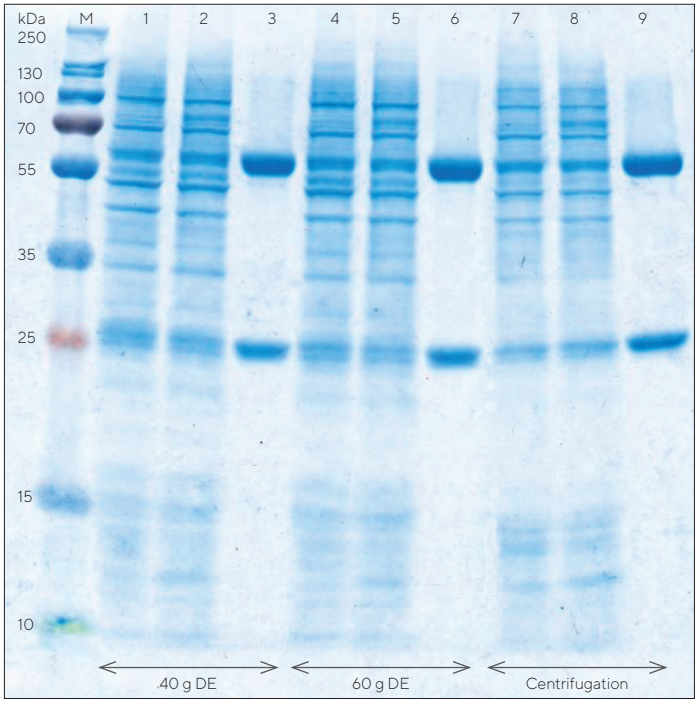


Figure 4: SDS-PAGE analysis of cell culture supernatants clarified by centrifugation and Sartoclear Dynamics® Lab, plus elution fractions after purification. Protein purity was 100% in all elution fractions, independently of the clarification method used. Samples 1, 4 and 7) supernatant; 2, 5 and 8) supernatant with BioLock and Buffer W; 3, 6 and 9) elution.

To test the effect of the Sartoclear Dynamics® system on product quality, samples were analyzed on SDS-PAGE (Figure 4) and by SEC analysis (Figure 6). Bands corresponding to the heavy and light chains of the IgG were present in the elution fractions of all samples, and no difference was observed between the centrifuged sample and samples clarified by Sartoclear Dynamics® Lab. The Twin-Strep-tag® heavy chain was detected in all samples by Western blot analysis (Figure 5), confirming the identity of the protein of interest. Moreover, the results of the SDS-PAGE analysis showed the efficiency of the

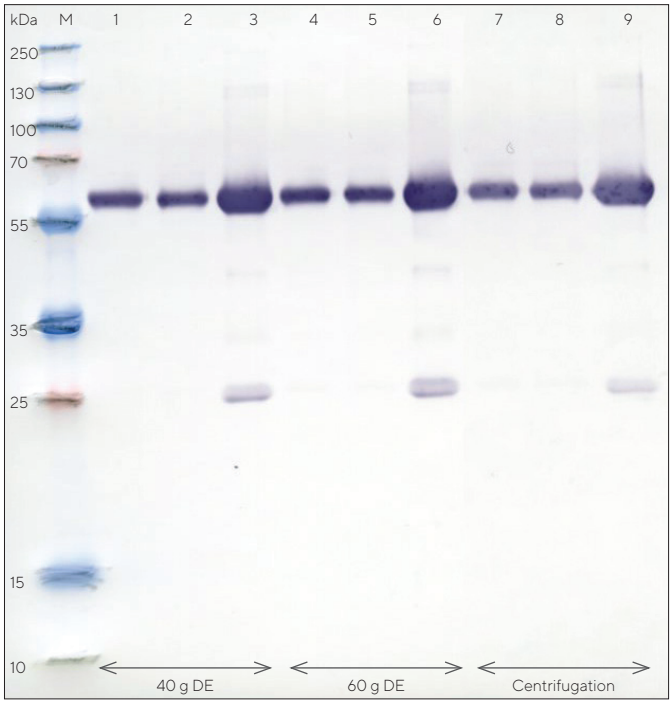


Figure 5: Western Blot analysis of cell culture supernatants clarified by centrifugation and Sartoclear Dynamics® Lab, plus elution fractions after purification. The Twin-Strep-tag® of the mAb's heavy chain was detected by the Strep-Tactin®-HRP conjugate. Samples 1, 4 and 7) supernatant; 2, 5 and 8) supernatant with BioLock and Buffer W; 3, 6 and 9) elution.

Twin-Strep-tag®:Strep-Tactin®XT Superflow® high capacity purification from mammalian cell supernatant in a simple, one-step purification process.

Results of SEC analysis revealed that regardless of the sample preparation method chosen, there was no effect on the aggregation rate (25%) of IgG. The main peak (75%) corresponds to monomeric IgG. It can be assumed that the high aggregation rate resulted from the low cell viability of the culture at the point of harvest. Manufacturers' protocols recommend harvesting cells before viability drops below 75%.

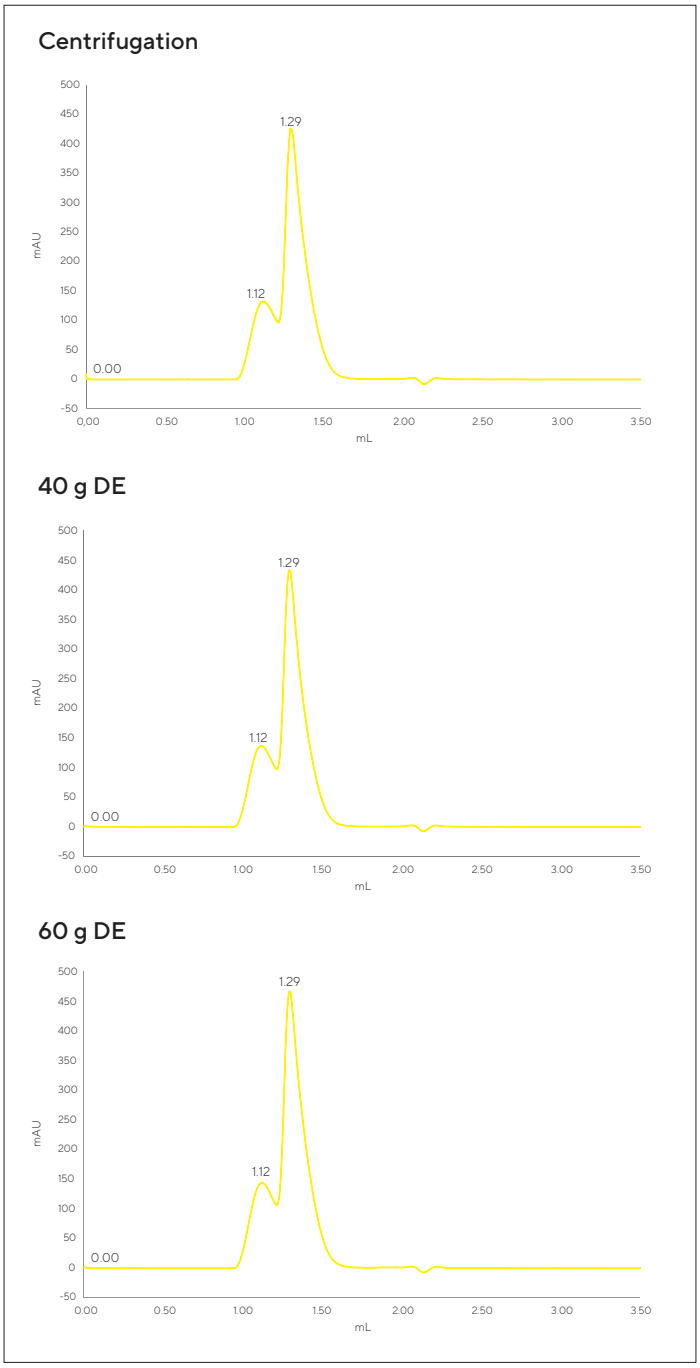


Figure 6: Size exclusion chromatograms of eluted mAb after purification from cell culture supernatants clarified by centrifugation, Sartoclear Dynamics® Lab with 40 g filter aid and 60 g filter aid, respectively. The clarification method did not have any influence on the mAb aggregation level. Peak 1.12 represents aggregated mAb (25%) and band peak 1.29 monomeric mAb (75%).

Conclusion

In summary, the efficient transient expression in the MEXi-293E (HEK293) cell line combined with simple, one-step Strep-Tactin®XT Superflow® high capacity purification generates more than 60 mg of highly pure IgG per liter of cell culture. The use of Sartoclear Dynamics® Lab significantly reduced the cell removal time compared with the standard centrifugation-based process, without affecting protein yield or product quality.

Abbreviations

HEK	Human embryonic kidney
PES	Polyethersulfone
DE	Diatomaceous earth
SEC	Size exclusion chromatography

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

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Application Note

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Keywords or phrases:
Syringe Filter, Protein Adsorption, mAb, RFP, RuBisCo, Design of Experiments (DoE)

How to Increase Recovery at Critical Protein Samples: Impact of Syringe Filter Membrane, Volume and pH

A. Croon, J. F. Buyel
Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany

Corresponding author
Tel.: +49 241 6085 13162
E-Mail address: johannes.buyel@ime.fraunhofer.de

Abstract

Protein loss during sample preparation can be an obstacle to reliable product quantitation in biological, biotechnological and biopharmaceutical settings. We compared four membranes typically used as part of syringe filters for sample preparation. In a design of experiments approach we quantified the recovery of four model proteins under different sample conditions and found that membranes composed of cellulose acetate or polyethersulfone adsorbed on average less than 5% of protein analyte. Even when only 0.5 mL sample with 0.01 g L^{-1} protein was filtered, the recovery was ~90% with these membranes. In contrast, nylon or polyvinylidene difluoride-based membranes exhibited adsorption of more than 30% of product under these conditions. Furthermore, adsorption was dependent on sample properties like pH which can facilitate a fine tuning of the sample conditions to improve product recovery during preparation.

Find out more: sartorius.com/en/products/lab-filtration-purification/syringe-filters

Introduction

Biopharmaceutical samples are often prepared from feedstocks containing insoluble particles like cell debris or protein aggregates and therefore require a solid-liquid separation before analysis to protect analytical instruments. Because separation by centrifugation requires a difference in density between solid and liquid phase, sample filtration can be advantageous and membrane filters offer absolute particle retention. However, filter membranes can adsorb analytes like proteins and thereby distort the results of the subsequent analyses. It is therefore important to select filter membranes with a minimal tendency to protein adsorption. But the latter does not only depend on the membrane type, yet is also affected by the sample and protein properties, like pH and surface charge respectively, as well as the specific handling steps including sample volume per unit filter area. Identifying conditions suitable to achieve minimal analyte loss can thus be a complex multi parameter problem with a work load that would be prohibitively high, especially for early development and screening approaches. We have therefore selected four typical syringe filter membranes and quantified the recovery of four model proteins including two different antibodies under various sample conditions representative for many biological, biotechnological and biopharmaceutical applications. The design of experiments (DoE) approach we used may provide guidance as to which conditions and membranes can help to minimize analyte loss during sample preparation.

Materials and Methods

Four model proteins were used to study protein adsorption to filter membranes (Table 1).

A split-plot I-optimal design with 120 runs containing four numerical and two categorical factors (Table 2) was set up to investigate protein binding to different membranes of syringe filters by a mixed linear-quadratic model. The numerical factor levels were selected based on typical sample conditions, for example in-process-controls during biopharmaceutical production. Proteins were dissolved in phosphate buffer (10 mmol L^{-1}, pH 5.5 or pH 7.5) containing 140 mmol L^{-1} (15 mS cm^{-1}) or 550 mmol L^{-1} (50 mS cm^{-1}) of sodium chloride according to the DoE approach. Sample preparation was carried out in glass containers and protein solutions were loaded to membrane filters using polypropylene syringes. Filtrates were collected in glass containers and filtration was performed at 22° C.

Table 1: Model proteins used for filter membrane testing

Protein name [-]	Protein type [-]	Molecular mass (monomer) [kDa]	Isoelectric point (pI) [-]	Oligomeric state	Purity [-]
DsRed	Red fluorescent protein (RFP)	27.15	7.4	4	0.84
Adalimumab	Monoclonal antibody (mAb1)	145.4	8.4	1[c]	>0.97
M12	Monoclonal antibody (mAb2)	144.8	7.9	1[c]	>0.97
RuBisCO[a]	Enzyme	52.9/20.3[b]	6.6	16[d]	0.92

a. Ribulose-1,5-bisphosphate carboxylase/oxygenase; b. values for large and small subunit respectively; c. composed of two heavy and two covalently linked heavy and light chains; d. composed of 8 small and 8 large subunits that are non-covalently attached.

Results and Discussion

RFP was diluted in 0.9% m/v sodium chloride and quantified by fluorescence spectroscopy with excitation at 559 nm and emission at 585 nm in black 96-well plates with a 7 mm measurement height and 50 flashes per sample using an EnSpire (Perkin Elmer) multimode plate reader. RuBisCO containing 10-µL samples were analyzed at 220 nm by ultra-high performance size exclusion chromatography (UHPSEC) using an Ultimate 3000 (Thermo Fischer Scientific). Proteins were separated isocratically on an Acquity UPLC Protein BEH SEC Column, 20 nm, 1.7 µm, 4.6 × 150 mm with 50 mmol L^{-1} sodium dihydrogen phosphate, 250 mmol L^{-1} sodium chloride, pH 6.8 at a column temperature of 30° C and a flow rate of 0.2 mL min^{-1}.

Monoclonal antibody samples of M12 and Adalimumab were analyzed by surface plasmon resonance (SPR) spectroscopy using a Biacore T200 (GE Healthcare). Samples were diluted and analyzed in 0.01 mol L^{-1} HEPES, 0.15 mol L^{-1} sodium chloride, 3 mmol L^{-1} EDTA and 0.005% v/v polysorbate-20 and loaded to a Protein A functionalized chip surface at 22° C with 0.03 mL min^{-1} and a contact time of 180 s. Injections of 45 µL 0.03 mol L^{-1} hydrochloric acid were used for surface regeneration.

A statistical experimental design (DoE) was used to quantify the binding of four model proteins to four different types of syringe filter membranes (all with a pore size of 0.2 µm), frequently used for sample preparation, for example in the context of in-process controls. The highest protein recovery of >98% was observed for a cellulose acetate (CA) membrane (Minisart® NML, Table 3) which was insignificantly higher than the average recovery achieved with a polyethersulfon (PES) membrane (Minisart® High Flow) (two-sided t-test with 0.05 alpha level). Also, both membranes exhibited a 3 to 8-fold lower standard deviation compared to a nylon or a polyvinylidene difluoride membrane, indicating that high recoveries were achieved with these membranes even for varying sample conditions and target proteins (Table 2).

When analyzing the DoE, sample volume and especially protein concentration had the strongest effects on protein recovery and the latter increased with higher concentrations and volumes (Figure 1). These observations were in good agreement with a saturation model for protein adsorption to surfaces, for example a Langmuir model. In such a model, a given surface will bind a certain absolute quantity of protein and accordingly the (relative) recovery increases as sample volume and concentration increase. Therefore, large volumes and high concentrations can reduce the percentage of product loss during sample preparation using syringe filters.

Table 2: Summary of the DoE setup used to study protein adsorption to filter membranes

Factor	Unit	Type	Level
Conductivity	mS cm^{-1}	Numeric	15; 50
pH	-	Numeric	5.5; 7.5
Protein concentration	g L^{-1}	Numeric	0.01; 0.10; 1.00
Specific sample volume	mL cm^{-2}	Numeric	0.5; 5.0
Protein	-	Categoric	[see Table 1]
Membrane	-	Categoric	[see Table 3]

a. Ribulose-1,5-bisphosphate carboxylase/oxygenase; b. values for large and small subunit respectively; c. composed of two heavy and two covalently linked heavy and light chains; d. composed of 8 small and 8 large subunits that are non-covalently attached.

Conclusion

The membrane type had a relevant effect as well and membranes composed of CA or PES exhibited substantially less protein adsorption (>95% recovery) compared to counterparts made of nylon or polyvinylidene difluoride (PVDF), especially when exposed to low product concentrations and sample volumes (<75% recovery) (Figure 1). Importantly, the recovery achieved with CA and PES membranes was largely independent of protein, sample conditions and handling, implying that a fine tuning may not be necessary for each new product to be investigated. Therefore, CA or PES-based membranes can help to limit product loss during sample preparation for analysis if a target protein is scarce. The pH-effect was strongly protein specific. For example, no substantial pH effect was observed for mAb1 at pH 5.5 (0.01 g L^{-1}, 0.5 mL cm^{-2}) but a recovery of only ~60% was observed for RuBisCO even when Minisart® NML was used under the same conditions. However, the low recovery of RuBisCO was linked to a known low-pH instability of the protein and therefore unlikely an effect of membrane adsorption.

Whereas the conductivity did not exhibit a significant influence on recovery within the parameter space investigated in this study, a salinity below 15 mS cm^{-1} or above 50 mS cm^{-1} may cause product losses as it can affect protein solubility and may trigger protein aggregation. The resulting aggregates in turn may interact with the membrane or, depending on their size, can be sterically retained by the latter. Therefore, care should be taken if conditions outside the reported parameter space are used.

Most sample manipulation or preparation is associated with some product loss. However, analytics during process development or monitoring require that such losses are kept to a minimum so that reliable results can be obtained. Minimal product loss during sample preparation can be achieved over a wide range of conditions by selecting an adequate filter membrane. For example, ~90% of product was recovered using Minisart® NML (CA) or Minisart® High Flow (PES) filter membranes even with sample volumes and concentrations as little as 0.5 mL cm^{-2} and 0.01 g L^{-1} respectively. The product recovery may be further improved by fine tuning the sample conditions for an individual product, e.g. by selecting a proper pH value. In contrast, if protein binding is beneficial for sample preparation, nylon-based membranes such as Minisart® NY can be used instead.

Table 3: Properties of 0.2 µm pore size filters and average protein recovery after filtration in dependence of membrane type. RFP, mAb1, mAb2 and RuBisCO samples were in a 5.5–7.5 pH range, conductivities of 15 or 50 mS cm^{-1}, concentrations between 0.01 and 1.00 g L^{-1} and loadings of 0.5 or 5.0 mL sample per cm^2 membrane area

Filter name [-]	Membrane type [-]	Housing material [-]	Filter area [cm^2]	Average recovery [%][a]	n
Minisart® NML	Cellulose acetate (CA)	Methacrylate butadiene styrene (MBS)	6.2	98.4 ± 7.4	15
Minisart® High Flow	Polyethersulfon (PES)	Methacrylate butadiene styrene (MBS)	6.2	98.2 ± 5.3	18
Minisart® NY	Nylon (NY)	Polypropylene (PP)	4.8	59.7 ± 41.4	20
Standard filter	Polyvinylidene difluoride (PVDF)	Polypropylene (PP)	4.2	81.7 ± 27.4	17

a. The variability is indicated as the standard deviation.

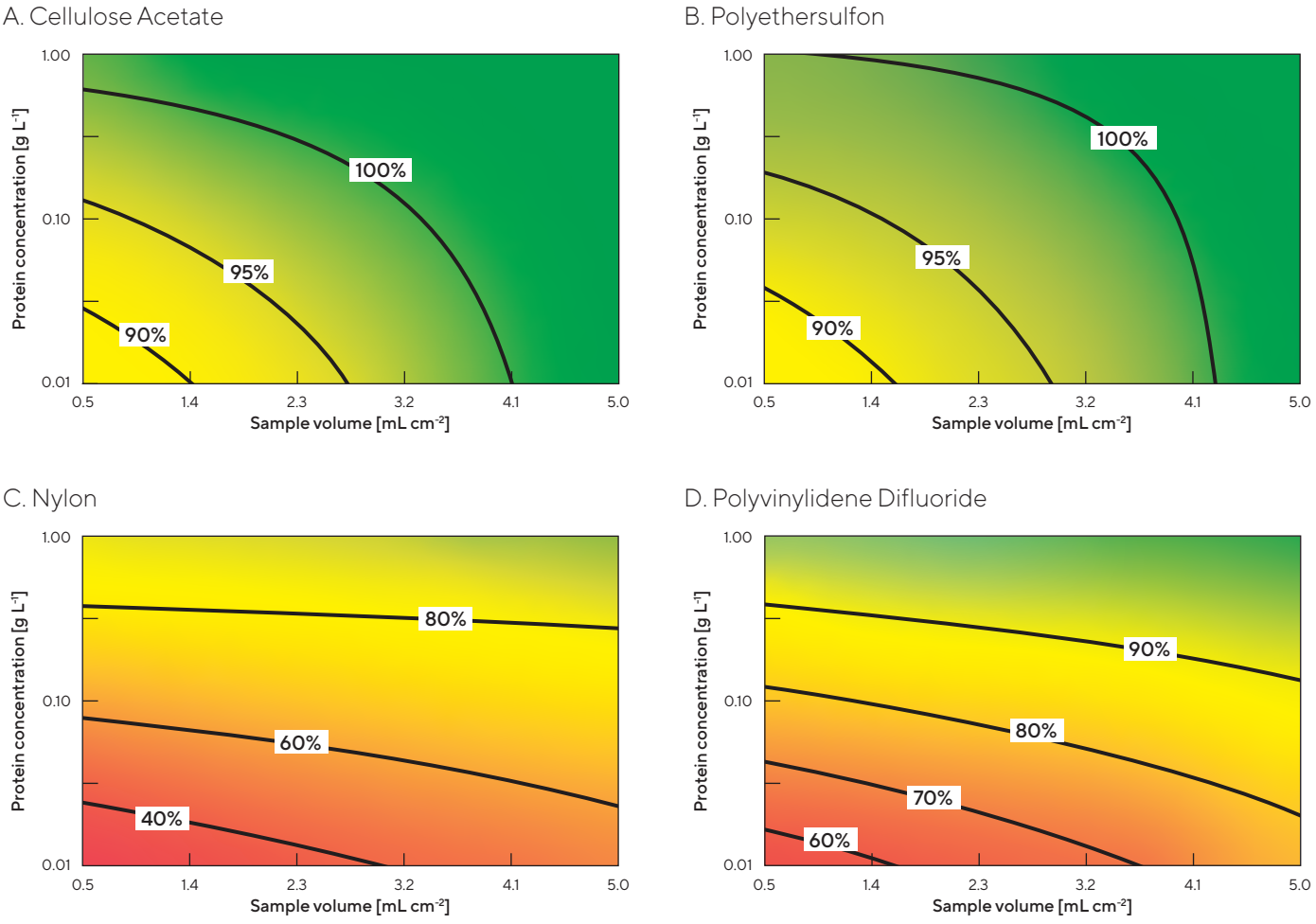

Figure 1: Average protein recovery with membrane-based syringe filters. Recovery was averaged over proteins RFP, mAb1, mAb2 and RuBisCO for a conductivity of 32.5 mS cm^{-1} at pH 6.5 using cellulose acetate (A), polyethersulfon (B), nylon (C) and polyvinylidene difluoride (D) membranes.

Acknowledgements

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Sales and Service Contacts

For further contacts, visit
www.sartorius.com

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
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Very often, the application requires weighing of very small amounts of samples into large flasks or containers. The smaller the sample quantities used, the greater the relative measuring errors become, and the larger the tare container size employed, the higher the influence of environmental conditions will be on weighing accuracy. External environmental influences or improper handling can lead to inaccurate results or poor weighing performance, which are not caused by the balance.

To ensure high accuracy during weight measurements and excellent repeatability of the results you need to observe certain basic rules and requirements.

When following the instructions and recommendations below, your balance will always provide the best weighing performance and highly reliable results.

1. Choose a Stable Weighing Table in a Quiet Place to Set Up Your Balance.

1. The table should be solid-built and, whenever possible, be made of stone or synthetic stone.

2. Avoid causing the tabletop to sag or deflect even slightly; for example, never use it to prop up your arm.

3. Set up the balance in a vibration-free location. Ensure that there are no machines or engines that generate vibrations or electromagnetic fields near the balance. Magnetism must be ruled out (e.g., tables may not be made of stainless steel).

4. Do not position the table in the middle of the room, but near a wall or, even better, in the corner of a room, as this is where the vibration amplitudes are generally at their lowest.

5. Avoid exposing your balance to sunlight and infrared radiation emitted by lamps or heaters.

6. The location may only be slightly ventilated. Exposure to drafts needs to be avoided, and the air flow rate should be below 0.2 m/s.

7. Cold air currents from air conditioners may not pass directly across or over the draft shield, as this can result in an inversion layer of air inside the draft shield. This, in turn, can cause unstable weight readouts.

2. Work in the Lab under Consistently Constant Climate Conditions.

1. Avoid significant temperature changes or spikes.

> 40%

2. Keep the relative humidity as constant as possible. Prevent the relative humidity from dropping below 40%, as this will significantly increase interference by static electricity.

°C
%
hPa

3. Use the Cubis® II climate sensor option (temperature, barometric pressure and relative humidity) to monitor climate conditions.

4. Use the Cubis® II ionizer option to eliminate electrostatic influences. Electrostatic charges on glass vessels dissipate only very slowly, particularly when these vessels have very clean surfaces, especially when they are used freshly from a laboratory glassware washer. Electrostatic influences are easy to detect by the continuous drift of weight readouts. Increase the air humidity to levels up to 60%, and use an ionizer to reduce these effects on the resulting weight readings.

3. Ensure That the Balance Is Leveled and Calibrated.

3. The Cubis® II Status Center shows all information about your balance and environmental conditions, e.g. calibration, leveling, temperature, humidity, air pressure and service, centralized in a dashboard.
In case of warnings or errors, you get detailed help and support.

isoCAL

1. All Cubis® II balances will support you in using the calibration | adjustment function isoCAL, and the Q-Level function implemented in the balance for leveling continuously maintains the accuracy of the weighing results within a narrow tolerance range.

2. Moreover, routinely check the balance using an external, certified weight.

4. During the Measuring Sequence, Ensure That ...

1. ... the vessels used are acclimatized next to your balance; i.e., have adapted to the temperature conditions in the same room.

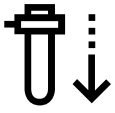
2. ... you do not touch the container with your hands when positioning it on the weighing pan or in a sample holder. Touching the sample vessel with your hand usually increases the temperature of the vessel. Buoyancy and air current effects influence weighing results. Remember that it takes ten minutes for these effects to subside. Use a pair of tweezers or forceps to position the vessel.

3. Avoid placing your hand inside the draft shield to ensure that no unnecessary interchange of air outside and inside the draft shield takes place and that no heat is transferred into the draft shield.

4. Avoid touching a vessel with your bare fingers at all times, as a single fingerprint can weigh up to 50 µg and therefore have a major impact on the accuracy of your weight measurement result.

5. When weighing, ensure that no powder falls onto the weighing pan next to the vessel, as this will mean that the displayed sample weight is not what is actually in the vessel.

6. Avoid the complete interchange of air when opening the draft shield by opening only one door, where possible. Optimal to use the draft shield learning capability to open the door only as far as actually necessary.



7. Carefully place the tare container on the weighing pan or in the sample holder. Avoid applying any excessive force.

8. Do not lean on or against the weighing table or rest your arm on it during the weighing procedure.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

Preparation of standards, also called reference samples, of known concentrations is a common routine procedure in analytical laboratories. Internal or external standards with very low concentrations are used in these laboratories for highly sensitive quantitative analytical methods to exactly determine the concentration of chemical components in samples using highly sensitive quantitative analytical procedures.

Standard Preparation

External standards are separate samples used for comparison to test samples, whereas internal standards are added to the samples to be analyzed. However, all standards have a defined concentration of one or several known component(s). The concentration of these standards must be as accurate as possible to prevent subsequent errors in determining unknown concentrations in samples.

Also the preparation of standards is routine work in analytical laboratories two problems can occur when standards are manually prepared from soluble solids:

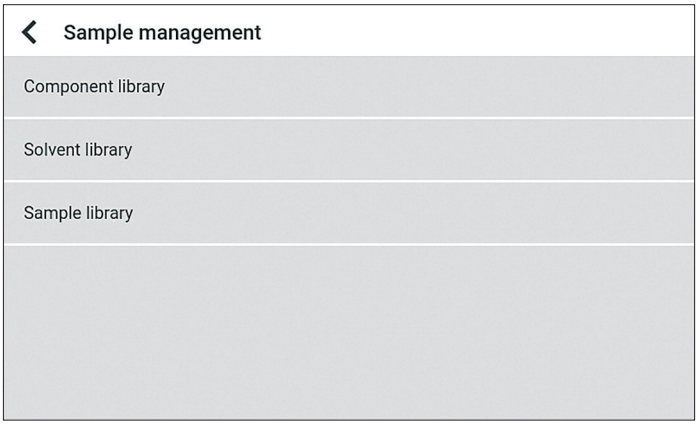
1. The required weight of the soluble component(s) is calculated based on the desired final component(s) concentration and the final solution volume. The decisive problem is the weighing process of the solid component(s). Normally, high-resolution laboratory balances with an accuracy of several decimal places are used to measure the exact weight and it is almost impossible to reach the target weight exactly to the last digit during weighing in a component. Most often the measured component weight exceeds the target weight because users don't want to weigh in less than required but don't hit exactly the target weight. Especially when preparing mixed standards it is not possible to remove excessive material from the vessel without impairing the component(s) final concentration(s).

2. If the component weight does not precisely equal the calculated weight, the volume of solvent has to be adjusted to reach the desired final concentration. Recalculation of the required solvent volume is time consuming and is a possible source of error as many factors must be considered. Depending on the type of concentration specified, various parameters need to be taken into account, such as the desired concentration, purity of the substance, amount actually weighed and possibly even the molecular weight. For inexperienced users, recalculation of the component weight is usually takes considerable time, whereas experienced users commonly find this a boring task so inadvertent errors can easily creep in.

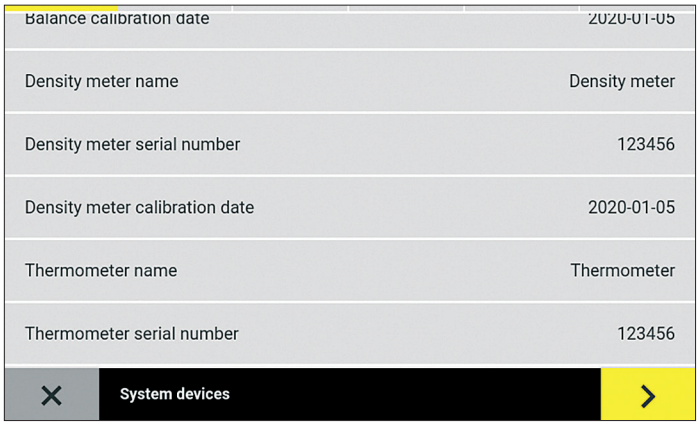
The Cubis® MCA software Standard preparation (QAPP001) is designed to eliminate the described problems.
The system guides the user automatically throughout the entire process of preparing standards, and the application software automatically takes care of all calculations in the background. There is no need to reach the target weight exactly as the software automatically calculates the required solvent volume based on the gravimetrically measured component(s) weight(s). After the user applied the solvent the added weight is gravimetrically checked and using this value the verified concentration(s) and the verified volume of the standard prepared is calculated.

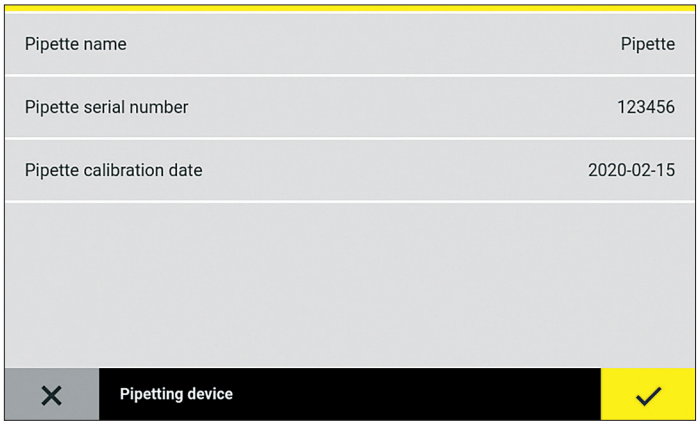
The Standard preparation application works with database to save components, solvents and samples. Components are defined by name, molecular weight and purity and solvents by name and density. For 58 frequently used solvents names and density values at standard room temperature are preset in the database and can be selected by users for defining samples.

Each sample consists of one solvent and at least one component and up to 20 components can be selected for each sample for the preparation of mixed standards.

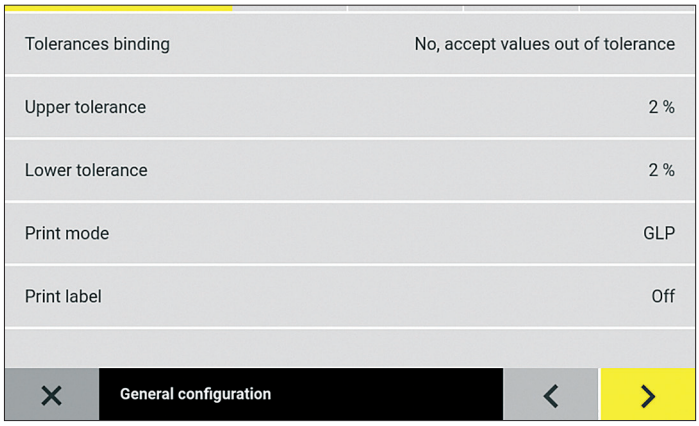


In addition to solvents, components and samples information for used system devices like the balance, thermometer, density meter, printer and pipette are saved to the database. Data for the balance, thermometer, density meter and printer is entered in the task management by a user with the right to edit tasks and pipette data is entered by the user during the task start. With the print mode GLP is activated set data for system devices is printed to reports for documentation.

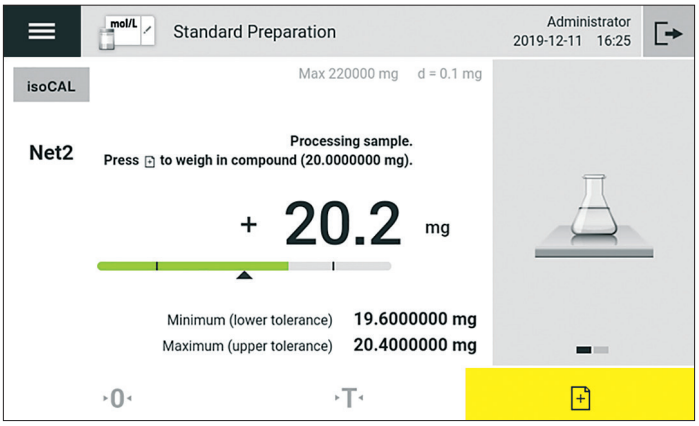




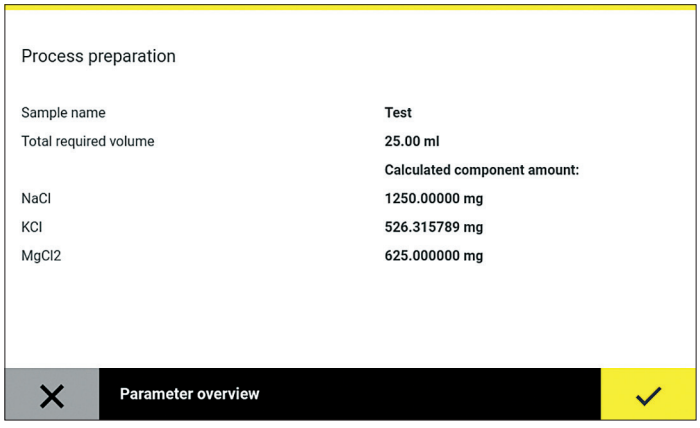
Additionally during the task setup the permissible sample and solvent weight tolerance and the mode for samples out of tolerance can be defined. If the sample weight is below or above the set tolerance the user either a) cannot take over the weight value, or b) must enter the set password to take over the weight value or c) can acquire any value even if the weight is out of tolerance. It is under control of the lab manager creating the task to select what the user is allowed to do with samples out of tolerance. As print mode GLP print inclusive all data or standard with measurement data and calculates results only can be selected and the label print can be set on or off.



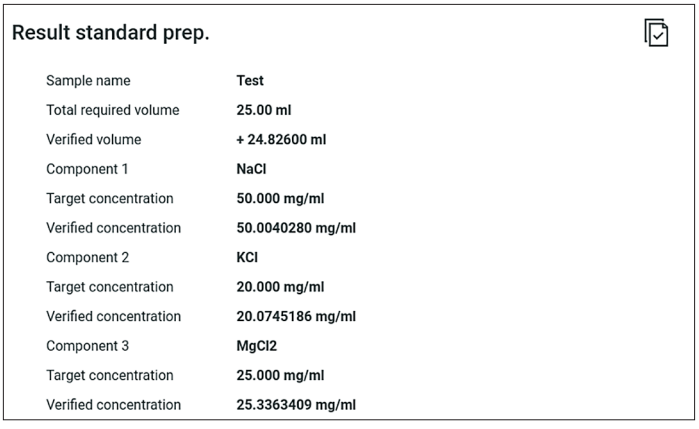
During the weighing process for each component the target weight and a tolerance bar are displayed. If the measured weight is within the permissible tolerances the tolerance bar is shown in green, for weight values out of tolerances it is shown in red. By the color code the user gets an immediate visual feedback if the measured weight value is within the tolerances or not. Depending upon the task settings for out of tolerance values the user either is allowed to accept the value and continue with the process, or can accept the value by entering the password set in the task management or cannot accept the value and the process is stopped.



During sample processing the user is guided through the complete process. The user enters the desired target component(s) concentration(s), selects the concentration unit and the required sample volume and the software application automatically calculates the required amount of component(s) and displays the values to the user. Using this comprehensive overview the user can check if sufficient amount of component(s) and solvent is available to prepare the desired standard solution.



Based upon the measured component(s) weight(s) the software calculates the required solvent volume. For mixed standard the calculated mean value is used. As for the components the software displays a tolerance bar with target weight and permissible tolerances. When the user adds the solvent the balance gravimetrically measures the solvent weight and the software application using the solvents density calculates applied solvent volume and the verified component(s) concentration(s) in the prepared sample. Results are printed either as comprehensive short report or as GLP report listing additionally the system devices used for sample processing. In addition, labels can be printed to label the used vessel.



Sales and Service Contacts

For further contacts, visit www.sartorius.com

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

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Application Highlight

May, 2020

Keywords or phrases:
Loss on Drying, Drying to Constant Mass or Weight, USP Chapter <731>, PhEur Chapter General Notices 1.2

Cubis® II Loss on Drying

For Determining Dry Weight of Tablets, Capsules, or Bulky Material according to USP or PhEur

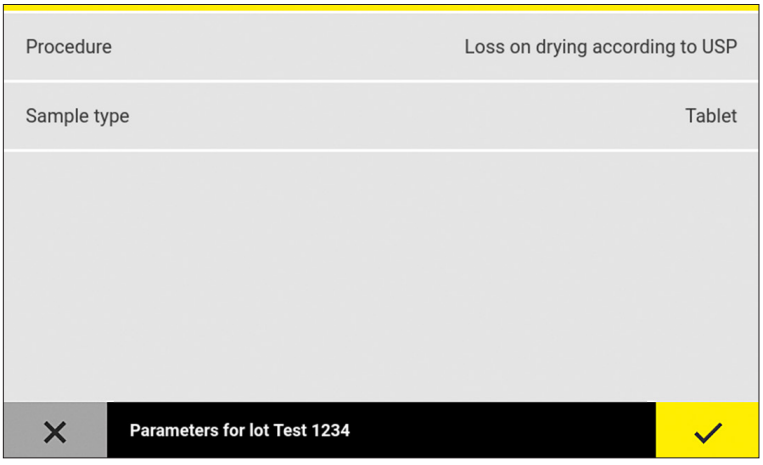
Loss on Drying is a back-weighing application to determine the amount of volatile matter in tablets, capsules, or bulky material. Samples are weighed before and after treatment, and the weight difference is measured.

According to the US Pharmacopoeia Chapter 731 (USP Chapter 731) 1–2 g of sample is mixed; for large particles the size is reduced to about 2 mm by quickly crushing. If tablets are to be tested, the powder of not less than 4 tablets must be used; For capsules, the mixed contents of not less than 4 capsules must be used. A glass-stoppered, shallow-weighing bottle that has been dried for about 30 minutes and cooled to room temperature in a desiccator is tared, the sample placed in the bottle, and the initial sample weight is measured. The sample is evenly distributed in the bottle by gentle shaking, the stopper is removed, and the bottle placed in a drying chamber to be incubated at elevated temperature. After heat treatment, the bottle is closed promptly, cooled down in a desiccator to room temperature, and then the back weight is measured.

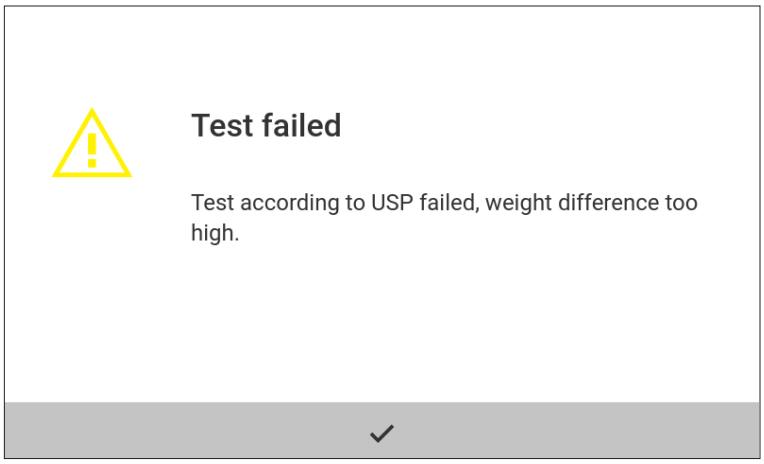
Find out more: www.sartorius.com/cubis-ii

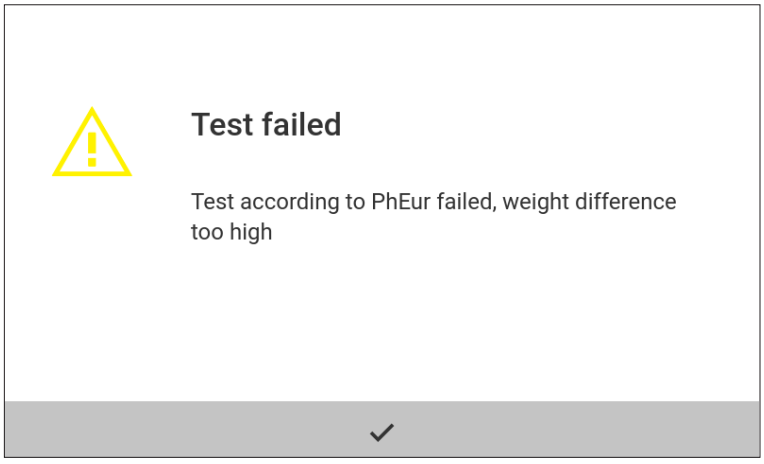
According to USP the “dry to constant weight” or according to European Pharmacopoeia (PhEur) the “dried to constant mass” or “ignited to constant mass” weight value of pharmaceutical products is to be measured. The USP defines that two consecutive weighings must not differ by more than 0.50 mg per g of sample, whereas the PhEur specifies that two consecutive weighings must not differ by more than 0.5 mg to consider the sample as dried to constant weight or mass. If the measured weight difference is out of the allowed limits, the drying shall be continued and the weight measured again.

In the Cubis® II software application for loss on drying, the administrator selects between the test procedure according to USP or PhEur, and the sample type—tablet or capsule. Due to the different definition of allowed weight difference, the selection between USP and PhEur determines the mode, or how the software application considers samples to have passed or failed the test.

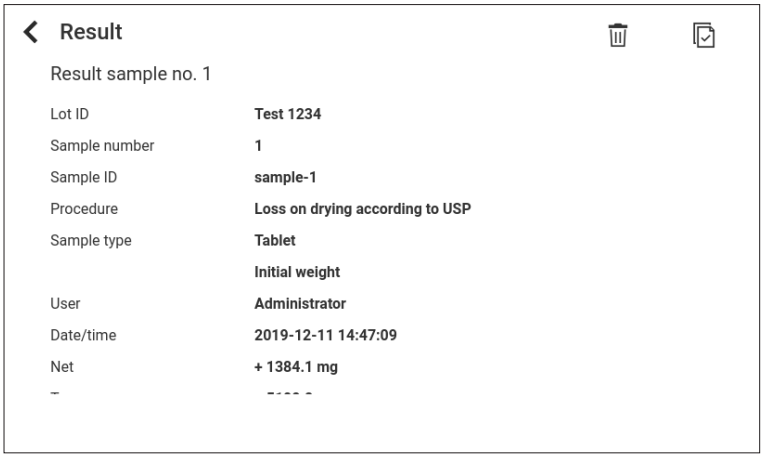


First the initial sample weight (with tare) and then up to three back weights are measured. In total, ten batches with up to 100 samples each can be processed. The software application calculates the difference between the initial and back weight for each sample, and determines if the weight difference is within the range allowed by USP Chapter 42 <731> or European Pharamacopoeia (PhEur). After weighing back a sample, the software evaluates the weight difference and, if it is out of limit, displays a corresponding message to the user. By this mechanism the user gets a direct feedback when the volatile matter of a sample is too high and the drying process must be prolonged.





The software application for each sample creates a comprehensive report, inclusive of the measured initial and back weight(s), and records if the test was passed or failed according to the applied pharmacopoeia.



The Cubis II software application Loss on Drying guides the user through the backweighing process and automatically evaluates the results according to USP or PhEur. If a test for a product fails the user gets a corresponding message displayed that cannot be overlooked.

Sales and Service Contacts

For further contacts, visit www.sartorius.com

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

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White Paper

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Measuring Uncertainty

A New Software Function for Dynamic Display of the Uncertainty of Measurement in Cubis® II Balances

Dr. Holger Densow, Dr. Julian Haller, Dr. Thomas Pertsch
Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Goettingen

Correspondence
E-Mail: lab-requests@sartorius.com

Abstract

The QApp "Measuring Uncertainty" is a new software function available in the QApp Pharma Package of Sartorius's Cubis® II premium balances. This QApp function provides a dynamic display of the uncertainty of measurement, based on the EURAMET Calibration Guide No. 18 "Guidelines on the Calibration of Non-Automatic Weighing Instruments"[1-3] (denoted as "EURAMET cg-18" in the following).

The attachment to Sartorius's calibration certificate according to EURAMET cg-18 also shows a determination of weighing uncertainty when the balance is in use. This global expanded software function "measuring uncertainty" is given in the form of a straight-line equation, containing both a constant contribution and a contribution proportional to the reading. In most cases, this represents a sufficiently good estimation of the uncertainty of weighing results of the balance in use.

For the benefit of the user, the software function allows entering the parameters (as shown in the calibration certificate according to EURAMET cg-18) of the straight-line equation directly into the device. Thus the software function always directly specifies the uncertainty for each measured value, as shown in the appendix to the calibration certificate.

Find out more: www.sartorius.com/cubis-ii

Why is Measurement of Uncertainty Important?

Today's high-performance laboratory balances support users in every respect, with impressive practical and regulatory functionality. Nonetheless, high-performance laboratory balances are complex and highly sensitive measuring devices. Therefore, depending on the device, many different influences, such as the installation location and the expertise of the user, can influence the displayed results.

Even if a balance is properly leveled, adjusted, and calibrated, one question for the user remains: How large is the uncertainty of the displayed weight value?

A new dynamic function displays the uncertainty for each measured value in the complete weighing range, from zero to the maximum capacity. This particularly benefits customers working in regulated environments, for whom documentation of weighing values is of critical importance; the function allows printing of each weighing value with its corresponding uncertainty.

The required parameters can be set easily by Sartorius Service. Values are taken from the calibration certificate, considering multiple parameters contributing to the uncertainty.

All influencing parameters are defined and described in detail in the EURAMET cg-18.

The EURAMET (European Association of National Metrology Institutes) is a collaborative alliance of national metrological organizations from member states of the European Union (EU) and of the European Free Trade Association (EFTA). EURAMET coordinates metrological activity at a European level, liaising with the International Organization of Legal Metrology (OIML) and the International Bureau of Weights and Measures (BIPM), where appropriate.

The software function's calculation for measuring uncertainty of weighing results includes uncertainty contributions for many aspects, for instance, rounding at zero and under load, repeatability, deviation from eccentric loading, possible changes to the balance, and buoyancy effects of temperature changes at the place of use. Moreover, the error of indication, and its interpolation determined during calibration.

Theoretical Background

The EURAMET cg-18 describes the complete metrological theory and background of the measuring uncertainty of weighing. In the following, we attempt to summarize this very comprehensive theory in its basic statements and try to clarify the essential relationships.

A general description of the possible influences on the weighing result W can be shown by the following equation, which takes various corrections into account:

$$W = W^* + \delta R_{\mathrm{instr}} + \delta R_{\mathrm{proc}}$$

In this general equation, W^* is the indicated weight value directly after the calibration of the balance. The term δR_{instr} represents a correction due to environmental influence when using the instrument after calibration. The term δR_{proc} includes all corrections due to the operation of the instrument.

To distinguish from the indications obtained during calibration and the weighing results obtained when weighing a load *L* after calibration on the instrument, the parameters R_0 and R_{L} must be taken into account.

R_0 = the reading without load on the calibrated instrument obtained after the calibration.

R_{L} = the reading when weighing a load *L* on the calibrated instrument obtained after the calibration.

The indicated weight value directly after the calibration, can then be expressed by the following equation

$$W^* = R_{\mathrm{L}} + \delta R_{\mathrm{digL}} + \delta R_{\mathrm{rep}} + \delta R_{\mathrm{ecc}} - \left(R_0 + \delta R_{\mathrm{dig0}}\right) - E$$

The following error contributions of the reading in use are taken into account:

δR_{dig0} = the rounding error at zero reading

δR_{digL} = the rounding error at load reading

δR_{rep} = the repeatability of the instrument

δR_{ecc} = the error due to eccentric positioning of a load

E = the error of indication for a reading, which is reported in the calibration certificate

Generally, users of an instrument should be aware that, in normal use, the weight is different from that at calibration, in some or, very often, all aspects.

The following term describes the errors regarding the individual environmental influences during weighing, after calibration of the instrument.

$$\delta R_{\text{instr}} = \delta R_{\text{temp}} + \delta R_{\text{buoy}} + \delta R_{\text{adj}}$$

Including these contributions

δR_{temp} = the possible change in the characteristic of the instrument caused by a change in ambient temperature

δR_{buoy} = the possible change in the buoyancy due to a variation of the air density

δR_{adj} = the possible change in the characteristics of the instrument since the time of calibration due to drift, or wear and tear (not taken into account in the calibration certificate)

The contributing terms for the errors resulting from the particular operation of the instrument are

$$\delta R_{\text{proc}} = \delta R_{\text{Tare}} + \delta R_{\text{time}} + \delta R_{\text{ecc}}$$

with

δR_{Tare} = the possible change due to a tare balancing operation

δR_{time} = possible effects of creep and hysteresis (not taken into account in the calibration certificate)

δR_{ecc} = the possible error due to eccentric positioning of a load.

Finally, the expanded global measurement of uncertainty $U_{gl}(W)$ can be calculated using the following equation which includes all the above described parameters

$$U_{\text{gl}}(W) = 2 \cdot \left[u^2(R) + a_1^2 \cdot R^2 + a_1^2 \cdot u^2(R) + R^2 \cdot u^2(a_1) + u^2(\delta R_{\text{temp}}) + u^2(\delta R_{\text{buoy}}) + u^2(\delta R_{\text{adj}}) + u^2(\delta R_{\text{Tare}}) + u^2(\delta R_{\text{time}}) + u^2(\delta R_{\text{ecc}})\right]^{0.5}$$

This includes an additional coefficient a_i and its uncertainty $u(a_i)$, resulting from a linear regression of the error of the result obtained during calibration.

Since all contributions are either constant or proportional to R, $U_{\text{gl}}(W)$, uncertainty can be approximated by a straight-line equation. According to the EURAMET cg-18, the contributions to the global expanded uncertainty equation can be grouped into the terms α_{gl}, which includes all constant uncertainties, and β_{gl}, which includes all proportional uncertainties. This can then be expressed in the simplified linear equation

$$U_{\text{gl}}(W) \approx \alpha_{\text{gl}} + \beta_{\text{gl}} \cdot R$$

with the interception $\alpha_{\text{gl}} = U_{\text{gl}}(W = 0)$, the slope $\beta_{\text{gl}} = \frac{U_{\text{gl}}(W=Max) - U_{\text{gl}}(W=0)}{Max}$ and the displayed weighing value R.

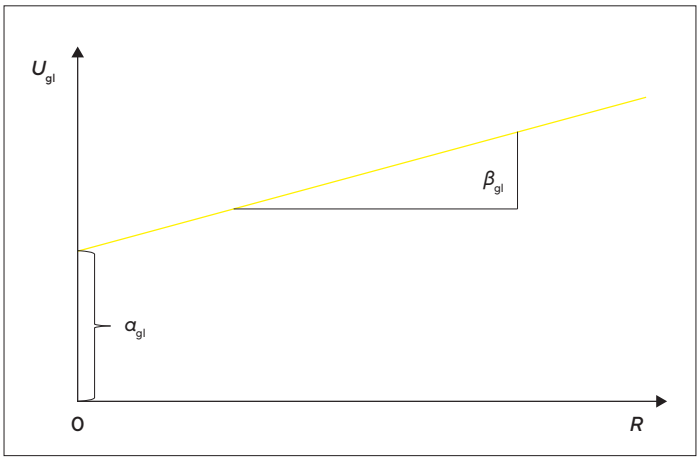

Figure 1: Graphical representation of the absolute measurement uncertainty as a straight line with interception α_{gl} and slope β_{gl}.

How to Enter Values and to Configure the QApp

Sartorius Service can create a calibration certificate based on the guidelines of the EURAMET cg-18 using the Sartorius calibration software Verical®. From the calibration certificate, the summand α_{gl} and the factor β_{gl} can be taken and entered in the QApp "Measuring Uncertainty" function to indicate the uncertainty of any load placed on the pan of the balance.

Since the factor β_{gl} usually is on the order of some millionth, it is given in scientific notation, i.e., $1.23 \cdot 10^{-6}$ as a better manageable notation than 0.00000123. For convenience, the value in the front (the so-called "mantissa" – "1.23" in this example) and the exponent must be entered separately. Since the latter is always negative, one need only enter the absolute value, i.e., "6" instead of "-6" in this example.

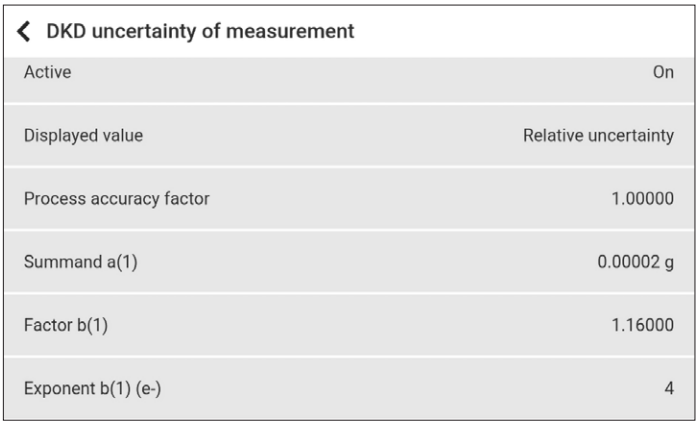

Figure 2: Menu of the QApp with the possibility to enter summand, factor and exponent from a straight line equation

For multi-interval balances (denoted with a "P" in the model name of the Cubis® II series, i.e., MCA225P), a separate equation is given on respective calibration certificates for each calibrated partial weighing range. Accordingly, these models feature the possibility of entering one set of parameters for each partial weighing range.

The settings of the QApp further allow setting the status Active on or off, i.e., to indicate the uncertainty or not.

Furthermore, the user can adjust whether the uncertainty shall be indicated as an absolute value, a relative value, or as a process accuracy.

Accordingly, the QApp automatically calculates the corresponding absolute uncertainty of measurement $U = a + b \cdot W$ (in the chosen unit, i.e., "g" or "mg") or the relative uncertainty of measurement $U^* = U/W \cdot 100\ \%$ (in %) for every indicated weight W. For process accuracy, the relative measurement uncertainty is multiplied with a safety factor | process accuracy factor that can be entered by the user: $PA = k_S \cdot U^*$.

This factor can be used to account for influences on the accuracy of a user's process that could not be considered during calibration and | or to increase the confidence interval of the stated uncertainty. For some advice on how to reasonably choose this factor, read our relevant Whitepaper [4].

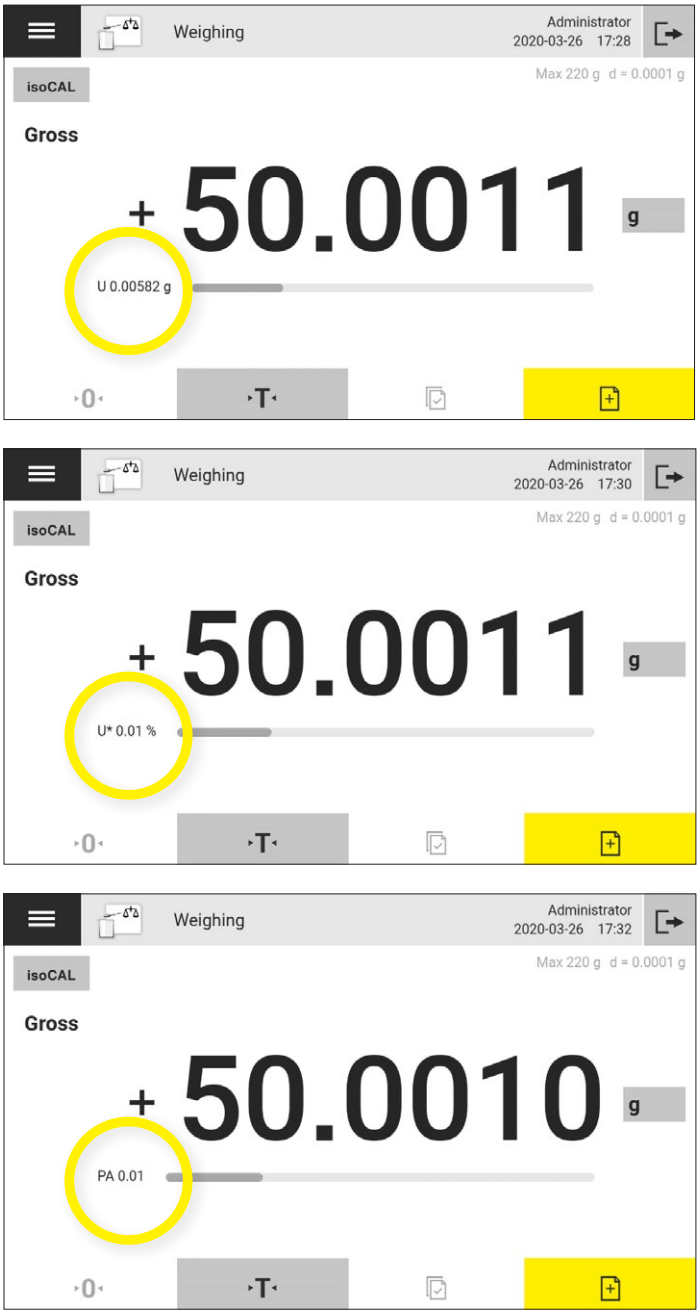

Figure 3: The indication of the measurement uncertainty can be given as an absolute value (top), a relative value (middle) or as a process accuracy (bottom).

How to Understand the Indicated Measurement Uncertainty Values

The determined measurement uncertainty according to EURAMET cg-18 is an expanded uncertainty – that means that for any reading *R*, the true value is within the interval [*R-U* ... *R+U*] with a probability of 95.45%. For the example in Figure 2, this would correspond to a probability of 95.45% for the interval [50.0011 g – 0.00582 g ... 50.0011 g + 0.00582 g] = [49.99528 g ... 50.00692 g].

Furthermore, the user can monitor for any weighing result, whether the process accuracy requirements are fulfilled. For example, let the user's requirement be a process accuracy of 0.1% for a sample preparation before a subsequent analytical method. The user will weigh-in 10 mg of sample in a weighing boat on a Cubis® II MCA semi-micro balance, with a maximum capacity of 220 g and a scale interval of *d* = 0.01 mg. This user will monitor this process permanently, and he is able to see when to increase the sample amount, so that the required process accuracy will not exceed 0.1%.

With regard to state-of-the-art good documentation practice, it is furthermore possible to choose in the printing settings whether the uncertainty shall be printed on print-outs. Furthermore, it is possible to enter additional information like the calibration certificate number, from which the values were taken, as well as the calibration date and whether the calibration was conducted by an accredited calibration laboratory. With GLP print settings it is possible to decide, if (and which of) the parameters and settings of the app shall be printed.

Gross	G	+637.86	g
Absolute uncertainty	U	0.083	g
Relative uncertainty	U*	0.01	%
Process accuracy	PA	0.01	
Gross	G	+739.76	g
Absolute uncertainty	U	0.096	g
Relative uncertainty	U*	0.01	%
Process accuracy	PA	0.01	
Gross	G	+930.19	g
Absolute uncertainty	U	0.121	g
Relative uncertainty	U*	0.01	%
Process accuracy	PA	0.01	

Figure 4: Example print-out of some weighing values and their respective absolute and relative uncertainties as well as their process accuracies.

Conclusion

With this new function available in the Cubis® II balance, Sartorius makes it much easier for users to determine the uncertainty of a measurement or the process accuracy of an initial sample weight, without needing to calculate it from a diagram of the calibration certificate. A calibration certificate is, thus, an important document containing relevant information to support the demands of monitoring testing and measuring equipment.

References

1. EURAMET Calibration Guide No. 18, Version 4.0. **Guidelines on the Calibration of Non-Automatic Weighing Instruments.** (2015)

2. **Sartorius White Paper: Calibration certificates according to EURAMET cg-18 (Understanding calibration certificates and practical application of the results)**. 2020 (planned)

3. **Sartorius White Paper: Calibration guide EURAMET cg-18 for electronic non-automatic weighing instruments (Specifications, options and implementation of the guideline by Sartorius).** (2020) (planned)

4. **Sartorius White Paper: Minimum net weight values according to USP <41>, OIML R76 and EURAMET cg-18 (What is the minimum weight of a sample to get reliable weighing results?).** (2020)

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Germany
Sartorius Lab Instruments
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Otto-Brenner-Strasse 20
37079 Goettingen
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USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

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How to Prevent Mycoplasma Contamination and Spread in Your Cell Culture Laboratory

Sandra Söderholm[1] and Emilia Varhimo[2]

[1] Lab Essentials Applications Development, Sartorius Biohit Liquid Handling Oy, Helsinki, Finland
[2] Pipetting and Dispensing, Sartorius Biohit Liquid Handling Oy, Helsinki, Finland

Correspondence | Email: LHinfo.Finland1@Sartorius.com

Introduction

Mycoplasma is a common bacterial contaminator in cell culture laboratories. In one study, researchers identified mycoplasma contamination in 11% of 10,000 cell lines that they tested (Olarerin-George *et al.* 2015). Moreover, mycoplasmas have been shown to even contaminate liquid nitrogen storage tanks where cell stocks are stored (Bajerski *et al.* 2020).

Mycoplasmas are resistant to commonly used antibiotics, and cannot be detected under the light microscope, because they lack a cell wall and are extremely small – only 0.2-0.4 µm in diameter. This increases the risk of failing to detect mycoplasma contamination in the laboratory. Mycoplasma contamination has been shown to induce cellular changes, e.g. susceptibility to drugs. Therefore, any results obtained from mycoplasma-contaminated tissue cultures potentially render the data invalid (Kim et al., 2015; Gedye *et al.*, 2016).

Pipettes are the most frequently used tools in the laboratory and therefore prone to contamination. Contaminated pipettes can cross-contaminate samples and cell cultures. Regular cleaning of pipettes is absolutely essential for contamination control. Some pipettes are fully autoclavable or have parts that can be autoclaved. Wrong pipetting technique can also cause cross-contaminations. Therefore, an ergonomic pipette and the right pipetting technique are absolutely essential to prevent mycoplasma contamination and to ensure clean samples and successful experiments.

In this study, we demonstrate that autoclaving the pipette is the most efficient method to remove mycoplasma from pipettes. We also show that the pipette and pipetting technique have a great impact on cross-contamination

1. Experimental setup

1.1 Surface Contamination and Decontamination of Mechanical Pipettes
Five to seven spots on mechanical pipettes were contaminated with *A. laidlawii* (ATCC 23206) liquid culture, 2.0-3.7x10^5 colony-forming units (CFUs) per spot (Figure 1). One Tacta pipette was not inoculated and served as negative control (Table 1). The pipettes were incubated in a closed laminar flow cabinet for 24 h at room temperature. The autoclave-resistant pipettes and parts were sterilized at 121 °C for 15 min. The non-autoclavable pipettes and parts were cleaned by wiping surfaces, seams, and grooves with 70% ethanol.

Samples were taken from each spot on the pipettes with cotton swabs soaked in 0.9% sterile saline solution. After sampling, the swabs were placed back into the container with saline solution (1.5 mL) and vortexed for 30 sec, after which the swabs were discarded.

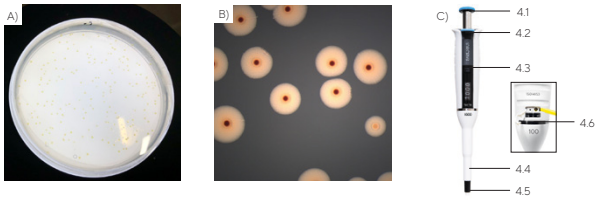

Figure 1: (A) Mycoplasma (*A. laidlawii*) colonies on Luria broth agar plates incubated for 72 h at 36°C. (B) *A. laidlawii* colonies after 5 days (10x magnification). (C) The pipettes were contaminated with *A. laidlawii* on five to seven different spots. In this picture the spots are indicated for the Sartorius Tacta.

Pipette ID	Pipette Type	Number of Contaminated Spots	Decontamination Procedure
1.	Pipette with autoclavable lower parts	7	70% ethanol and autoclave
2.	Non-autoclavable pipette	6	70% ethanol
3.	Non-autoclavable pipette	5	70% ethanol
4.	Sartorius Tacta (0.1-3 µl)	6	Autoclave
5.	Non-autoclavable pipette	6	70% ethanol
6.	Fully autoclavable pipette	5	Autoclave
7. Positive control	Sartorius Tacta (0.5-10 µl)	6	No decontamination
8. Negative control	Sartorius Tacta (20-200 µl)	0	Autoclave

Table 1: Mechanical pipettes included in the surface contamination and decontamination study and their decontamination procedure.

1.1.1 Detecting Live Mycoplasma
Agar plates (M55, Mycoplasma Experience) were inoculated with 200 µl of sample. The plates were sealed with parafilm and incubated at 36°C for 72 h. The CFUs were counted and the total CFUs in the original samples were determined.

1.1.2 Detecting Mycoplasma DNA
Total DNA was extracted from 200 µl of sample with the Sartorius Microsart® AMP Extraction Kit (Cat No. SMB95-2003). The procedure for low complexity aqueous samples was applied, adding 80 µl Microsart® AMP Coating Buffer (Cat No. SMB95-2002) to the sample before extraction procedure. The eluate (possibly containing mycoplasma DNA) was analyzed with RT-qPCR applying Sartorius Microsart® ATMP Mycoplasma kit (Cat No. SMB95-1003) and the LightCycler 480 Real-Time PCR Instrument (Roche).

1.2 Cross-contamination Test for Mechanical Pipettes and Bad Pipetting Technique
One milliliter of *A. laidlawii* culture (1.9x10^6 CFU/ml) was pipetted in total 25 aspiration-and-dispensing steps according to three different pipetting conditions. The tip was changed after every 5th step. After completion, 1 mL of sterile PBS was pipetted under the same pipetting condition. Sartorius Tacta 100-1000 µl pipette and two other manufacturers' mechanical 1000 µl pipettes (Pipette A and B) were tested (Figure 4). Agar plates were inoculated with the PBS sample and CFUs were determined after 72 h of incubation at 36°C.

2. Results – Autoclaving Eliminates Mycoplasma

In comparison to mechanical non-autoclavable pipettes, the fully autoclavable Tacta pipettes can be 100% decontaminated of mycoplasma. Autoclaving is the only way to ensure that the surface of your pipette is fully decontaminated of infectious mycoplasma (Figure 2).

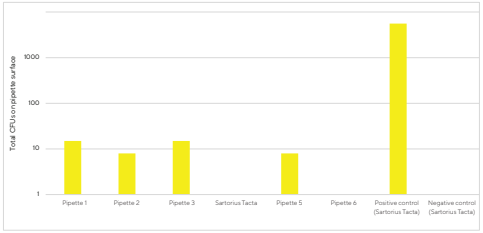

Figure 2: Autoclaving ensures complete decontamination of mycoplasma. The surface of the pipettes (except negative control) were contaminated with mycoplasma. Only autoclaving (Sartorius Tacta and Pipette 6) completely eliminated mycoplasma. After 24 h, ca. 0.5% of the total inoculated number of mycoplasma could still be recovered when no decontamination procedure was applied (Positive control).

3. Results- Autoclaving or Ethanol Do Not Remove Mycoplasma DNA

Neither autoclaving nor 70% ethanol removes mycoplasma DNA - samples taken from every pipette, except the pipette that was not contaminated, were positive for mycoplasma when analyzed with RT-qPCR (Figure 3).

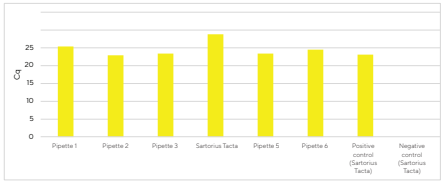

Figure 3: Mycoplasma DNA is not removed by autoclaving or 70% ethanol. The DNA extracted from the samples taken from the surface of the pipettes were analyzed with RT-qPCR with the the Microsart® ATMP Mycoplasma kit. The average quantification cycle (Cq) values for are shown. All the pipettes, except for the negatve control, were positive for mycoplasma DNA.

4. Results - Pipette and Pipetting Technique Matter

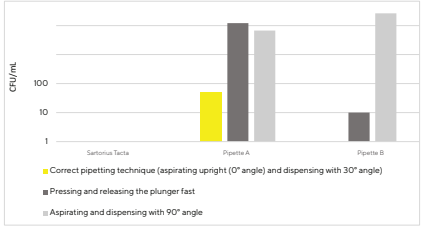

Figure 4: Bad pipetting technique, in particular in combination with mechanical pipettes with stiff plungers, increases the risk for cross-contamination. Sartorius Tacta pipettes have soft plungers, preventing retraction of contaminating droplets into the pipette. In comparison to the two pipettes from other manufacturers, cross-contamination was not observed with the 100-1000 µl Tacta pipette.

5. Conclusion

Pipettes are potential sources of mycoplasma contamination, as mycoplasmas can survive for at least 24 h on their surface. Autoclaving eliminates infectious mycoplasma. Sartorius Tacta pipettes can be completely autoclaved, significantly reducing the risk of contaminating your cell cultures. However, for removing mycoplasma DNA from the pipette we suggest sodium hypochlorite solution, DNA AWAY™, or PCR Clean™ Wipes (Minerva Biolabs). Additionally, correct pipetting technique and pipettes with soft plungers such as Sartorius Tacta are essential for preventing contamination and spread of mycoplasma.

Tips and tricks to prevent mycoplasma contamination in your laboratory:
- Wear protective clothes and gloves
- Frequently clean the laminar flow cabinet and surfaces where you work with cells
- Set up a regular cleaning-schedule for your pipettes
- Use pipettes in one single laboratory – do not move them around
- Add Safe-Cones to the tip cones of your pipettes if you are not using filter tips
- Use Safetyspace™ Filter tips
- Use Sartorius Tacta ergonomic pipettes with soft plunger and tip ejector, avoiding splashes and retraction of droplets into the pipette while pipetting
- Aspirate with the pipette upright (0° angle) and dispense with 30° angle
- Test your cell cultures and cell lines on a regular basis – use Sartorius Microsart® Mycoplasma PCR kits to detect any traces of mycoplasmas in your cell cultures

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Gedye et al. Stem Cell Rev Rep (2016); 12(1).
Kim et al. Biomaterials Research (2015); 19(6).
Olarerin-George AO & Hogenesch JB. Nucl Acid Res (2015); 43(5).

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Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further contacts, visit
www.sartorius.com

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