A Sponsored Supplement to *Science*

Your Practical Guide to Basic Laboratory Techniques

Sponsored by

sartorius

Produced by the *Science*/AAAS Custom Publishing Office

Science AAAS

BIOLOGY ▪ EARTH ▪ ENVIRONMENT ▪ LIFE ▪ INTERDISCIPLINARY ▪ PHYSICAL ▪ MATERIAL ▪ SOCIAL SCIENCES

Science Advances | AAAS

OPEN ACCESS, DIGITAL, AND FREE TO ALL READERS

Pushing the Boundaries of Knowledge

As AAAS's first multidisciplinary, open access journal, *Science Advances* publishes research that reflects the selectivity of high impact, innovative research you expect from the *Science* family of journals, published in an open access format to serve a vast and growing global audience. Check out the latest findings or learn how to submit your research: **ScienceAdvances.org**

Your Practical Guide to Basic Laboratory Techniques

Cover Image: © abscent/shutterstock.com

Original content (pages 4-9) has not been peer-reviewed or assessed by *Science*.

This booklet was produced by the *Science*/AAAS Custom Publishing Office and sponsored by Sartorius.

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

© 2018 by The American Association for the Advancement of Science.
All rights reserved. 26 October 2018

Introductions

2 **Ready . . . Set . . . Pipet!**
Sean Sanders, Ph.D.
Science/AAAS

3 **Taking the First Steps**
Ferencz Paldy, Ph.D.
Head of Segment Marketing Academia, Sartorius

Fiona Coats, Ph.D.
Head of Life Science Research Marketing, Sartorius

Feature Articles

4 **Building Skills in Basic Lab Techniques: Useful Tips from the Experts**
Mike May, Ph.D.

7 **The Paperless Lab**
Chris Tachibana

Technical Notes

10 **How to Avoid Contamination in Pipetting**

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

15 **Cell Culture Expansion in Fully Closed Erlenmeyer Shake Flasks Outside the Biosafety Cabinet with MYCAP™ CCX**

20 **How to Achieve Optimal Weighing Performance**

24 **Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories**

29 **Minimizing Syringe Filter Consumption for Monoclonal Antibody Harvest from CHO Cell Culture Supernatants**

34 **Correlation Between Colony Forming Units and Genome Copies of 9 Different *Mycoplasma* Species Using Quantified CFU and GC Standards for Validation**

38 **Scouting Protein Purification Conditions Using Vivapure Centrifugal Ion Exchange Membrane Absorbers**

42 **Additional Resources**

Ready . . . Set . . . Pipet!

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

"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 Life Science Research 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. "It 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. "This 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."

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.

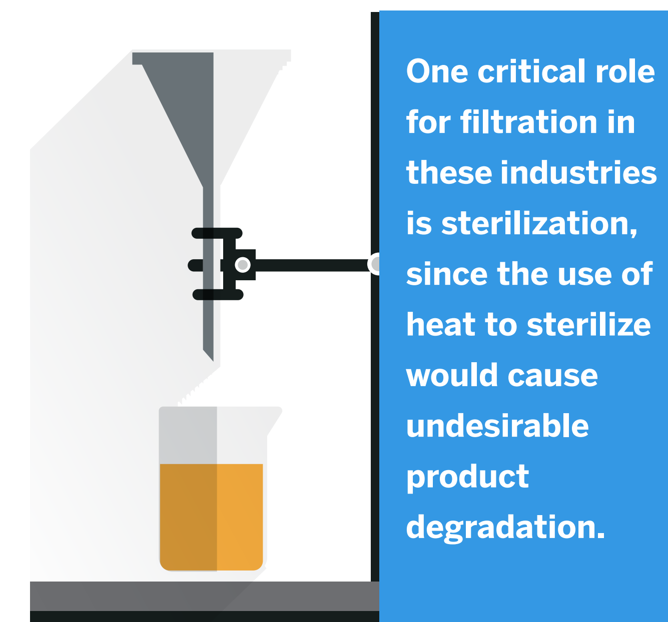
Purifying the processes

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.

IMAGES: (FROM TOP) © M.LORAINE/SHUTTERSTOCK.COM; MAKYZZ/SHUTTERSTOCK.COM



Filtering fluids

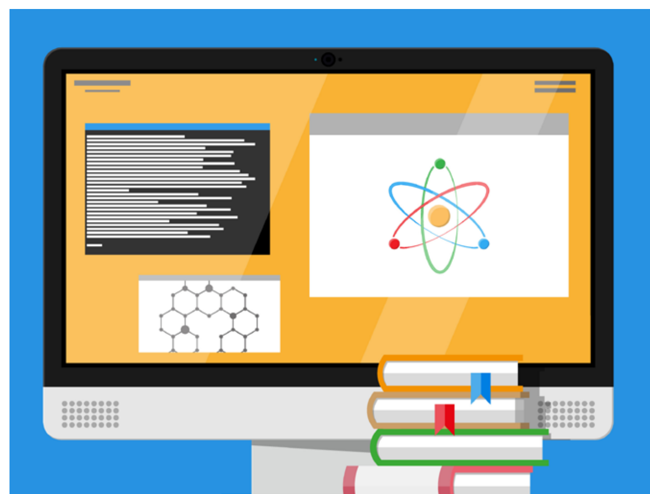
To remove unwanted solids from a sample and increase purity, scientists often use various forms of filtration, which extend from a simple piece of filter paper in a funnel to advanced membrane-based devices. Many molecular methods include filtration to concentrate a sample. Filtration is used extensively 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.

PHOTO: © ABSCENT/SHUTTERSTOCK.COM

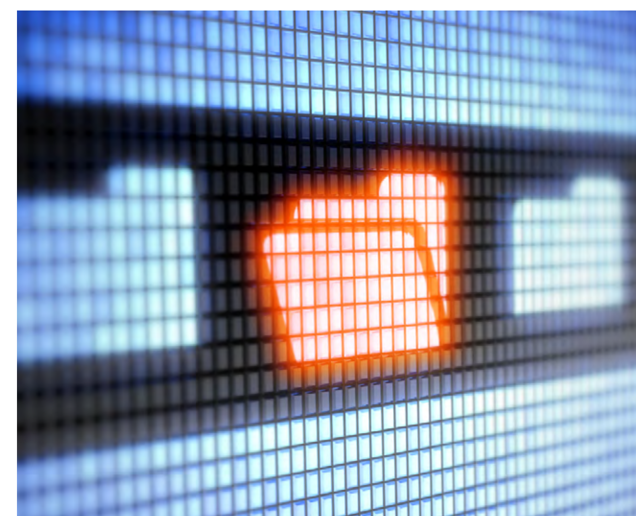


PHOTO: PAVEL IGNATOV/SHUTTERSTOCK.COM

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: At an Australian university, 30 years of notebooks became a pile 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 by reviewers. In a positive example of going paperless, a Swiss contract manufacturing organization wowed clients with real-time, 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. 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 web-based research management system. As a postdoc, Morrish

Originally published 25 July 2014 in *SCIENCE*

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

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 time-saver. 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.

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.

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. "Don'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 cloud-based 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 informatics 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."

PHOTO: BRUCE ROLFF/SHUTTERSTOCK.COM

Originally published 25 July 2014 in *SCIENCE*

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."

Featured Participants

Accelrys
www.accelrys.com

Atrium Research & Consulting
www.atriumresearch.com

LabArchives
www.labarchives.com

Labguru
www.labguru.com

LabKey Software
www.labkey.com

LabLynx
www.lablynx.com

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
www.openwetware.org

PerkinElmer
www.perkinelmer.com

Thermo Fisher Scientific
www.thermofisher.com

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 far-flung 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 OpenWetWare 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 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.

Chris Tachibana is a science writer based in Seattle, USA, and Copenhagen, Denmark.

DOI: 10.1126/science.opms.p1400087

Originally published 25 July 2014 in *SCIENCE*

How to Avoid Contamination in Pipetting

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 non-filter 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-to-pipette 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 or positive displacement 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.

Sample-to-Sample Contamination

Sample-to-sample contamination (or carryover 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 carryover contamination:
- Use filter tips or positive displacement 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.

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

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 Göttingen, Germany
[*] Correspondence
E-Mail: hannes.landmann@sartorius.com

Abstract

This short Application Note describes how you can use Vivaspin® Turbo 15, Vivaspin® Turbo 4 and Vivaspin® 500 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, Vivaspin® Turbo 4 and Vivaspin® 500 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 10 kDa MWCO
– Vivaspin® Turbo 4 10 kDa MWCO
– Vivaspin® 500 10 kDa 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 labeled with Bromophenol blue

Methods
1. Add defined amount of water to the filtrate tube (see table below).
2. Put the concentrator insert into the filtrate tube and add sample solution.
3. Close the concentrator screw cap (for Vivaspin® Turbo 15 or Vivaspin® Turbo 4) or close the cap (Vivaspin® 500) 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

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 x g	1.50 ± 0.02 mL
9.5 mL	15 mL	20 min @ 4,000 x g	0.96 ± 0.01 mL
7.5 mL	15 mL	20 min @ 4,000 x g	0.53 ± 0.02 mL

Results for Vivaspin® Turbo 4

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 x g	0.34 ± 0.03 mL
1.5 mL	4 mL	20 min @ 4,000 x g	0.15 ± 0.02 mL
1.2 mL	4 mL	20 min @ 4,000 x g	80 ± 10 µL

Results for Vivaspin® 500 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 x g	103 µL ± 13 µL
380 µL	500 µL	15 min @ 15,000 x g	51 µL ± 11 µL
250 µL	500 µL	15 min @ 15,000 x g	30 µL ± 5 µL
200 µL	500 µL	15 min @ 15,000 x g	23 µL ± 7 µL

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

Cell Culture Expansion in Fully Closed Erlenmeyer Shake Flasks Outside the Biosafety Cabinet with MYCAP™ CCX

Charles Meadows[1*] (Senior Product Manager), Gregory Bremer[1] (Upstream Engineer, Research & Development), Dr. Michael Zumbrum[1] (Director of Research & Development, New Oxford)

1. Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen
* Correspondence
E-Mail: charles.meadows@sartorius-stedim.com

Abstract
Expansion of suspension cell culture from cell banks to seed bioreactor is performed through passages of successively larger Erlenmeyer shake flasks. The traditional cap of an Erlenmeyer flask is unscrewed for each fluid transfer. Risk of contamination is mitigated by performing these fluid transfers in a biosafety cabinet (BSC) or laminar flow hood.

Work in a BSC is not preferred because of high maintenance and operating costs, intensive cleaning and decontamination procedures, and the risk and inconvenience of performing operations in the BSC.

Despite working in a BSC, expansion processes include passages with backup flasks to be used in the case of contamination. Backup flasks are a material waste and multiply labor-intensive BSC work.

Sartorius' MYCAP™ CCX includes integral tubing and a specially designed gas exchange cartridge. Integral tubing supports good aseptic technique to prevent contamination. All fluid transfers are done outside the BSC. The gas exchange cartridge has a high filter surface area to support passive gas exchange and vibrant cell growth in the incubator.

Introduction

Bottle closures with integral tubing are widely used in bioprocessing because they reduce or eliminate the risk of contamination from poor aseptic technique. Good aseptic technique is especially important upstream where preserving axenic, or monoculture conditions is compulsory.

Tubing materials of the cap closure are commonly thermoplastic elastomer (e.g., C-Flex®), which can be aseptically welded to another tube of the same size. Alternatively, aseptic connecting devices (Sartorius Opta®, Colder Aseptiquik®, Pall Kleenpak®, etc.) may be installed at the tube ends. In either case, the bottle can be aseptically connected to receive or dispatch fluids in non-classified spaces without the risk of introducing a contaminant.

Key customers approached Sartorius to improve aseptic technique in cell expansion with the following objectives:
– Eliminate contamination risk
– Enable fluid transfers in non-classified spaces
– Reduce waste from requisite backup passages
– Achieve comparable culture growth rates & doubling times to incumbent expansion methods

Cellular respiration consumes O_2 and produces CO_2 as a byproduct. Cell cultures starved of O_2 will not propagate. Cultures with an overabundance of CO_2 become acidic and impair cell viability. The exchange of O_2 and CO_2 across the filter membrane is critical to cell growth.

It is customary to attach a disc filter to integrated tubing in a cap for air venting during fluid transfer. Early testing of closures on Erlenmeyer flasks with a 50 mm disc filter showed slowed or fully halted cell growth. Despite the large filter surface area (3 in² | 20 cm²) of the 50 mm disc filter, the gas exchange across the membrane was inadequate for cell growth. Air flow through the membrane is restricted at the 8 in. (3.2 mm) orifice of the hose barb on the filter housing. Cell growth resumed once the culture was moved to a flask with the traditional flask cap.

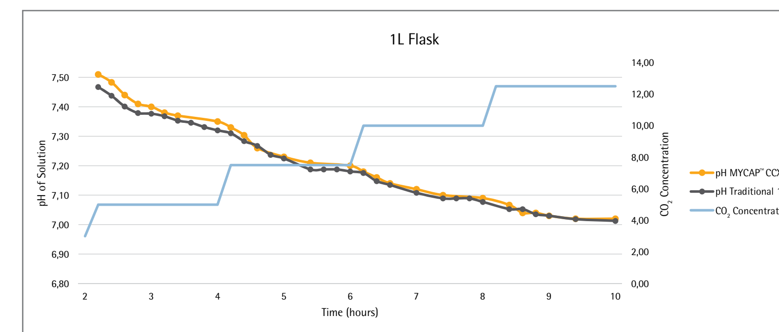
Traditional flasks have a filter membrane embedded in the cap. The arrangement allows for unrestricted air flow across the entire filter surface. However, the filter membrane occupies the entire cap surface, leaving no room for integral tubing for aseptic fluid transfers.

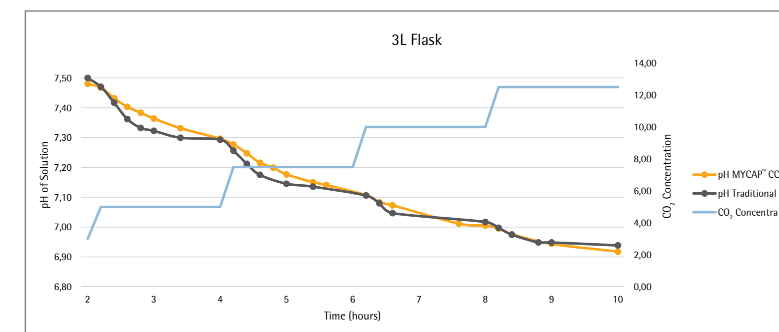
Sartorius' Solution

The manufacturing process of the patented MYCAP™ bottle closure is an enabling technology. Components, usually tube assemblies, are inserted into pre-formed holes. Silicone elastomer is dispensed into the cap to hermetically seal the installed components in place and to create the highly compliant, plasticizer-free bottle closure.

Inserted components are not restricted to tube assemblies. Sartorius developed the MYCAP™ CCX gas exchange cartridge with the following objectives:
– Provide adequately large filter surface area
– Allow unrestricted air flow across filter membrane
– Reduce the filter footprint allowing space for integral tubing

The MYCAP™ CCX gas exchange cartridge is a three-dimensional, stadium-shaped part. Two generous 0.2 µm, hydrophobic filter membranes extend into the neck of the flask. The orientation of the filter membranes protects and places them in position for unrestricted gas exchange between the culture and the incubator environment. The stadium shape conserves space on the cap for integral tubing for media addition, inoculum addition, sampling and transfer.





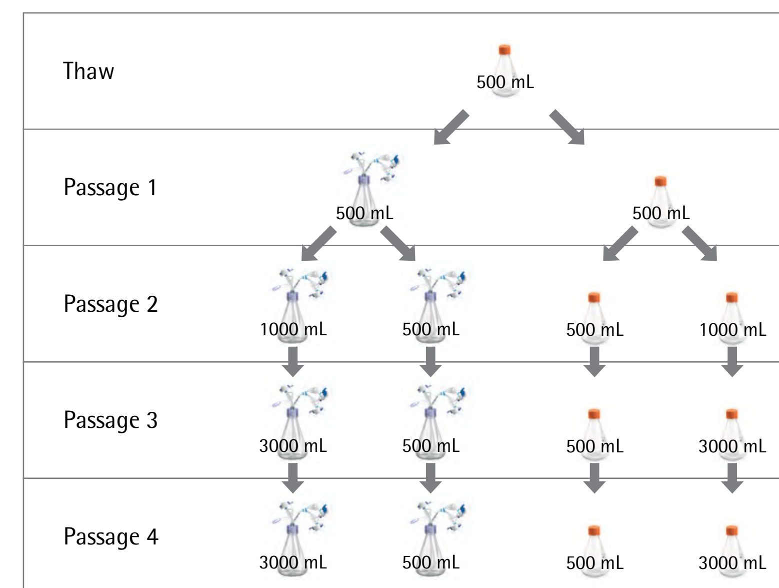


Figure 1: Cell Growth Study Process Diagram

Table 1: Process Parameters

Incubator Parameter	Description | Set Point	
Temperature	36.8°C	
Carbon Dioxide %	7.5%	
Agitation	500 mL, 1000 mL 3000 mL	120 rpm 80 rpm

Gas Exchange Study

Sartorius performed an evaluation to compare gas exchange across the MYCAP™ CCX cap closure and the traditional vented cap closure.

1L and 3L flasks were modified to accept a pH probe in the side wall so that the probe would be in direct contact with solution to read pH changes. Flasks were filled with phosphate buffered saline (PBS) solution containing sodium bicarbonate buffer. Test articles were placed in an incubator and CO_2 concentrations changed every two hours.

Change in pH of the solution indicates gas exchange across the filter membrane.

The pH change of the solution on flasks with the MYCAP™ CCX cap and flasks with the traditional vented cap are virtually identical.

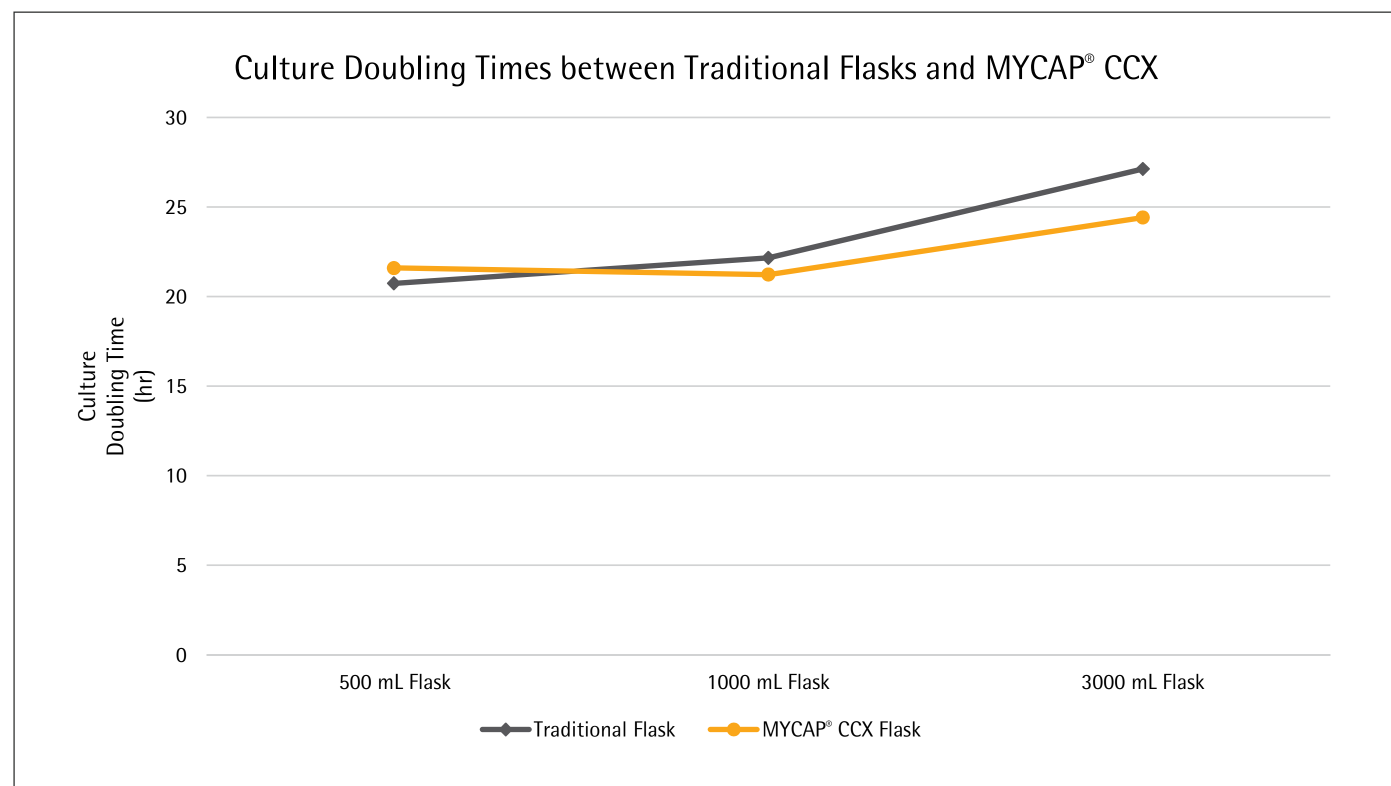
Cell Growth Study

Sartorius performed a study comparing cell growth in flasks with the MYCAP™ CCX cap to flasks with the traditional vented cap.

CHO DG44 cells were directly thawed into a traditional flask and then split into two trains: Train 1 utilized MYCAP™ CCX flasks; and Train 2 utilized traditional flasks. Cells were sub-cultured consecutively for three additional passages in various size flasks up to 3000 mL.

Two-tailed T-Tests were performed comparing the doubling times between MYCAP™ CCX and traditional flasks of the same size. There was no statistically significant difference in growth rates between the two systems, with a 95% confidence level.

Average culture doubling times for each flask size were graphed. The graph (next page) illustrates the comparability of doubling times for MYCAP™ CCX flasks and traditional flasks.



Conclusion

Expansion of suspension cell cultures using Erlenmeyer flasks in a BSC is a labor-intensive process. The flask's cap is removed at each passage and fluid transfers including media addition, inoculation and sampling are done, typically by hand-pipetting. These operations are performed under laminar flow in the BSC to prevent contamination. Yet, contamination risk persists, so backup flasks are maintained for use in the event of a contamination. In a GMP seed expansion process, a typical passage requires three to four operators; the hood technician, hood assistant and data/batch record recorder(s).

MYCAP™ CCX has integral tubing allowing for aseptic fluid transfers in the open space of a workbench. The number of operators is cut in half, contamination risk is eliminated and wasteful backup flasks are not necessary.

Carefully controlled conditions for cell growth in a shake flask in an incubator are required. In particular, the unrestricted exchange of CO_2 and O_2 between the cell culture and the incubator environment is critical to achieving targeted cell density and viability.

Gas exchange, as measured by a change in pH of solution in response to a change in CO_2 concentration, was compared between MYCAP™ CCX and traditional flasks and found to be substantially equivalent.

Successful passages in an expansion process are benchmarked by cell growth rates and cell culture doublings. A comparison of cell culture doublings was compared between MYCAP™ CCX and traditional flasks across 4 passages were found to be equivalent.

MYCAP™ CCX should be considered a suitable replacement for traditional Erlenmeyer flasks to reduce waste, eliminate contaminations and streamline cell expansion operations.

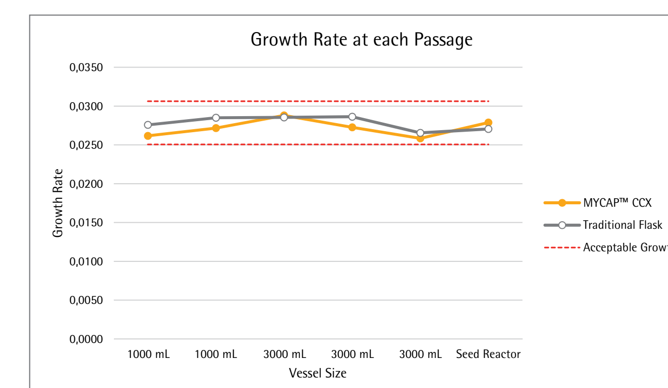
Recommendations

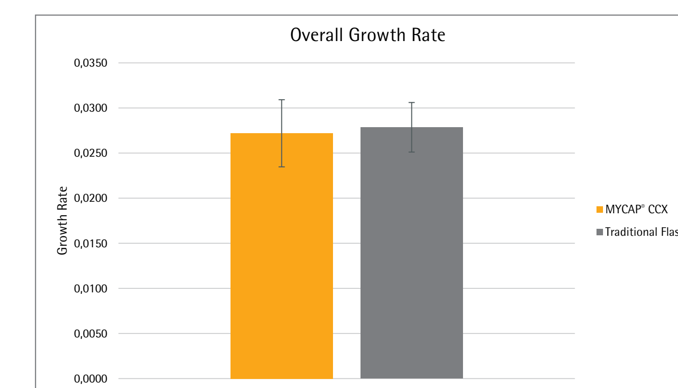
A validation study comparing rates of growth in MYCAP™ CCX flasks with traditional flasks should performed before implementing. Sartorius offers the MYCAP™ CCX Validation Template Tool to streamline experimental design, data collection and data analysis.

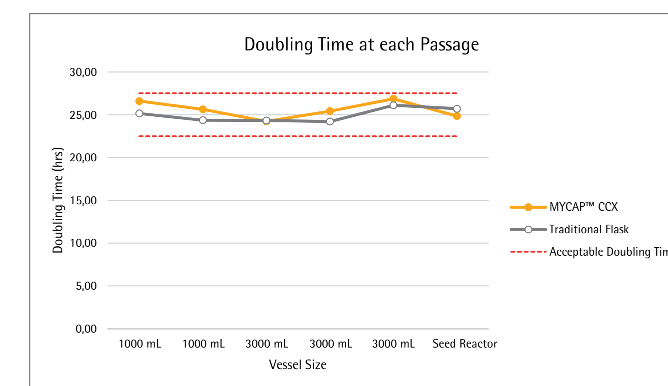
The Tool generates charts to visualize growth rates and performs the Student's T-Test to compare the datasets.

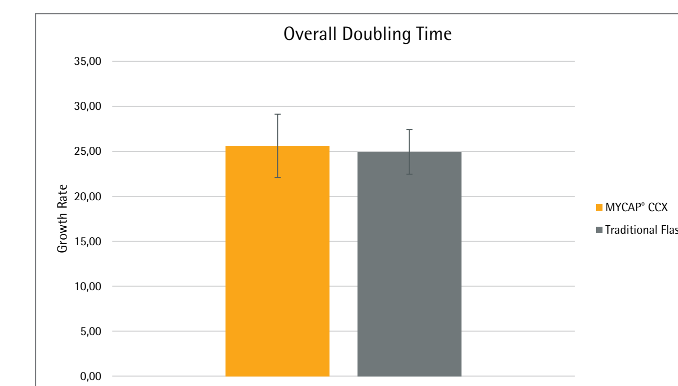
MYCAP™ CCX Validation Template Tool makes it quick and easy to make a scientifically sound and informed decision if MYCAP™ CCX is an acceptable replacement of incumbent technology for use in a production process.

MYCAP™ CCX Validation Template Tool:
– Supports up to 6 Passages
– Complete MYCAP™ CCX materials list including "Where Used Guide"
– Record and maintain experimental conditions; flask size, culture volume, shaker speed, incubator temperature, CO_2 concentration
– Compare against required performance criteria; growth rate, cell count targets, cell viability
– Visual and Statistical Analysis including:
  – Doubling Time and Growth Rate Graphs at each Passage
  – Overall Doubling Time and Growth Rate Graphs
  – Student's T-Test









MYCAP™ CCX and Opta® are registered trademarks of Sartorius-Stedim Biotech
AseptiQuik® is a registered trademark of Colder Products Company
Kleenpak® is a registered trademark of Pall Corporation
C-Flex® is a registered trademark of St. Gobain Performance Plastics

How to Achieve Optimal Weighing Performance

Introduction

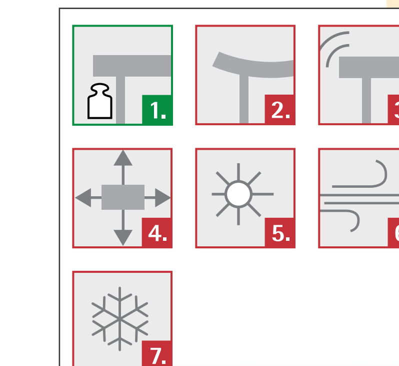
With full-resolution 1 µg readability up to 61 g, the new Sartorius high-capacity microbalances are pushing back the limits of what is possible in weighing technology: They set a new record in accuracy with 60 million divisions. Their exceptional weighing performance and the impressive quality of their weighing results are clearly revealed when they are checked with certified weights.

But perfect measurement of weights is not the application this balance was designed for. Sartorius high-capacity microbalances enable optimal minimum weights within the USP 41 operating range to be measured in heavy glass vessels, such as long-necked, volumetric flasks.

Direct weighing of even the smallest quantities of a substance in large glass flasks enables straightforward, accurate and efficient preparation of stock solutions and reference standards, e.g., for HPLC analysis. This eliminates the need for transferring a microsample from a weighing boat into a volumetric flask, which can result in errors. Weighing directly in a large container reduces both sample loss and contamination.

This application requirement that a balance needs to meet poses an even greater challenge to its weighing technology. The reason is that 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. To ensure high accuracy during weight measurements and excellent repeatability of the results, you need to observe certain basic rules and requirements.

External environmental influences or improper handling can lead to inaccurate results or poor weighing performance, which are not caused by the balance.



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.

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.

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

4. Use the Sartorius 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

1. Sartorius high-capacity microbalances 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. Opt for using 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.

Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories

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.

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.

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 were 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-to-use laboratory crossflow cassette in an acrylic housing, which allows caustic cleaning and 4-5 reuses. 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® 200 setup before and during the concentration process.

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.

Figure 1a and 1b: Vivaflow® 200 setup before (1a) and during (1b) the sample concentration process.

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

Vivaflow® 200 (5 kDa MWCO)

Filtrate Volume (mL)	Time taken (h:min:s)
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

Figure 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.

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 setup. 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 readjust 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.

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

Filtrate Volume (mL)	Time taken (h:min:s)
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

Part 3 – Purification of Lysozyme, the Protein of Interest

The purification of lysozyme was performed using a Vivapure® cation exchange membrane adsorber device (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 (Figure 3).

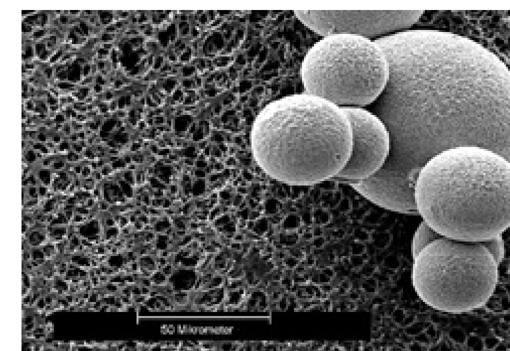


Figure 3: The electron microscopic image of chromatography gel beads (upper right) in comparison to a Q ion exchange membrane adsorber (background) reveals 100-fold larger pore sizes of the membrane adsorber.

Two Vivapure® Maxi H S type devices (Figure 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 x 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 x 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.

Figure 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), shown in Figure 5, and centrifuged at 5000 x g for 10 min or until approximately 2 mL of concentrate had been collected. The device was then refilled with 18 mL 50 mM 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.

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

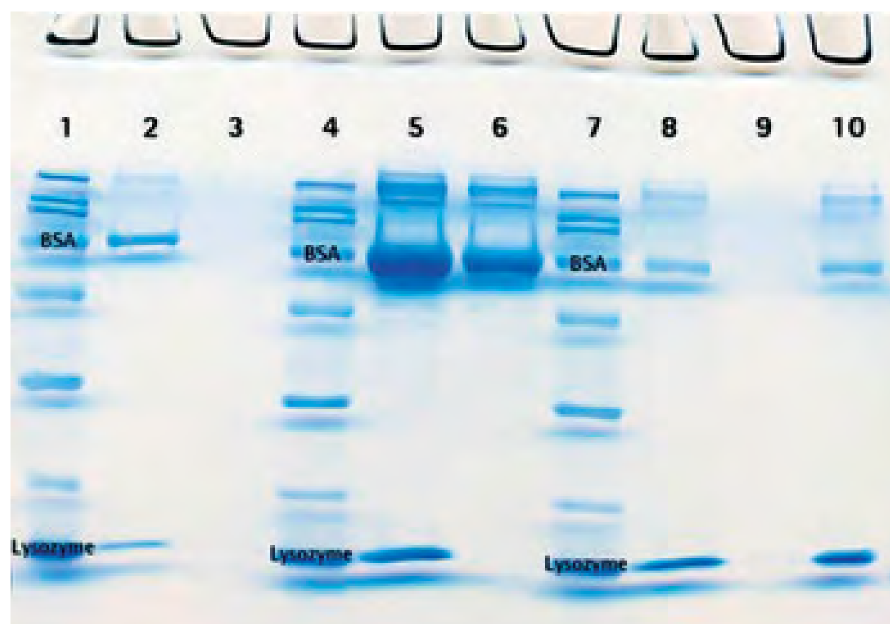


Figure 6: Coomassie stained 12% Tris-HCl SDS gel loaded with 20 µL sample preparations. Lane 1: Marker (SDS Broad Range marker); Lane 2: Original sample; Lane 3: Original sample filtrate (Part 1); Lane 4: Marker; Lane 5: Buffer exchange concentrate (Part 2); Lane 6: Filtrate after binding (Part 3); Lane 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.

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 (Figure 6).

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 setup and completion of protein purification takes approx. 3.45 h using this method, starting from 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.

Table 3

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

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

Minimizing Syringe Filter Consumption for Monoclonal Antibody Harvest from CHO Cell Culture Supernatants

Michael Grauf[1*], Klaus Schöne[2]

1. Sartorius Stedim Cellca, Erwin-Rentschler-Straße 21, 88471 Laupheim
2. Sartorius Lab Instruments GmbH & Co.KG, Otto-Brenner-Str 20, 37079 Göttingen
* Correspondence
E-Mail: michael.grauf@sartorius.com

Abstract

The clarification of cell culture supernatants with volumes < 25 mL for harvesting monoclonal antibodies by using syringe filters is often a laborious and sometimes an exhausting work step. Therefore, a proper selection of the suitable filter model could be paramount. In this work, we compared two syringe filter models with a similar effective filtration area from two suppliers regarding their clarification characteristics of CHO cell culture supernatant samples. To obtain robust results we examined 10 combinations of cultivation methods and monoclonal antibody products like IgG1, IgG2, fc fusion proteins, and bispecific antibodies with regard to turbidity, mAb recovery, relative yield, and throughput. As a result, we found that syringe filter model Minisart® High Flow shows an average throughput of 18.0 mL compared to 9.3 mL of another premium brand at cell densities between 38.3 and 163.6 x 10^5 cells/mL. For the other parameters, we could not find any significant differences. This finding emphasizes the importance of carefully selecting the syringe filter model that reduces the number of both devices needed and thus the workload.

Introduction

Clarification of mammalian cell culture samples for preparative or analytical purposes is a necessary step to enable both a subsequent purification step and a smooth operation of analytical instruments. The overall aim of the clarification step is to remove cells, cellular debris and other particles from the cell culture while simultaneously allowing the target product to be recovered with a sufficient yield. The conventional procedure for clarification of small volumes (approx. < 25 mL) is a combination of centrifugation of the cell culture sample followed by a microfiltration of the supernatant obtained. While centrifugation removes coarse and high-density particles, microfiltration is frequently necessary to pull out small or low-density particles from the centrifugation supernatant. Microfiltration can serve as a simultaneous sterilization step by using 0.2 or 0.22 µm rated sterile filters.

Figure 1: Clarification and sterile filtration of cell culture supernatants under aseptic conditions by using the syringe filter Minisart® High Flow with a pore size of 0.22 µm.

Even though centrifugation removes the vast majority of particles, clogging of filters is often a problem and can lead to an increased consumption of filter devices or to ergonomic handling issues. However, both the reduction of filter consumption and the associated operation time can be achieved by a well-considered choice of the right filtration device.

In the present work, we show that a proper choice of the syringe filter device for the clarification of CHO cell cultures can improve sample throughput and filter consumption without having a negative impact on turbidity, recovery of mAb product, and total yield. Two common sterile syringe filters available in the market were chosen with a pore size of 0.22 µm, slight difference in effective filtration areas, and a polyethersulfon membrane.

Methods and Materials

In an attempt to facilitate the filtration of cell culture supernatants for the development of cell lines, a comparative study was performed. The study was executed by using CHO cell culture samples from real projects spread over a period of 3 months. The syringe filter models Minisart® High Flow (Sartorius, order no. 16532-K, 0.22 µm PES membrane, EFA = 6.2 cm²) and another premium brand (0.2 µm PES membrane, EFA = 5.8 cm²) and were examined regarding their filtration performance by means of the parameters: turbidity, mAb recovery, relative yield, throughput, and filter consumption.

In 13 cultivation batches, 10 combinations of target proteins and cultivation methods were used (Table 1).

In addition to 125 and 1000 mL shaking flasks, 5 L stirred tank reactor (UniVessel, Sartorius) were also used. The cell density and viability was examined with the Vi-CELL XR from BeckmanCoulter. As target proteins, CHO cell lines were selected with mAb from different IgG1 types, IgG2, fc fusion protein and a bispecific antibody. The specific designation has been anonymized, due to confidentiality agreements.

All cell culture batches were harvested after 14 days. From every batch, two samples were taken (max. 31 mL per sample), one sample destined for clarification with Minisart® High Flow and one for another premium brand. The samples were clarified by centrifugation for 60 min at 4000 g, and the supernatants were filtered with the respective syringe filters (Figure 1).

The mAb titer was determined in the unharvested and harvested cell culture fluid using the Octet QK^e system equipped with a protein A Biosensor (ProA) from FortéBio without any interfering sample preparation.
The recovery was calculated by the values determined.

$$\frac{volume\ filtrate\ [mL] \times mAb\ titer\ filtrate\ \left[\frac{mg}{mL}\right]}{volume\ sample\ volume\ CCF\ [mL] \times mAb\ titer\ CCF\ \left[\frac{mg}{mL}\right]} \times 100\% = yield\ [\%]$$

Equation 1: Calculation formula of the relative mAb yield [%].This was necessary to compare the results from different sample volumes ranging from 24 mL–31 mL. CCF = cell culture fluid (= cell culture broth).

As samples with different volumes between 25 and 31 mL were compared, the relative mAb yield was calculated (Equation 1).

Turbidity values were measured before and after clarification by using the TurbiCheck WL turbidimeter from Lovibond (white light source). Afterwards the reduction of turbidity was determined by calculation of the ratio of values from harvested and unharvested samples.

Results and Discussion

The goal of the study was to compare the suitability of two different syringe filter models for clarification of mAb supernatants in regard to particle reduction, mAb recovery, yield, and consumption of filter units.

For the experiments, we used both various cultivation systems and expression vectors. With this approach, we generated a heterogeneous range of characteristics with respect to viable cell count, viability, turbidity, mAb product, and titer (Table 1, next page). In particular, the turbidity of the cell culture at harvest ranged from 457 to 1431 NTU, the viable cell count ranged from 4 x 10⁶ to 16 x 10⁶ cells/mL, a viability from 48 to 89%, and mAb titers (cell culture) between 0.4 and 8.8 mg/mL. This diversity was the prerequisite for a robust statement in terms of the syringe filter suitability.

To determine the particle reduction, we examined the turbidity of the cell culture and the filtrate. We found that both filter models removed particles efficiently from the supernatant. The filtrate of the Minisart® High Flow showed an average of 17.6 NTU, and other premium brands showed an average turbidity of 17.7 NTU. Considering the entire clarification process, including centrifugation and filtration, this leads to a relative reduction in turbidity between 93.8 and 98.8%. Remarkably, the turbidity in the filtrate does not depend on the initial turbidity of the cell culture (Figure 3).
The various cell culture samples had titers of monoclonal antibodies in a range of 0.2 to 8.8 g/L. The mAb titers of the filtrate ranged from 0.2 to 8.2 g/L for both manufacturers, resulting in recovery rates between 89.9 and 103.9% (average: 97.7%) for Minisart® High Flow and 86.9 and 107.3% (average: 98.2%) for the other premium brand. It should be emphasized that the recovery was independent of the cell culture titer (Figure 3). This is important when regularly monitoring mAb titers during cultivation with different levels of product concentrations.

Table 1: Overview of various sample types (expression vectors| mAb products) and their parameters such as cultivation system (STR = stirred tank reactor and SF = shake flask), viable cell count (VCC) and viability after 14 days, and turbidity of the cell culture at harvest. Clarification tests were run with both syringe filters, so that the respective volume was clarified with both variants to obtain an objective comparison.

		[10^5 cells/mL]		cell culture [NTU]	cell culture [mg/mL]	volume [mL]
V1 | IgG1	STR (5 L)	86.9	58%	1431	7.8	31
V1 | IgG1	STR (5 L)	155.2	78%	1355	6.0	31
V1 | IgG1	STR (5 L)	163.6	89%	828	8.8	31
V2 | fc fusion protein	SF (25 mL in 125 mL)	121.0	71%	1031	0.2	25
V3 | IgG1	SF (25 mL in 125 mL)	73.0	64%	508	0.9	25
V4 | fc fusion protein	SF (25 mL in 125 mL)	47.7	67%	457	0.4	24
V5 | IgG2	SF (25 mL in 125 mL)	112.6	67%	873	0.7	23
V6 | fc fusion protein	SF (300 mL in 1 L)	42.2	69%	701	1.8	25
V6 | fc fusion protein	SF (300 mL in 1 L)	43.5	62%	834	1.2	25
V7 | IgG2	SF (300 mL in 1 L)	38.3	48%	821	0.4	25
V8 | IgG1	SF (300 mL in 1 L)	69.9	73%	558	1.6	25
V9 | IgG1	SF (300 mL in 1 L)	52.3	59%	669	0.3	25
V10 | bispecific antibody	SF (300 mL in 1 L)	46.1	69%	671	0.6	25

The relative yield per sample was the same for both syringe filter models, despite differences in housing design and number of filters used per sample (Figure 4).

In terms of throughput and filter consumption, we determined for each sample the volume of the supernatant, the volume of the total filtrate, and the required number of filter units. The average throughput for Minisart® High Flow was 18.0 mL and for the other premium brand was 9.3 mL and (Figure 5). This 100% discrepancy cannot be explained by the small difference in the effective filtration area (Minisart® High Flow: 6.2 cm², other premium brand: 5.8 cm²). More likely, differences in the structural design of the polyethersulfon membrane utilized in the devices could be the reason for this observation. In consequence, we found an average filter consumption per sample of 2.5 pcs for Minisart® High Flow and 1.4 pcs for the other premium brand.

These results demonstrate that the Minisart® High Flow allows the filtration of larger volumes before clogging, while other parameters like turbidity, mAb recovery, and relative yield showed the same high performance.

Conclusion

Microfiltration is most commonly an indispensable step after centrifugation of a cell culture sample. When processing a small number of samples with volumes < 25 mL, syringe filters are often the perfect choice. Using the right filter can significantly facilitate the task and reduce the number of devices needed. In this study, we compared two different filter models with slight differences in filtration areas (Sartorius Minisart® High Flow: EFA = 6.2 cm² and other premium brand: EFA = 5.8 cm²). However, no significant difference in terms of turbidity, recovery, and yield per sample could be found. What could be observed is a clear impact of the filter model on the filtration performance. With its 7% larger filtration area, the Minisart® High Flow achieved a 94% higher filtrate volume per device and thus halved the number of filter units per sample. This finding emphasized that the efficiency of supernatant clarification can be improved substantially without impairment of other relevant parameters.

Acknowledgment

Special thanks go to Dr. Noushin Delmdahl and Dr. Andrea Friße for reviewing the manuscript and for their constructive discussion on this subject.

Abbreviations

CHO Chinese hamster ovary
mAb monoclonal antibody
EFA effective filtration area
PES polyethersulfon
NTU nephelometric turbidity units

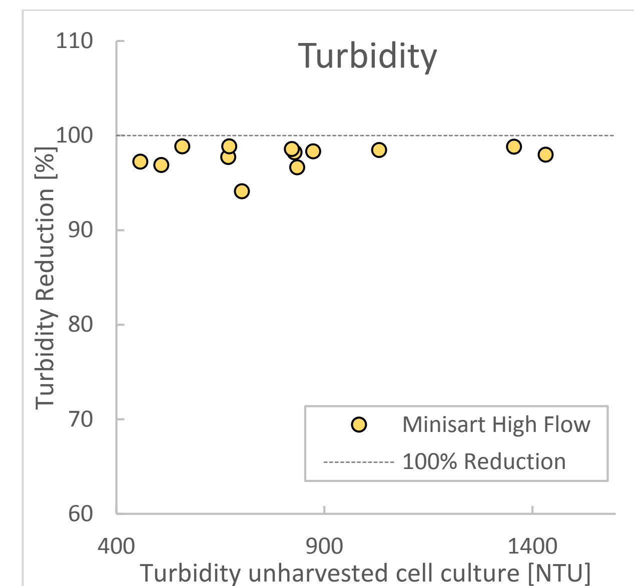


Figure 2: Turbidity reduction [%] in relation to the turbidity of the unclarified cell culture. The clarification procedure comprises a centrifugation and a microfiltration step. The reduction of the turbidity does not depend on the turbidity of the cell culture. This is valid for the Minisart® High Flow as well as for the other premium brand (figure not shown because the data points are virtually the same).

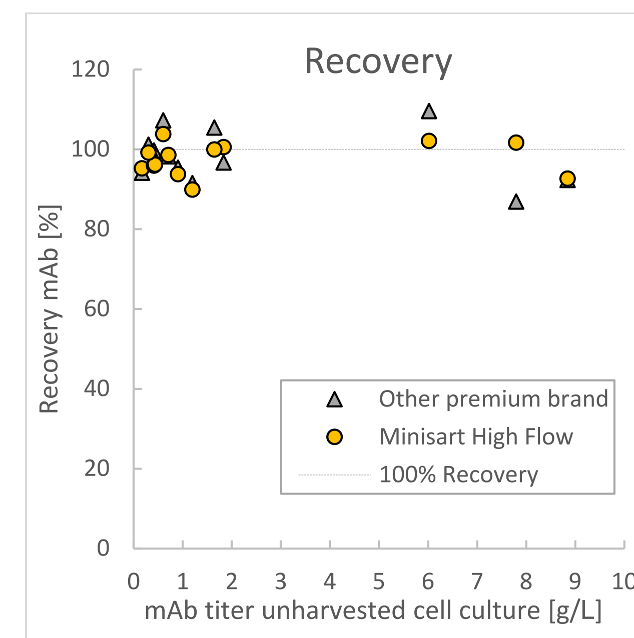


Figure 3: The recovery [%] of mAb products in relation to the mAb titer of the unclarified cell culture. The recovery was not influenced by the syringe filter model and was on average at 97.7% for the Minisart® High Flow and at 98.2% for the other premium brand. No impact of the syringe filter used on the mAb recovery was observed in a range of 0.3 to 8.8 g/L of the cell culture titer.

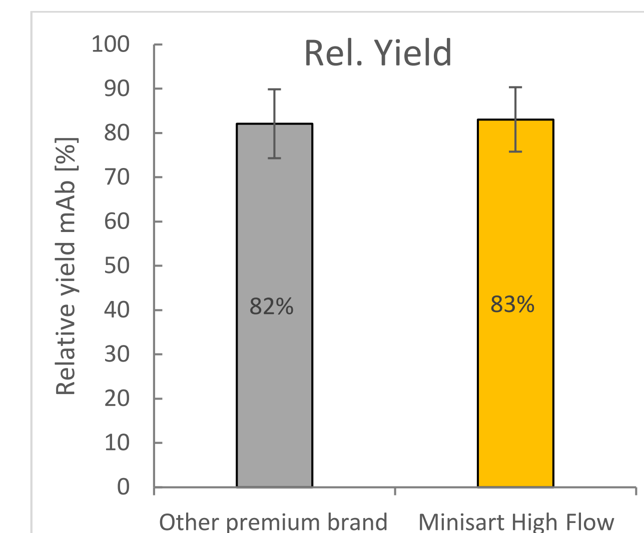


Figure 4: Average values of the relative yield [%] of various mAb products. The relative yield of a sample is the relation of the total mAb amount in the filtrate and in the unharvested cell culture. As a result, we found no difference between both filter models irrespective of the syringe filter design and consumption.

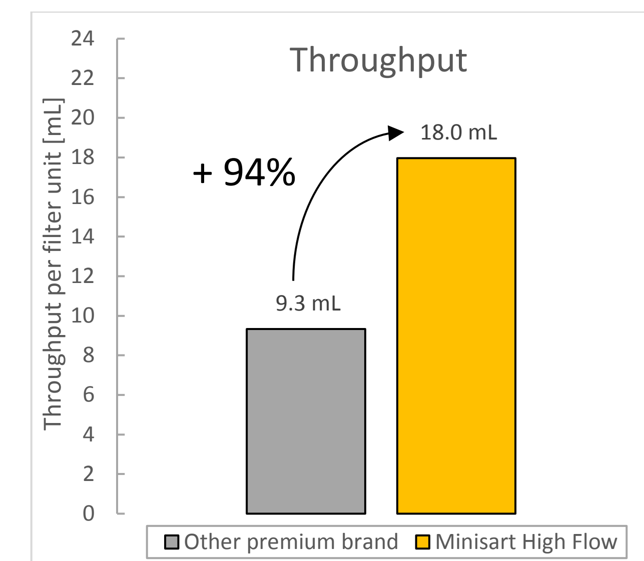


Figure 5: CHO cell culture supernatants were filtered through two different syringe filter models: Sartorius Minisart® High Flow and other premium brand. The average throughput per filter unit was determined. Differences between both filter models in throughput were probably not caused by the deviation in effective filtration area (Minisart® High Flow: 6.2 cm², other premium brand: 5.8 cm²) but most likely by differences in structural membrane design.

Correlation Between Colony Forming Units and Genome Copies of 9 Different *Mycoplasma* Species Using Quantified CFU and GC Standards for Validation

Caroline Paeschke[1], Dr. Alexandra Mueller-Scholz[1*], Dr. Susanne Roederstein[2], Kai Nesemann[2]

1.Research and Development Department, Sartorius Stedim Biotech, Göttingen, Germany
2. Department Microbiology, Sartorius Lab Instruments, Göttingen, Germany
*Correspondence:
E-mail: PCR@Sartorius.com

Abstract

In this study, the correlation between genome copies (GC) and colony forming units (CFU) of 9 different *Mycoplasma* species has been investigated using Sartorius' quantified CFU and GC standards. As PCR technology only detects genome copies (GC), a correlation between colony forming units (CFU) and GC is required by different authorities, i.e., the Korean Food and Drug Administration (KFDA) or the Pharmaceuticals and Medical Devices Agency (PMDA), Japan, before an assay is accepted to be used in quality control of cell culture products. The results of this study show that the number of genome copies vary between *Mycoplasma* species, but have successfully been correlated to 20 CFU and 40 CFU respectively.

Introduction

Mycoplasma are the smallest free-living organisms. They belong to the bacterial class Mollicutes, which are distinguished by their lack of cell wall. For that reason they are unaffected by many commonly antimicrobial agents such as beta-lactam antibiotics [1]. Mycoplasma are widespread contaminants in cell culture. In fact, depending on the laboratory, 10% to 85% of cell lines may be contaminated [2]. Due to their extremely basic genomes, mycoplasma live as parasites. They compete with host cells for biosynthetic precursors and nutrients and can alter DNA, RNA, and protein synthesis and induce chromosomal alterations [2]. Given their tiny size (~0.3 µm–0.8 µm) mycoplasma contamination cannot be visualized with a light microscope [1]. Moreover, altered growth rates and morphological changes in infected cell cultures can be minimal or unapparent. Furthermore, mycoplasma-contaminated products represent a human health risk [2]. All these facts show clearly the high demand of routine mycoplasma detection.

Microsart® ATMP Mycoplasma enables a reliable and sensitive detection of mycoplasma DNA in cell cultures and cell culture derived biologicals, like autologous transplants, according to European Pharmacopeia 2.6.7. Regulations require comparability studies with compendial growth based methods. As PCR technology only detects genome copies (GC), a correlation between colony forming units (CFU) and GC is required by different authorities, i.e., the Korean Food and Drug Administration (KFDA) or the Pharmaceuticals and Medical Devices Agency (PMDA), Japan.

In this study, the correlation between CFU and GC of 9 different *Mycoplasma* species was investigated using Sartorius' quantified CFU and GC standards to facilitate implementation and approval of qPCR-based *Mycoplasma* detection methods.

Materials and Methods

DNA Extraction of Microsart® Validation Standard

Each package of Microsart® Validation Standard contains 3 vials, each containing 10 CFU of the chosen *Mycoplasma* species. 250 µL Dulbecco's Modified Eagle Medium (DMEM) + 10% fetal bovine serum (FBS) were added to two vials to prepare a suspension with a concentration of 40 CFU/mL. 500 µL DMEM +10% FBS were added to one vial to prepare a suspension with a concentration of 20 CFU/mL. The DNA of the cell suspensions was extracted with Microsart® AMP Extraction Kit in duplicates according to the protocol. The eluate was used directly for Microsart® ATMP Mycoplasma qPCR.

Microsart® ATMP Mycoplasma qPCR

All lyophilized components were rehydrated. For one reaction, 15 µL of Mycoplasma Mix were mixed with 1 µL Internal Control DNA. 15 µL of this mix were added to each PCR tube. Each test was carried out with at least two Non-Template Controls (NTC) and samples in duplicate. Therefore 10 µL of sample or NTC were added to the PCR tubes with Master Mix respectively.

Standard Curve with Microsart® Calibration Reagent

To quantify the DNA extracts of Microsart® Validation Standards, it is necessary to generate a standard curve with known concentrations of genome copies (GC). Therefore Microsart® Calibration Reagents were used. The calibration reagents contain 10^6 GC/µL of the specific organism after rehydration. Dilution series havebeen prepared in Tris-buffer to achieve final concentrations of 5 GC/10 µL to 500 GC/10 µL.

Microsart® ATMP Mycoplasma qPCR was performed on the CFX96 Touch Cycler (Bio-Rad; 45 cycles, 3 min 95°C, 30 s 95°C, 30 s 55°C, 45 s 60°C). The mycoplasma DNA is indicated by an increasing fluorescence signal in the FAM® channel. Internal Control DNA is detected in the ROX® channel in the same tube to indicate a successful reaction in every individual PCR tube. The analysis of the reaction was done with the CFX Manager Software (Bio-Rad). The limit of detection of all *Mycoplasma* species listed in the EP/USP is ≤ 10 CFU/mL.

Table 1: Product overview of Microsart® Validation Standards and Microsart® Calibration Reagents for different *Mycoplasma* species.The Validation Standard contains 10 CFU per vial, the Calibration Reagent contains 10^6 GC/µL after rehydration.

Mycoplasma species	NCTC code	ATCC code	Catalog No. Microsart® Validation Standard	Catalog No. Microsart® Calibration Reagent
Mycoplasma arginini	10129	23838	SMB95-2011	SMB95-2021
Mycoplasma orale	10112	23714	SMB95-2012	SMB95-2022
Mycoplasma gallisepticum	10115	19610	SMB95-2013	SMB95-2023
Mycoplasma pneumoniae	10119	15531	SMB95-2014	SMB95-2024
Mycoplasma synoviae	10124	25204	SMB95-2015	SMB95-2025
Mycoplasma fermentans	10117	19989	SMB95-2016	SMB95-2026
Mycoplasma hyorhinis	10130	17981	SMB95-2017	SMB95-2027
Acholeplasma laidlawii	10116	23206	SMB95-2018	SMB95-2028
Spiroplasma citri	10164	27556	SMB95-2019	SMB95-2029

Results and Discussion

Figures 2 and 3 show exemplary amplification plots of *A. laidlawii*. On basis of the ct values (FAM® channel) and concentrations of the standards, the CFX Manager software created a standard curve (Figure 1) with a linear equation. A regression coefficient of 0.983 is an indication for a good standard curve. The efficiency of an optimal qPCR is 100%. In that case the amplicon DNA will be doubled in each cycle. According to the analysis of the CFX Software, the exemplary qPCR run of *A. laidlawii* ran highly efficient with an efficiency of 101% (see efficiency in Figure 1).

Each sample and Non-Template Control (NTC) showed an amplification of the internal control DNA and consequently fluorescence signal in the ROX® channel (Ct<40; data not shown). A successful PCR without inhibition was indicated. The NTC did not show a fluorescence signal in the FAM® channel, as expected (see Figures 2 and 3). Consequently a mycoplasma-free preparation of the PCR reactions without cross-contamination was indicated.

20 CFU/mL and 40 CFU/mL of each *Mycoplasma* species have been detected successfully in all samples (Assay LOD is ≤ 10 CFU/mL; determined during kit validation).

Based on the linear equation of the standard curve, the software calculated the GC concentrations of the mycoplasma samples (20 CFU/mL and 40 CFU/mL extracts). In Table 2, the average GC to CFU ratios of 9 different *Mycoplasma* species are shown.

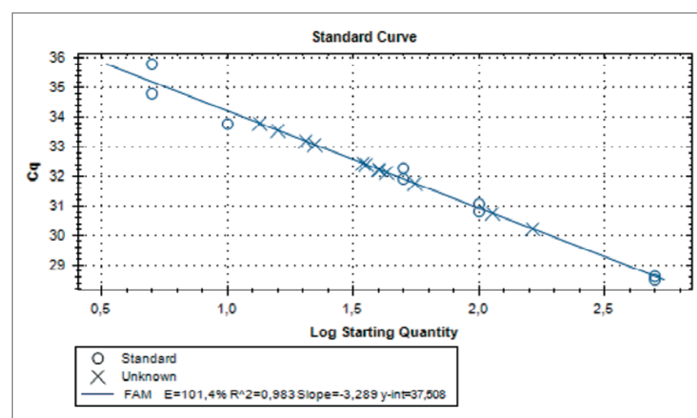


Figure 1: Exemplary standard curve of *Acholeplasma laidlawii* (Microsart® Calibration Reagent), using final genome copy (GC) concentrations of 5 GC/10 µL to 500 GC/10 µL generated with Microsart® ATMP Mycoplasma.

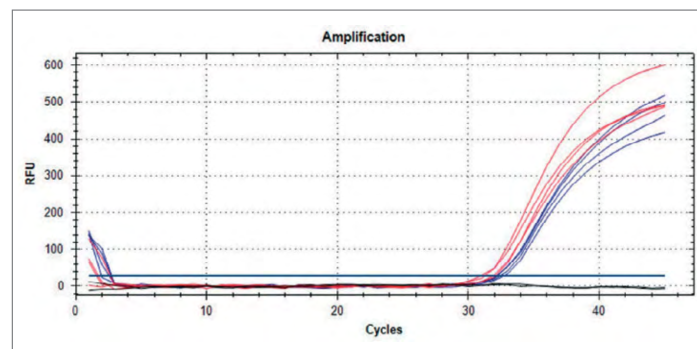


Figure 2: Exemplary amplification plot of *Acholeplasma laidlawii*, generated with Microsart® ATMP Mycoplasma qPCR. Fluorescence signals in FAM® channel. Black Lines: Non-Template Control (NTC). Blue Lines: 20 CFU/mL of *A. laidlawii*. Red Lines: 40 CFU/mL of *A. laidlawii*.

The study indicated that the GC/CFU ratio varied from species to species and lies within a range of 9 GC/CFU to 68 GC/CFU after DNA extraction.

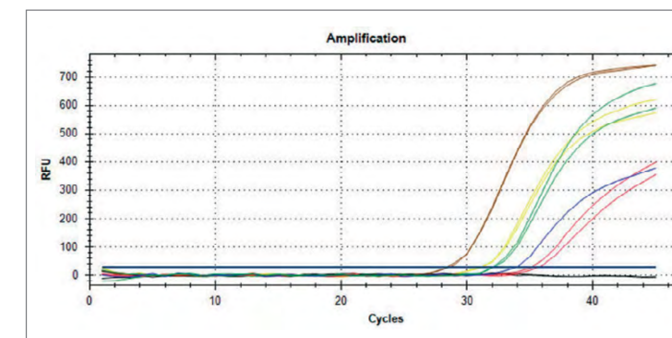


Figure 3: Exemplary amplification plot of *Acholeplasma laidlawii*, generated with Microsart® ATMP Mycoplasma qPCR. Fluorescence signals in FAM® channel. Black Lines: Non-Template Control (NTC). Red: 5 GC/PCR. Blue: 10 GC/PCR. Green: 50 GC/PCR. Yellow: 100 GC/PCR. Brown: 500 GC/PCR.

Table 2: Average GC to CFU ratio of 9 different *Mycoplasma* species.

Mycoplasma Species	Average GC to CFU ratio
Mycoplasma arginini	1.1 × 10 GC/CFU
Mycoplasma orale	3.5 × 10 GC/CFU
Mycoplasma gallisepticum	1.7 × 10 GC/CFU
Mycoplasma pneumoniae	4.3 × 10 GC/CFU
Mycoplasma synoviae	0.9 × 10 GC/CFU
Mycoplasma fermentans	1.2 × 10 GC/CFU
Mycoplasma hyorhinis	0.9 × 10 GC/CFU
Acholeplasma laidlawii	5.6 × 10 GC/CFU
Spiroplasma citri	6.8 × 10 GC/CFU

Discussion

In this study, the correlation between genome copies (GC) and colony forming units (CFU) of 9 different *Mycoplasma* species has been investigated using Sartorius' quantified GC and CFU standards. A correlation between CFU and GC is required by different authorities, i.e., the Korean Food and Drug Administration (KFDA) or the Pharmaceuticals and Medical Devices Agency (PMDA), Japan, for assay approval. These results demonstrate a good data basis. Nevertheless it should be kept in mind that significant variations within the GC:CFU ratio might be observed if other media | matrices are used for the CFU spikes, which affects the DNA isolation efficiency, or if other conditions are used for correlation. The results of this study indicate a higher GC than CFU number, as expected. The theoretical GC:CFU ratio should be 1:1, as 1 GC per cell should ideally be detected. Practically, this ratio is not realizable even if mycoplasma cultures are harvested during early logarithmic growth to prevent detection of DNA from dead cells in the preparation. This non-equal ratio arises because a significant number of the mycoplasma cells would not grow to a colony in culture and remain undetected (i.e., stressed or viable but non-culturable cells). Furthermore, mycoplasma cells tend to form agglomerates, which would be detected as 1 CFU, but in fact combine several cells and therefore several GC. Both scenarios lead to a significant underestimation of the realistic mycoplasma cell number in the sampled cell culture, as only a portion of the cells would grow to form a CFU. Non-culturable species or viable but non-culturable cells could lead to false-negative results using a growth-based method. Undetected mycoplasma contamination because of false-negative results in growth-based methods can result in unsafe products with potential infection risks, especially for patients with immunodeficiency. This study shows that the correlation between GC and CFU can successfully be demonstrated and easily be implemented during validation. Furthermore, detection of GC by PCR shows a more realistic result of the real contamination level in the respective sample and therefore directly contributes to drug safety.

References

1. Drexler HG, Uphoff CC. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 2002;39(2):75-90. doi:10.1023/A:1022913015916.
2. Olarerin-George AO, Hogenesch JB. Assessing the prevalence of mycoplasma contamination in cell culture via a survey of NCBI's RNA-seq archive. Nucleic Acids Research. 2015;43(5):2535-2542. doi:10.1093/nar/gkv136.

Scouting Protein Purification Conditions Using Vivapure Centrifugal Ion Exchange Membrane Absorbers

C. Naumann and N. Kashani-Poor

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.

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 x 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

Procedure

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 reached. IPTG was added to a final concentration of 1 mM and incubated for further 4 h with shaking at 150 rpm. Cells were harvested by centrifugation at 4000 x 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 were removed by centrifugation at 10000 x g for 30 min at 4°C.

Sample Preparation

4 x 200 µL of the cell lysate were 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 x Q and 4 x 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 x 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 x g.

Afterwards, Vivapure Mini spin columns were reloaded with 400 µL sample and spun again for 5 min at 2000 x 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 x g. Flow-through and wash fractions were collected for subsequent detection of the target protein.

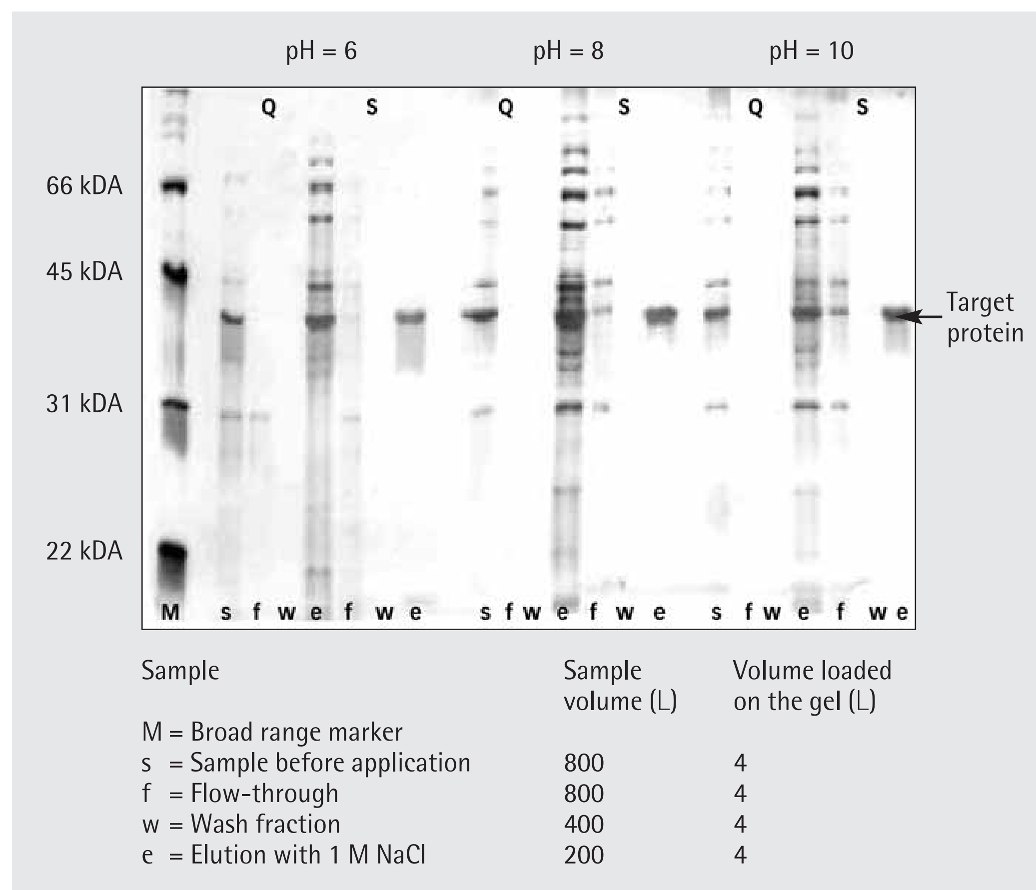


Figure 1: Scouting for optimal binding conditions of a SH2 domain expressed in *E. coli.* SDS gel (reducing, 12%), silver stained. Shown are sample before loading, flow-through, wash, and elution fractions (1 M NaCl) from Vivapure Q and S Mini spin columns, at the various pH values tested.

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 x 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").

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 flow-through, 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.

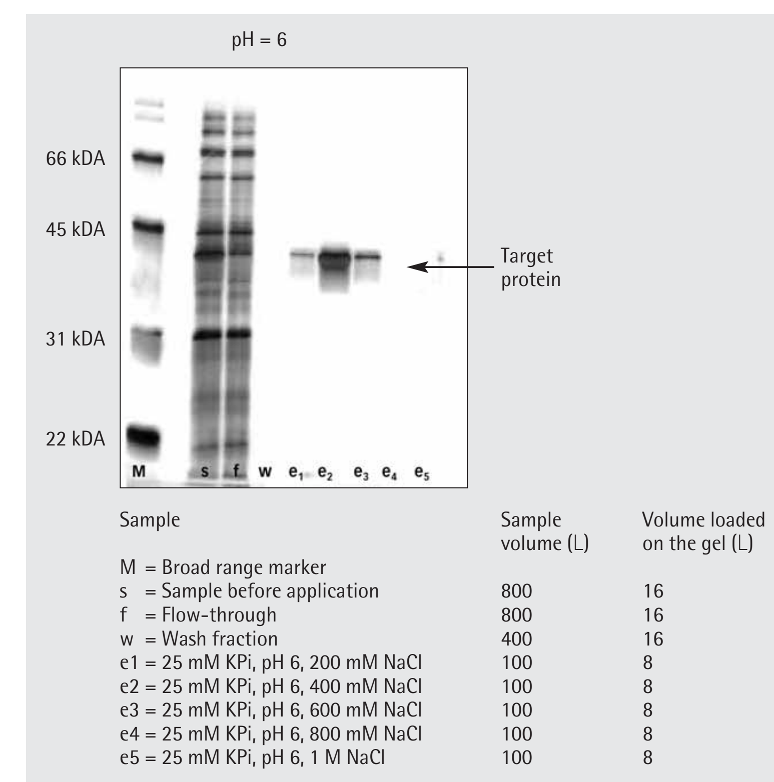


Figure 2: Scouting for optimal elution conditions of a SH2 domain expressed in *E. coli.* SDS gel (reducing, 12%), silver stained. Sample before loading, flow-through, wash, and elution fractions from Vivapure S Mini spin column at pH 6 are shown.

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 x 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 x g. Afterwards, the Vivapure S Mini spin column was reloaded with 400 µL sample and spun again for 5 min at 2000 x g.

Loosely bound proteins were washed away by application of 400 µL binding buffer to the column and spun for 5 min at 2000 x 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 x 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 x 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 dueto the low elution volume.

Discussion

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.

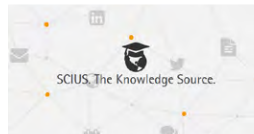
Trainings

Customized Training for Lab Professionals. With our Lab Academies we strive to offer a beneficial selection of training courses in pipetting, filtration, lab weighing and lab water purification. For the most effective learning, our training sessions are custom-tailored to target your specific needs and to enable you to excel in your daily tasks. Contact us today to advance your expertise. https://promotions.sartorius.com/passionforscience/trainings

Sartorius Pipetting Academy. Sartorius Pipetting Academy offers training and content aimed at not only beginners to pipetting but also to experienced lab professionals. Pipetting Academy modules are a balance of theory and hands-on sessions. They are packed with tips and tricks that help you get the most out of your pipetting. Join the Pipetting Academy now. https://promotions.sartorius.com/pipetting-academy

Life Science & Biopharma Training Courses. At different training sites we designed methodical training courses in the life sciences and biopharma sectors. The theoretical part will help you better understand the background on your application and Sartorius products. In the practical part, you can apply what you have just learned and quickly assimilate procedural methods. https://www.sartorius.com/en/knowledge/trainings

The Knowledge Source



Concentrated Scientific Knowledge at a Glance. Take your tour through "SCIUS" and take advantage of our comprehensive source of scientific expert knowledge. Discover scientific publications, application notes, expert lectures, webinars, and many more valuable resources to support your big ideas and small daily routines alike. Start your SCIUS search now. https://promotions.sartorius.com/passionforscience/scius-the-knowledge-source

Prize

The Sartorius & *Science* Prize for Regenerative Medicine & Cell Therapy. The Sartorius & *Science* Prize for Regenerative Medicine & Cell Therapy is an annual prize aimed at supporting and encouraging scientists focused on basic or translational research that advances medical progress in regenerative medicine and cell therapy. Are you a representative of the upcoming generation of thought leaders in your field? Then go ahead and apply in April 2019. https://promotions.sartorius.com/passionforscience/sartorius-and-science-prize

Guides

Sartorius Cubis® Premium Balances. In this guide you will find an overview of various weighing applications from the fields of biopharma, pharma, experimental medicine and chemical engineering published in peer-reviewed academic journals. https://goo.gl/JneXLA

Products

Avoid Sample Transfer and Loss with Cubis® High-Capacity Micro Balances. Weigh minimum amounts of sample directly into heavy flasks: With Cubis® High-Capacity Micro Balances, you can add your small samples directly to large containers of up to 250 mL and avoid sample losses and errors in your experiments. https://promotions.sartorius.com/cubis-high-capacity-micro-balances

Speed Up Your Clarification and Sterile Filtration up to 70%. Sartoclear Dynamics® Lab kits have been designed for harvesting 15 mL to 1000 mL volumes of cell cultures in the lab. They enable clarification and sterile filtration to be performed in one step, quickly and easily. https://promotions.sartorius.com/sartoclear-dynamics-lab

sartorius

arium® mini: The Only Ultrapure Water System with Integrated Bagtank Technology

Easy to operate with touch screen activated color display, the compact arium® mini delivers reliable and reproducible results.

Its unique bagtank technology can save valuable time in cleaning and prevent permanent biofilm, ensuring the highest water quality for your applications.

To find out more, visit:
www.sartorius.com/arium-mini

Take Your Microwell Plate Pipetting Back to the Comfort Zone

Looking for ways to speed up Microwell plate pipetting without errors?

Experience the Picus® with the unique Plate Tracker feature that keeps track of where you need to pipette next. In combination with the multi-dispensing mode, plate work has never been easier and faster!

To find out more, visit:
www.sartorius.com/picus