Volume 33 Number 5

BioPharm INTERNATIONAL®

May 2020

The Science & Business of Biopharmaceuticals

www.biopharminternational.com

GENE THERAPIES PUSH VIRAL VECTOR PRODUCTION

UPSTREAM PROCESSING
SYNTHETIC BIOLOGY

DOWNSTREAM PROCESSING
FLUID HANDLING CONSIDERATIONS

MANUFACTURING
CLOSED FILLING SYSTEMS

ANALYTICS
BIOASSAY DEVELOPMENT

QUALITY/REGULATIONS
REMOTE AUTOMATION PRACTICES

OUTSOURCING
CONTINUOUS BIOPROCESSING

PEER-REVIEW RESEARCH
IR SPECTROSCOPY FOR BIOCHARACTERIZATION

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

AVTR
LISTED
NYSE

QUALITY

MOVES NEW THERAPIES ***FORWARD***

Whether it's a vaccine, recombinant protein, or gene therapy, Avantor® can help transform how these therapies are brought to market, combining our unique workflow expertise with advanced biologic products engineered to satisfy the highest quality standards and regulatory requirements.

We help enable breakthroughs in life-changing biologics.

avantorsciences.com/moves-biopharma-forward

ADVERTISING

Publisher **Mike Tracey** MTracey@mjhlifesciences.com
National Sales Manager **Scott Vail** SVail@mjhlifesciences.com
European Sales Manager **Linda Hewitt** LHewitt@mjhlifesciences.com
European Senior Sales Executive **Stephen Cleland** SCleland@mjhlifesciences.com
VP/Managing Director, Pharm/Science Group **Dave Esola** DEsola@mjhlifesciences.com
C.A.S.T. Data and List Information **Michael Kushner** MKushner@mjhlifesciences.com

AUDIENCE DEVELOPMENT

Audience Development **Christine Shappell** cshappell@mjhlifesciences.com

MJH LIFE SCIENCES™

Chairman and Founder **Mike Hennessy, Sr**
Vice Chairman **Jack Lepping**
President and CEO **Mike Hennessy, Jr**
Chief Financial Officer **Neil Glasser, CPA/CFE**
Executive Vice President, Operations **Tom Tolvé**
Executive Vice President, Global Medical Affairs and Corporate Development **Joe Petroziello**
Senior Vice President, Audience Generation and Product Fulfillment **Joy Puzzo**
Senior Vice President, Content **Silas Inman**
Senior Vice President, I.T. & Enterprise Systems **John Moricone**
Vice President, Human Resources & Administration **Shari Lundenberg**
Vice President, Business Intelligence **Chris Hennessy**
Executive Creative Director, Creative Services **Jeff Brown**

© 2020 MultiMedia Pharma Sciences LLC All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical including by photocopy, recording, or information storage and retrieval without permission in writing from the publisher. Authorization to photocopy items for internal/educational or personal use, or the internal/educational or personal use of specific clients is granted by MultiMedia Pharma Sciences LLC for libraries and other users registered with the Copyright Clearance Center, 222 Rosewood Dr. Danvers, MA 01923, 978-750-8400 fax 978-646-8700 or visit http://www.copyright.com online. For uses beyond those listed above, please direct your written request to Permission Dept. Alexa Rockenstein, arockenstein@mjhlifesciences.com. **Reprints:** Contact Michael Tracey, mtracey@mjhlifesciences.com.

MultiMedia Pharma Sciences LLC provides certain customer contact data (such as customers' names, addresses, phone numbers, and e-mail addresses) to third parties who wish to promote relevant products, services, and other opportunities that may be of interest to you. If you do not want MultiMedia Pharma Sciences LLC to make your contact information available to third parties for marketing purposes, simply email mmhinfo@mjhlifesciences.com and a customer service representative will assist you in removing your name from MultiMedia Pharma Sciences LLC lists.

BioPharm International does not verify any claims or other information appearing in any of the advertisements contained in the publication, and cannot take responsibility for any losses or other damages incurred by readers in reliance of such content.

BioPharm International welcomes unsolicited articles, manuscripts, photographs, illustrations, and other materials but cannot be held responsible for their safekeeping or return.

To subscribe, email mmhinfo@mmhgroup.com

EDITORIAL

Editorial Director **Rita Peters** RPeters@mjhlifesciences.com
Senior Editor **Agnes M. Shanley** AShanley@mjhlifesciences.com
Managing Editor **Susan Haigney** SHaigney@mjhlifesciences.com
European Editor **Felicity Thomas** FThomas@mjhlifesciences.com
Science Editor **Feliza Mirasol** FMirasol@mjhlifesciences.com
Manufacturing Editor **Jennifer Markarian** JMarkarian@mjhlifesciences.com
Assistant Editor **Lauren Lavelle** LLavelle@mjhlifesciences.com
Senior Art Director **Marie Maresco**
Graphic Designer **Maria Reyes**

EDITORIAL ADVISORY BOARD

BioPharm International's Editorial Advisory Board comprises distinguished specialists involved in the biologic manufacture of therapeutic drugs, diagnostics, and vaccines. Members serve as a sounding board for the editors and advise them on biotechnology trends, identify potential authors, and review manuscripts submitted for publication.

K. A. Ajit-Simh
President, Shiba Associates

Madhavan Buddha
Freelance Consultant

Rory Budihandojo
Director, Quality and EHS Audit
Boehringer-Ingelheim

Edward G. Calamai
Managing Partner
Pharmaceutical Manufacturing and Compliance Associates, LLC

Suggy S. Chrai
President and CEO
The Chrai Associates

Leonard J. Goren
Global Leader, Human Identity Division, GE Healthcare

Uwe Gottschalk
Vice-President,
Chief Technology Officer,
Pharma/Biotech
Lonza AG

Fiona M. Greer
Global Director,
BioPharma Services Development
SGS Life Science Services

Rajesh K. Gupta
Vaccinnologist and Microbiologist

Denny Kraichely
Associate Director
Johnson & Johnson

Stephan O. Krause
Director of QA Technology
AstraZeneca Biologics

Steven S. Kuwahara
Principal Consultant
GXP BioTechnology LLC

Eric S. Langer
President and Managing Partner
BioPlan Associates, Inc.

Howard L. Levine
President
BioProcess Technology Consultants

Hank Liu
Head of Quality Control
Sanofi Pasteur

Herb Lutz
Principal Consulting Engineer
Merck Millipore

Hanns-Christian Mahler
Head Drug Product Services
Lonza AG

Jerold Martin
Independent Consultant

Hans-Peter Meyer
Lecturer, University of Applied Sciences and Arts Western Switzerland, Institute of Life Technologies

K. John Morrow
President, Newport Biotech

David Radspinner
GE Healthcare

Tom Ransohoff
Vice-President and Senior Consultant
BioProcess Technology Consultants

Anurag Rathore
Biotech CMC Consultant
Faculty Member, Indian Institute of Technology

Susan J. Schniepp
Executive Vice President of Post-Approval Pharma and Distinguished Fellow
Regulatory Compliance Associates, Inc.

Tim Schofield
Consultant
CMC Sciences, LLC

Paula Shadle
Principal Consultant,
Shadle Consulting

Alexander F. Sito
President,
BioValidation

Michiel E. Ultee
Principal
Ulteemit BioConsulting

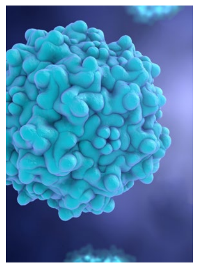
Thomas J. Vanden Boom
VP, Biosimilars Pharmaceutical Sciences
Pfizer

Krish Venkat
Managing Partner
Anven Research

Steven Walfish
Principal Scientific Liaison
USP

FOR PERSONAL NON-COMMERCIAL USE

BioPharm International integrates the science and business of biopharmaceutical research, development, and manufacturing. We provide practical, peer-reviewed technical solutions to enable biopharmaceutical professionals to perform their jobs more effectively.



COVER STORY

12 Gene Therapies Push Viral Vector Production

Viral vectors show promise as a delivery mechanism for gene therapy, but which virus types are commercially viable?

Cover Design by Maria Reyes. Images: Kateryna_Kon - Stock.adobe.com

FEATURES

UPSTREAM PROCESSING

Biopharmaceutical Manufacturing and the Power of Synthetic Biology
David McElroy
Synthetic biology has advanced the scope and scale with which biologically derived therapeutics can be developed. 22

DOWNSTREAM PROCESSING

Fluid Handling Considerations
Jennifer Markarian
Pumps and other components meet the demands of single-use systems in biopharmaceutical downstream processing. 28

MANUFACTURING

Straight Talk on Closed Aseptic Systems
James Agalloco
The industry's aversion to risk has led to its treating closed aseptic processing systems as miniature cleanrooms, resulting in redundant and expensive practices. 32

ANALYTICS

Building Better Bioassays
Cynthia A. Challener
Next-generation therapeutics and regulatory requirements create demand for complex, fit-for-purpose tests. . . . 37

PEER-REVIEW RESEARCH

Shaping IR Spectroscopy into a Powerful Tool for Biopharma Characterizations
Dipanwita Batabyal, Libo Wang, Jeffrey Zonderman, and Mats Wikström
The authors present experimental studies showcasing the performance of microfluidic modulation spectroscopy in the secondary structure characterization of biopharmaceutical products 42

QUALITY/REGULATIONS

Good Automation Practices for Remote Operations
Lauren Lavelle
Having remote operations in place is crucial to maintaining good automation practices. 48

OUTSOURCING

Biomanufacturing: Demand for Continuous Bioprocessing Increasing
Eric S. Langer
But are innovations sufficient to increase adoption? CMOs are demanding better continuous bioprocessing options. . . . 51

COLUMNS AND DEPARTMENTS

FROM THE EDITOR
Will moving at "warp speed" to develop a vaccine impact efficacy or safety?
Rita Peters. 6

REGULATORY BEAT
Policy makers seek to ensure supplies of new therapies and to limit shortages.
Jill Wechsler 8

CELL THERAPIES PIPELINE 10

PRODUCT SPOTLIGHT 56

AD INDEX. 57

ASK THE EXPERT
Having a better understanding about compliance will be of benefit when looking for a job or for furthering one's career.
Siegfried Schmitt 58

SPONSORED CONTENT

BIOPHARMA INSIGHTS

Challenges in Bulk Drug Substance Management
Michael Eder
Closing the gap between downstream and fill–finish.. 20

FOR PERSONAL NON-COMMERCIAL USE

BioPharm International is selectively abstracted or indexed in: • Biological Sciences Database (Cambridge Scientific Abstracts) • Biotechnology and Bioengineering Database (Cambridge Scientific Abstracts) • Biotechnology Citation Index (ISI/Thomson Scientific) • Chemical Abstracts (CAS) • Science Citation Index Expanded (ISI/Thomson Scientific) • Web of Science (ISI/Thomson Scientific)

BioPharm International ISSN 1542-166X (print); ISSN 1939-1862 (digital) is published monthly by MultiMedia Healthcare LLC 2 Clarke Drive, Suite 100, Cranbury, NJ 08512. Subscription rates: $82.95 for one year in the United States and Possessions; $112.35 for one year in Canada and Mexico; all other countries $159.60 for one year. Periodicals postage paid at Trenton, NJ 08650 and additional mailing offices. Postmaster Please send address changes to BioPharm International, PO Box 457, Cranbury, NJ 08512-0457, USA. PUBLICATIONS MAIL AGREEMENT NO. 40612608, Return Undeliverable Canadian Addresses to: IMEX Global Solutions, P. O. Box 25542, London, ON N6C 6B2, CANADA. Canadian GST number: R-124213133RT001. Printed in U.S.A.

What lies ahead isn't as concerning if you're prepared.

You set your commercial manufacturing scale and supply forecast long before you have final clinical data knowing you might over or under estimate your commercial demand. But when you leverage WuXi Biologics' scale-out manufacturing approach, which by design easily adapts to market demand fluctuations, you'll exit process validation ready for virtually any outcome. This scale-out paradigm, coupled with our industry-leading expertise, world-class quality standards and harmonized global supply chain across 4 countries greatly reduces your risk and provides the flexibility to adjust to market changes.

To learn more about our scale-out manufacturing approach:

wuxibiologics.com/scale-out

WuXi Biologics
Global Solution Provider

Rita Peters is
the editorial director of
BioPharm International.

Will moving at "warp speed" to develop a vaccine impact efficacy or safety?

How Fast Is Too Fast?

The rapid spread of the novel coronavirus is a reminder of the interconnected nature of global populations and economies. A few other realities also should be obvious. First, no single individual, company, or country can solve the pandemic crisis alone. Second, while getting a vaccine to patients quickly is crucial, decisions about quality and efficacy should be based on science, not political or public pressure.

The competition for vital medical resources in the early days of the pandemic illustrated how uncontrolled or misguided competition and anxiety can quickly overwhelm response to an emergency. Fortunately, collaborative efforts are shaping the development of treatments and vaccines. For example, the World Health Organization (WHO) is coordinating global efforts for vaccine development and clinical trials for treatment options.

In the United States, the Accelerating COVID-19 Therapeutic Interventions and Vaccines partnership is a public-private effort to develop a framework to prioritize vaccine and drug candidates, streamline clinical trials, coordinate regulatory processes, and leverage assets to respond to the COVID-19 and future pandemics. More than a dozen biopharmaceutical companies have joined the National Institutes of Health (NIH), Foundation for the NIH, Health and Human Services Office of the Assistant Secretary for Preparedness and Response, FDA, Centers for Disease Control and Prevention, and the European Medicines Agency in an effort to advance the most promising vaccine and therapeutic candidates (1).

While groups are collaborating on development and clinical trial phases; the need for a coordinated manufacturing strategy cannot be overlooked.

For an industry used to development timelines counted in years, the COVID-19 vaccine and therapy development process is moving very fast. Sponsors of the leading vaccine candidates now in early clinical trials are expressing confidence that the product can reach patients by the end of 2020, months or years earlier than predicted by experts just one week ago.

To achieve that goal, manufacturing capacity for unproven vaccines must be created now, a risky prospect for the companies developing the capacity and for patients. Drug companies and contract manufacturers are accepting the challenge; governments and taxpayers may bear the financial risk.

In April 2020, AstraZeneca and the University of Oxford announced a development and manufacturing partnership. Johnson & Johnson announced manufacturing agreements with Catalent and Emergent BioSolutions for its vaccine candidate, as did Lonza with Moderna for its mRNA vaccine. Both Johnson & Johnson and Moderna have received funding from the Biomedical Advanced Research and Development Authority.

BioPharm INTERNATIONAL
FOR PERSONAL, NON-COMMERCIAL USE

PRUDENT PATIENCE

In late April, media outlets reported a Trump Administration plan—dubbed "Operation Warp Speed"—to have 300 million doses of coronavirus vaccine available by the end of 2020 (2). Details for this program—including which vaccines would be manufactured—were not available at press time.

After weeks of stay-at-home orders, social distancing, closed restaurants, high unemployment numbers, and bad economic news, optimism about the early arrival of a vaccine, as well as treatments such as Gilead Sciences' remdesivir, offers some good news and excitement. With anxiety for a resolution to the pandemic driving vaccine development activity, however, it is crucial that science-based information—from the global bio/pharma experts—should drive development, approval, and manufacturing decisions.

REFERENCES

1. NIH, "NIH to Launch Public-Private Partnership to Speed COVID-19 Vaccine and Treatment Options," Press Release, April 17, 2020.
2. J. Jacobs and D. Armstrong, "Trump's 'Operation Warp Speed' Aims to Rush Coronavirus Vaccine," *Bloomberg.com*, April 29, 2020. ◆

Coming soon from USP Biologics

Monoclonal Antibodies helping to drive the biologics revolution

That's why we're expanding our family of Monoclonal Antibody Reference Standards (mAb RS) by adding 3 new options that are part of the same IgG1 subclass, each with unique quality attributes. These Reference Standards can be used for many non-compendial purposes that a laboratory may need, including:

- As an independent control material for method development, training, and method transfer
- As an internal assay control
- For standardization of physico-chemical testing, such as intact mass, charge heterogeneity, size variants, purity and glycan analyses

Currently Available:

USP Monoclonal IgG System Suitability RS
Catalog #: 1445550

Vial contains:
2 mg of IgG lyophilized Monoclonal Antibody

Coming Soon:

USP mAb 001 RS, Monoclonal IgG1

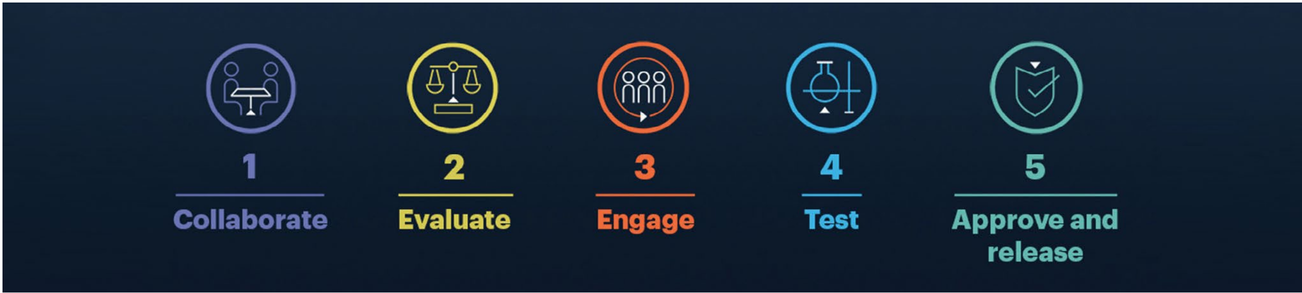
USP mAb 002 RS, Monoclonal IgG1

USP mAb 003 RS, Monoclonal IgG1

Coming soon from USP Biologics: 3 new IgG1 monoclonal antibody Reference Standards

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

When you choose USP Biologics, you're choosing quality and reliability that can only come from our rigorous and collaborative testing and development process.



Explore all 4 of our mAb RS and our full biologics catalog at
usp.org/biologics/reference-standards/igg
To contact someone directly about USP Reference Standards please email RSTech@usp.org

Regulatory Beat

Modern Drug Manufacturing Key to COVID-19 Response

Policy makers seek to ensure supplies of new therapies and to limit shortages.

Concerns about access to medicines and diagnostics critical to containing the coronavirus pandemic (COVID-19) and treating infected patients have broadened support for advanced pharmaceutical manufacturing and test methods. FDA officials have long pressed industry to adopt continuous manufacturing and online testing methods able to scale up quality production quickly and efficiently. The current pandemic has boosted support for such initiatives, as global health organizations and US policy makers recognize the importance of being able to produce millions of doses of any promising new treatments.

While the lack of personal protective gear and respirators has dominated the headlines since the pandemic outbreak, potential difficulties in ensuring sufficient supplies of newly discovered therapies and anticipated vaccines have moved to center stage. China's prominence in producing key APIs for antibiotics and many common medicines, plus a recent move by the Indian government to halt the export of multiple drugs and drug ingredients, heightened concerns about the United States' dependence on global supply chains. Rumors that an existing malaria treatment might be effective against COVID-19, for example, created a run on the drug, leading to notable shortages at some manufacturers.

Jill Wechsler is *BioPharm International's* Washington editor, jillwechsler7@gmail.com.

These concerns have spurred calls for greater US investment in high-tech biopharma production systems. In announcing the COVID-19 Therapeutics Accelerator in early March to advance treatments for the pandemic, the Bill and Melinda Gates Foundation cited the need to quickly build up manufacturing capacity to test a variety of drugs. Gates further emphasized that the federal government should provide suppport for building production facilities now, as making such investments before knowing that a product will be used is excessively risky for manufacturers.

> Potential difficulties in ensuring sufficient supplies of newly discovered therapies and anticipated vaccines have moved to center stage.

Funds to advance efficient drug manufacturing systems to ensure access to vital therapies and prevent shortages due to supply disruptions from China and elsewhere were included in the initial $8.3-billion emergency coronavirus funding package enacted March 5, 2020. FDA gained $61 million to support the development of new medical countermeasures and vaccines, of advanced manufacturing for medical products, and for monitoring medical product supply chains (1). The subsequent $2-trillion coronavirus aid and relief package approved March 27, 2020 similarly provided an added $80 million to FDA to advance the development and approval of medical countermeasures and vaccines (2). The legislation also directed FDA to use some of the funds to further the adoption of advanced manufacturing systems for medical products and to monitor supply chains for potential threats to access to medicines and APIs imported from abroad. To help FDA anticipate looming supply problems,

W.Scott McGill - Stock.adobe.com

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Congress instructed manufacturers to send FDA information on where interruptions in supply affect APIs as well as drugs and extended such requirements to medical device makers during this public health emergency. To further address these issues, Congress provided $1.5 million for the National Academies of Sciences, Medicine, and Engineering (NASEM) to prepare a study on ways to strengthen the manufacturing supply chain for drugs and devices to avoid shortages.

MILLIONS FOR MANUFACTURING

Added support for establishing advanced biopharma manufacturing facilities is found in sections of the pandemic relief legislation, which provides some $30 billion for federal health agencies to develop countermeasures and vaccines, plus platform technologies to advance US production of new therapeutics, diagnostics, vaccines, and medical supplies. Congress specified that a portion of $3.5 billion for the Biomedical Advanced Research and Development Authority (BARDA) should be used to construct or renovate US-based next-generation manufacturing facilities. Similar directions are included in providing added funds for the Defense Research Advanced Projects Agency (DARPA) in the Department of Defense, building on its research programs related to advancing biopharma manufacture. Among other things, these initiatives have supported the development of RNA and DNA vaccines to fight infectious diseases such as Chikungunya and Ebola and to overcome manufacturing challenges to faster scale-up.

The pandemic legislation also provides $27 billion for the Public Health & Social Services Emergency Fund managed by the Secretary of Health and Human Services (HHS) to develop countermeasures and vaccines in response to the pandemic. This includes building the Rapid Aseptic Packaging of Injectable Drugs (RAPID) consortium with a network of up to eight domestic facilities to rapidly fill and finish millions of prefilled syringes for delivering vaccines and therapies for COVID-19 (3). An innovative syringe developed by ApiJect Systems utilizes existing blow-fill-seal technology plus an interlocking needle hub to provide low-cost, easy-to-use injectables for the Strategic National Stockpile.

Industry is rising to the challenge, with pharma companies partnering with smaller biotechs and federal agencies.

Similarly, additional funding for the National Institute of Standards and Technology (NIST) supports programs to accelerate production of critical materials, build additional production facilities, ensure supply chains for vital ingredients, develop and train manufacturing workers, and return to the US the manufacture of critical conventional drugs (4). NIST has worked with biotech firms for several years to address challenges in developing more efficient and reliable ways to produce high quality cell and gene therapies.

These and other projects stand to assist manufacturers on accelerated timelines for testing promising COVID-19 therapies and vaccines looking to establish systems for fast, reliable manufacturing scale-up capabilities. At a "virtual summit" in March sponsored by the Biotechnology Innovation Organization (BIO), industry leaders cited the challenge in needing to expand manufacturing capabilities before knowing they have a viable product, and the fast launch of clinical trials for candidate vaccines aggravates those difficulties.

On many fronts, industry is rising to the challenge, with pharma companies partnering with smaller biotechs and federal agencies that offer innovative drug and vaccine candidates for established firms to test and produce. In March, Johnson & Johnson announced that a $1-billion partnership of its Janssen unit with BARDA planned to rapidly scale up vaccine manufacturing capacity to be able to supply over one billion doses of vaccine globally (5). Such fast expansion of production capacity will be needed for clinical trials slated to begin this fall and then to provide emergency use access to any promising product.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

REFERENCES

1. H.R. 6074, 116th Congress, Public Law 116–123—Mar. 6, 2020, Coronavirus Preparedness and Response Supplemental Appropriations Act, 2020.
2. Amendment H.R. 748 "An Act to amend the Internal Revenue Code of 1986 to Repeal the Excise Tax on High Cost Employer-Sponsored Health Coverage," US Senate, March 21, 2020.
3. HHS, "HHS Announces New Public-Private Partnership to Develop U.S.-Based, High-Speed Emergency Drug Packaging Solutions," Press Release, March 18, 2020.
4. NIST, "NIST Funding Manufacturing Institutes to Support Pandemic Response," Press Release, March 31, 2020.
5. J&J, "Johnson & Johnson Announces a Lead Vaccine Candidate for COVID-19; Landmark New Partnership with U.S. Department of Health & Human Services; and Commitment to Supply One Billion Vaccines Worldwide for Emergency Pandemic Use," Press Release, March 30, 2020. ◆

Researchers Tap Scorpion Venom for CAR T Cell Therapy

Scientists from City of Hope, a not-for-profit clinical research center, developed and tested the first chimeric antigen receptor (CAR) T-cell therapy using chlorotoxin (CLTX), an element in scorpion venom, to encourage T cells to pinpoint brain tumor cells (1).

Throughout the study, researchers found that, when using tumor cells in resection samples from a group of patients with glioblastoma (GBM) to compare CLTX attachment with targeted antigens, the CLTX attached to a large number of tumors and cells in the GBM patients. The researchers concluded that the CLTX–CAR T cells selected and killed large populations of GBM cells in cell-based assays and in animal models while disregarding non-tumors and toxicity.

"Our chlorotoxin-incorporating CAR expands the populations of solid tumors potentially targeted by CAR T cell therapy, which is particularly needed for patients with cancers that are difficult to treat, such as glioblastoma," said Christine Brown, PhD, City of Hope's Heritage Provider Network professor in Immunotherapy and deputy director of the T Cell Therapeutics Research Laboratory, in a press release. "This is a completely new targeting strategy for CAR T therapy with CARs incorporating a recognition structure different from other CARs."

"Much like a scorpion uses toxin components of its venom to target and kill its prey, we're using chlorotoxin to direct the T cells to target the tumor cells with the added advantage that the CLTX–CAR T cells are mobile and actively surveilling the brain looking for appropriate targets," added Michael Barish, PhD, City of Hope professor and chair of the Department of Developmental and Stem Cell Biology, in the press release. "We are not actually injecting a toxin but exploiting CLTX's binding properties in the design of the CAR. The idea was to develop a CAR that would target T cells to a wider variety of GBM tumor cells than the other antibody-based CARs."

FDA recently granted the cell therapy first-in-human clinical trial privileges and the study team is currently screening potential GBM patients for participation.

Reference

1. City of Hope, "From Scorpion to Immunotherapy: City of Hope Scientists Repurpose Nature's Toxin for First-Of-Its Kind Car T Cell Therapy To Treat Brain Tumors," Press Release, March 4, 2020. ◆

Daiichi Sankyo Seeks Approval for Lymphoma Car T Cell Therapy

On March 30, 2020, Daiichi Sankyo Company submitted a new drug application (NDA) to Japan's Ministry of Health, Labor and Welfare (MHLW) for the chimeric antigen receptor (CAR) T cell therapy, axicabtagene ciloleucel, for adult patients with relapsed/refractory diffuse large B-cell lymphoma and related lymphomas (1).

The NDA was submitted after the cell therapy received positive results from a global trial and a Phase II study conducted in Japan. The trials focused on individuals with four types of relapsed/refractory B-cell lymphomas, including diffuse large B cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBCL), transformed follicular lymphoma (TFL), and high-grade B cell lymphoma. The Phase II study met its primary endpoint for objective response rate. In 2018, axicabtagene ciloleucel received Orphan Drug Designation from the MHLW for the treatment of DLBCL, PMBCL, TFL, and high-grade B-cell lymphoma.

The cell therapy targets CD19, a cell membrane protein, while using the patient's immune system to fend off B-cell lymphoma on its own. Daiichi Sankyo received exclusive development, manufacturing, and commercialization rights for the cell therapy in Japan from Kite, a Gilead company, in 2017. Kite received approval for axicabtagene ciloleucel under the brand name Yescarta in the United States in October 2017 (2) and in the European Union in August 2018 (3). Yescarta had $456 million in 2019 sales (4).

"We are pleased to confirm submission of the NDA for axicabtagene ciloleucel following positive topline results from the [Phase II] bridging study in Japan," said Wataru Takasaki, PhD, corporate officer, head of Oncology Function and head of the R&D Division in Japan, Daiichi Sankyo, in a company press release. "We will continue to work with regulatory authorities to develop this important new cell therapy for eligible patients in Japan who need additional treatment options for relapsed or refractory DLBCL and related lymphomas."

References

1. Daiichi Sankyo Company, "Daiichi Sankyo Submits Application for CAR T Therapy Axicabtagene Ciloleucel for Treatment of Patients with Certain Relapsed/Refractory B-cell Lymphomas in Japan," Press Release, March 30, 2020.
2. FDA, "FDA Approves CAR-T Cell Therapy to Treat Adults with Certain Types of Large B-Cell Lymphoma," Press Release, Oct. 18, 2017.
3. Gilead Sciences, "Yescarta (axicabtagene ciloleucel) Receives European Marketing Authorization for the Treatment of Relapsed or Refractory DLBCL and PMBCL, After Two or More Lines of Systemic Therapy," Press Release, Aug. 27, 2018. ◆

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Boost your Host Cell Protein Analytics

Help ensure clinical success

INTERNATIONAL BioPharm
FOR PERSONAL NON-COMMERCIAL USE

Antibody Affinity Extraction™ and Mass Spectrometry Services from Cygnus HCP experts.

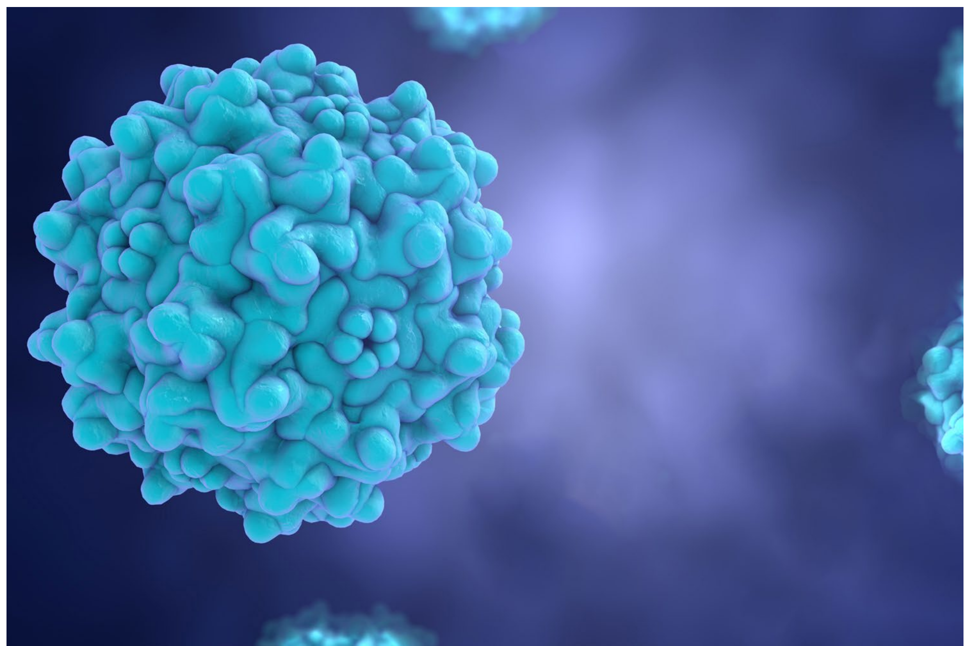
When it comes to outsourcing your critical Host Cell Protein analytical projects, it is essential to work with a dependable partner that has pioneered advanced orthogonal methods and HCP analysis solutions you can trust. Avoid delays in clinical trials due to an unforeseen need to optimize the purification process, adverse patient reactions, poor drug shelf life and reduced drug efficacy. **Save time and resources on your journey to a quality drug substance.**

- Assure that your HCP ELISA is fit for purpose of process monitoring and product lot release
- Enrich and identify hitchhiker HCPs and improve MS resolution
- Optimize your purification process to remove problematic HCPs
- Provide comprehensive HCP characterization data to regulatory agencies

With you all the way.

cygnustechnologies.com

© 2020 Cygnus Technologies. All rights reserved.
For research use only. Not intended for animal or human therapeutic or diagnostic use.



Gene Therapies Push Viral Vector Production

Viral vectors show promise as a delivery mechanism for gene therapy, but which virus types are commercially viable?

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

FELIZA MIRASOL

Several virus types have been studied for use as viral vectors in gene therapy, including adenovirus, lentivirus, retrovirus, and adeno-associated virus (AAV). Of these, AAVs have gained much attention as a gene delivery vehicle and are being explored for their commerical viability. The commercial viability of other virus types also continues to be explored, particularly for treatments that require a larger payload, transient expression, or insertion of a gene of interest in the genome.

The potential opportunity of a vector depends on the type of gene therapy being developed, says Gaurav Chaudhary, CEO of Roots Analysis, a business research and consulting firm. In general, there are two types of gene therapies, namely *ex-vivo* and *in-vivo*, Chaudhary points out. "In *ex-vivo*, cells are modified outside the patient's body and the corrected version is transplanted back into the patient. Opposite of *ex-vivo* is what we call *in-vivo*, where cells are treated inside the patient's body. The corrected copy of the genes is transferred into the body of the patient."

In both cases, cells may be treated either with a viral or non-viral vector carrying the corrected copy of the gene. Generally, AAV and adenoviruses are primarily used for creating *in-vivo* gene therapy. Compared to adenovirus and AAV, retrovirus-based vectors show relatively low transduction efficiency *in-vivo*, but their advantage lies in their ability to integrate stably and confer long-term transgene expression in dividing tissues. Retroviruses and lentiviruses, therefore, are most suitable for *ex-vivo* gene transfer, Chaudhary states.

Each viral vector system is characterized by an inherent set of properties that affect its suitability for gene therapy or other specific applications, says Florence Vicaire, global gene therapy business development leader, Cytiva (formerly part of GE Healthcare). "There is no one-fits-all multipurpose viral vector appropriate for all applications; each of the vectors has its own advantages, limitations, and range of applications," Vicaire says.

THE VIRAL-VECTOR MECHANISM

Viral vector systems are gutted viruses where the packaging signals (e.g., the genetic signals that target the wrapping into the viral particles) are fused to the genes of interest (the payload), and the remaining necessary elements are placed on separate genetic elements, often three or more, says Carsten Carstens, senior scientist, R&D, Agilent Technologies.

Kateryna_Kon/Stock.Adobe.com

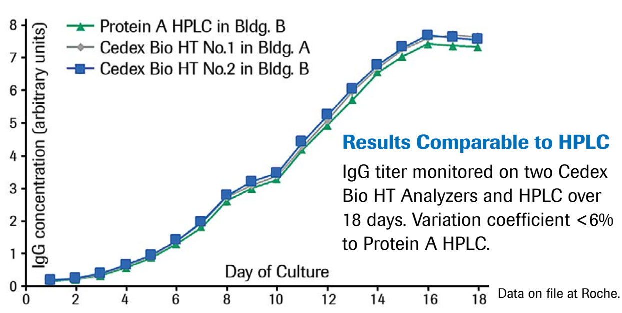
Take control of your bioprocess

Cedex Bio and Bio HT Analyzers

Quantify IgG in Real Time

- Accuracy over a wide range (10 mg/L to 8 g/L*)
- Customizable to your human IgG molecule
- No sample filtration or pretreatment required
- Also available: Human Ig Fab and Mouse IgG Assays

*Additional on-board dilution capability



Data on file at Roche.

Reliable Photometric Technology

- High accuracy and precision
- Broad range and sensitivity

Broad and Expandable Menu

- Glucose, Lactate, Ammonia, Glutamate, Glutamine, IgG, Pyruvate, Ala-Gln, Acetate, Phosphate **+17 more**

For more information, visit **go.roche.com/cedex-info** or contact **custombiotech.ussales@roche.com**

For use in quality control/manufacturing process only.
Glutamine, Ala-Gln, Iron, LDH: For quality control/manufacturing of IVD/ medical devices/pharmaceutical products only.
CEDEX is a trademark of Roche. © 2020 Roche. MC-US-06401-0420

BioPharm INTERNATIONAL
FOR PERSONAL, NON-COMMERCIAL USE

"The objective here is to prevent formation of a replication-competent viral particle," Carstens emphasizes. "The non-payload elements are generally referred to as the packaging system. If performed properly, packaging results only in infectious particles carrying the payload."

The primary objective of these viral vector systems is to achieve the highest possible titers without forming replication-competent variants. "For lentiviral packaging, there are iterative cycles of safety improvements. Agilent vectors, for example, are suitable and compatible with generation-four packaging systems (i.e., the latest version of packaging systems)."

For all practical purposes, a viral vector is a genetic element wrapped in its own transfection reagent, which makes transfection titrations possible (e.g., dosage responses) and highly effective. "In an R&D setting, this becomes preferable if the same reagent is transfected (for example, CAS9 delivery vectors) since all the work is in the generation of the viral stock, not in the infection," Carstens says.

Carstens points out that the greatest limitation of viral vector systems is their tropism, that is, the type of cells that a virus will naturally infect. Infectivity, he explains, is usually dictated by the envelope proteins present in a virus and the cellular surface components to which those proteins bind. "Depending on the application, the ideal vector either has an extremely broad tropism or extremely narrow if the objective is gene therapy of a very specific cell type (in this case this is a safety precaution)," Carstens elucidates.

Some vectors, such as adenoviruses, have a fairly broad tropism, and the target range is often modified by engineering part of the envelope proteins. Carstens cautions, however, that biomanufacturers need to be aware that such modifications are properties of the packaging system, not the viral vector. "The process of changing the tropism of the vectors is referred to as pseudotyping. For instance, Agilent has retroviral and lentiviral vector systems that are pseudotyped to achieve a wide target range. The upside of pseudotyping is a tailored target range. The downside is that some modifications of the envelopes lead to destabilization of the viral particle and that pseudotyping requires engineering of the packaging system that is often not accessible to most customers," Carstens states.

Lentiviral vectors, meanwhile, are a subgroup of retroviral vectors. Lentiviral vectors have displaced traditional retroviral vectors because lentiviral vectors do not require dividing host cells for integration, Carstens further explains. This characteristic broadens the utility of lentiviral vectors compared to traditional retroviral systems. Upsides to using a lentiviral vector include the ability of the system to carry a relatively large payload and genetic elements that become active only after integration. This makes it relatively straightforward to achieve desired titers and to achieve a single integration element, Carstens emphasizes. Downsides include the fact that it is more difficult to achieve large titers. In addition, each integration event constitutes a mutation in its own right, which becomes a safety concern when it comes to gene therapy, Carstens notes.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

In comparison, adenoviral vectors do not integrate into the host genome and are thus a transient delivery system. "They were once thought to have potential in gene therapy approaches, but there are potentially strong immune responses when used *in vivo*, which is why they are typically not used in gene therapy approaches anymore," Carstens states.

Finally, AAV is a "defective" virus that is not replication competent by itself. "[AAV] is famous as being the only known vertebrate virus to form a lysogen, meaning it integrates into the genome in a dormant state from which it can be rescued by superinfection with certain viruses, most notably the adenovirus (hence its name) or by some forms of chemical treatments (butyric acid and others)," notes Carstens. The natural defectiveness of AAV makes it an inherently safe delivery system.

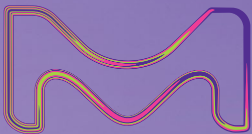
Most packaging systems will result in infectious particles that will not, or at least infrequently, integrate into the genome, and, thus, infection results in transient expression such as the adenoviral vectors. "The virus can integrate into the genome at a very specific site (e.g., the 'lysogenic' form). However, this requires modification to the packaging system," says Carstens. Upsides to AAV include the relative safety of these vectors (versus lentiviral), relatively low immune responses, and reasonably broad tropism. Some of the downsides include a limited payload, difficulty achieving high titers and functional titers, and difficulty in pseudotyping, Carstens reports.

THE PROMISE OF AAV

Of the different virus types that have been explored for gene therapy, AAV is the most commonly used because it offers certain advantages that make it a promising vector compared to other vector types. Chaudhary points out some of the advantages that AAV offers:

- AAV possesses characteristics that allow efficient manipulation of the vector (as required).
- AAV possesses the ability to be easily purified, as they are not readily degraded by shear forces, enzymes, or solvents.
- AAV exhibits reduced risk of adverse inflammatory reactions because of its non-pathogenic nature and because it has less immunogenic properties.
- AAV allows for the delivery of genetic sequences of up to approximately 4 kb.
- AAV exhibits reduced risk of ectopic integration of the therapeutic DNA.

"Adenovirus (AV) was the first viral vector investigated for gene therapy use; however, after the death of a patient in 1999 due to immunogenic reaction against AV (1), researches on gene therapy using AV were put to a halt, and other vectors were investigated, including AAV, especially due to AAV's lower immunogenicity. The latest approvals of gene therapies using AAV (e.g., Luxturna, Zolgensma) have led to a surge in AAV



INTERNATIONAL BioPharm
FOR PERSONAL NON-COMMERCIAL USE

An ever-changing journey needs an agile partnership

The dynamic nature of drug development and your world, means you need an adaptable partner. By joining forces with you, we provide a highly flexible, expert service covering contract development and manufacturing services for mAbs and viral vectors, product characterization, biosafety testing and toxicology testing services. So whatever direction your project needs to take, we'll help guide you there.

To find out more, visit:
SigmaAldrich.com/BioReliancePartner

MilliporeSigma, the vibrant M and BioReliance are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2020 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

The Life Science Business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

BioReliance®
Pharma & Biopharma Manufacturing & Testing Services

use as it has proven safe and effective," adds Vicaire.

Based on recent approvals and the benefits of AAV, including its low immunogenicity, the fact that it does not cause any disease in humans—something other viral vectors have the potential to do—its ability to confer long-term gene expression *in vivo*, its potential to infect replicative as well as non-replicative cells, and its potential to transduce a wide range of tissues, AAV is currently considered the best viral vector for *in-vivo* gene therapy, and is also considered as a potential delivery vehicle for other applications, such as gene editing, asserts Vicaire.

By now, a wide variety of AAV serotypes have been studied, adds Kai Touw, technical lead at Batavia Biosciences, a Netherlands-based contract development and manufacturing organization (CDMO). Some of the serotypes have been successful preclinical and clinical followed by regulatory approval in both Europe and the United States. "This makes it a popular starting point for gene therapy, although many serotypes continue to struggle with manufacturing challenges, such as low production yields and therefore high cost of goods," Touw says.

"Two distinct advantages of AAV vectors is the safety profile at large doses and the long-term expression (durability) of a gene of interest. This means they can be used to treat genetic disorders, such as spinal muscular atrophy and hemophilia," Chris Murphy, vice-president and general manager, Viral Vector Services, Thermo Fisher Scientific, further adds.

"Most packaging systems with AAV have been engineered so that it does not integrate into the host genome, and, as a result minimizes any potential non-intended mutations, versus lentiviral vectors, which are only active after integration. The relatively low immune response compared with adenoviral vectors makes AAV a more favorable approach," concurs David Weiss, product manager, Genome Engineering, Agilent Technologies.

MANUFACTURING BOTTLENECK

Manufacturers, meanwhile, are contending with the challenges of manufacturing viral vectors. Among the main manufacturing challenges today for AAV serotypes is the use of the adherent human embryonic kidney-293 (HEK-293) cell line. The current AAV manufacturing process using the HEK-293 cell line can only be increased by a "scale-out" method, notes Touw. This is carried out by implementing more hyperstacks, or cell stacks, for production. "Scale-out of these systems result in more laboratory handlings and, subsequently, a higher failure rate due to contaminations,"Touw says.

"This can be prevented by implementing a novel fixed-bed bioreactor system for adherent cell production like the iCELLis (Pall) or scale-X system (Univercells)," Touw continues. "Batavia managed to collaborate with the suppliers to optimize these systems and make them more suitable for process development and clinical manufacturing, specifically for viral vectors, leading to lower failure rates from significantly less contaminations and, therefore, lower cost of goods."

With a better understanding of the upstream process for the various AAV vectors, productivity can be increased, but this could give rise to new challenges downstream, such as changes in the impurity profile of the cell harvest, Touw adds. "Because each serotype has its own challenges, it's not possible to simply add in available plug-and-play technologies," he states.

More recent innovations, such as novel membranes and monoliths, are suitable as a capture step, Touw notes, while at the same time lowering buffer consumptions. Other innovations, such as affinity ligands for specific AAV serotypes are being developed for use with these technologies to further enhance the resolution of the capture step. A major challenge remains, however, and that is to control the amount of empty and hence non-infectious particles during manufacturing.

"For this, we also should not forget Industry 4.0 and the use of data analytics and machine learning within biopharma (such as multivariate data analysis). This will influence the production of AAV and other viral vectors. Learning how process parameters in the upstream process influence the purification process is fundamental for a good quality-by-design strategy. Furthermore, it will be interesting to see the developments in the intensification of monoclonal antibody (mAb) production processes. Lessons learned and technologies developed here could have the same benefits for the production of viral vectors," says Touw.

"After production, vectors need to be recovered from large volumes of cell lysate or medium which includes: the harvesting of the producer cells, cell lysis procedures to release AAV vector, the clarification and removal of cellular impurities, vector separation and purification, and vector formulation and sterile filtration," adds Chaudhary.

Most downstream approaches to viral vector separation and purification are based around traditional laboratory processes that are not scalable or suitable for clinical-grade manufacture because the viral vector field is still an emerging sector in biomanufacturing, Chaudhary also cautions. Despite the fact that the outcome of such conventional purification approaches would result in pure product, the lengthy processing time in manufacturing and the complexity of such a process results in a more expensive downstream purification procedure and ultimately, cumulative yield losses. "Therefore," Chaudhary explains, "the lack of scalable platform technologies for purification has proven to be a bottleneck in downstream processing. In addition, it is important to maintain AAV vector biological activity when removing impurities and contaminates present in a feedstock that originates from host cells or culture media. Next to this, the vast majority of described AAV purification processes rely on the specific serotype produced. This requires the design of a

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

new process for each different serotype." Chaudhary further explains that the presence of empty capsids presents yet another challenge associated with AAV vector purification.

The gene therapy manufacturing process is an overall exhaustive and complex task, presenting manufacturing challenges such as the following:

- Sterility and avoidance of adventitious agents. It is difficult to maintain a complete sterile environment using open systems that are extensively used in the manufacturing units.
- Live viral vector. Gene therapies involving the use of a live viral vector need extra care during handling and manufacturing processes to maintain the potency of the virus. Improper handling leads to loss of viral potency.
- Raw materials. Raw materials, such as growth factors and cytokines, that are required for manufacturing of gene therapy products are neither easily available nor affordable. In addition, the growth factors need to be continuously and manually added/replenished in the medium. This cumbersome process leads to an increase in the chances of errors.
- Cell expansion environment. Growing a large number of cells is a challenging task due to lack of technologies to monitor the cell-expansion phase. Moreover, some of the cells are sensitive to chemical transfection reagents. Furthermore, because a cell-harvesting process involves several steps, the probability of committing mistakes is much higher.
- Cell preservation. Freezing the cells for preservation is one of the major challenges as only a limited number of vessels are available for use in a cryopreservation environment, directly impacting the cell viability and quality.

"For certain manufacturing processes, downstream processing can be a bottleneck, for example, when ultracentrifugation is employed," agrees Murphy. "Thermo Fisher has developed downstream processes using scalable technology (e.g., column chromatography) that can handle large volumes of intermediate while separating out impurities. This eliminates the downstream bottleneck often making upstream production the yield limiting step in the process," he states. Another manufacturing challenge in viral vector production, Murphy points out, is the development and qualification of robust analytical methods to measure product quality.

"At the present time, there is currently a shortage of vector supply driven by the rapid growth of gene therapies both *in vivo* and *ex vivo*," adds Vicaire. The shortage in viral vector supply is also under pressure from inefficient manufacturing process and the need for specific facilities with increased biosafety level (BSL-2), which are currently lacking, Vicaire points out.

The solution to increasing viral vector capacity involves both structural and technical issues. Vicaire states that a combination of structural evolution on the CDMO side and development of new technical solutions on equipment suppliers' side can solve production challenges. "We must bring robust, scalable, industrialized manufacturing processes as fast as possible (less manual and more automated processes) and GMP solutions for both small-scale and large-scale production," Vicaire says.

"The CDMO market is currently fragmented with more new players offering to manufacture vectors. However, the capacity is still not covering the current needs. Capacity increases should resolve the supply shortage, which will be followed by the consolidation of CMOs [contract manufacturing organizations] with smaller players being acquired," Vicaire adds.

"On the technical side we must improve titer upstream (high titer stable producer cell line and/or cell line and media optimization) and for AAV specifically, improve ratio of full versus empty capsids; as well as improve downstream recovery (currently at best 30%, new nanofiber technologies coupled with next generation of ligands could significantly improve recovery)," Vicaire further explains.

At Cytiva, the company offers a structural solution to increasing viral vector manufacturing capacity through its KUBio box, a modular biomanufacturing environment. The company has tailored its KUBio product line to include a standardized bioproduction environment designed specifically for viral vector production. In addition, Cytiva has developed a technical solution that involves new fiber-based purification products (Fibro technology) that increases downstream recovery levels, according to Vicaire.

EXPLORING NON-VIRAL GENE DELIVERY VEHICLES

Viruses, however, are not the only type of delivery vehicle being explored for gene therapy. One area that is showing promise is with gene editing of cells that can then be re-administered to the patient to correct a genetic mutation, says Murphy. He highlights the fact that some innovators are using guide RNA with an endonuclease (e.g., Cas9) to modify cells *ex vivo* and make the edits. "I expect we'll see some products approved in the future using this technology," he asserts.

While there is a significant amount of work going into viral vectors to improve their tropism, rate of infection, and adverse immunological response, there is also much attention on developing other gene therapy mechanism options. "If we look beyond vector-based approaches, there are new CRISPR technologies that are moving into this space without some of the drawbacks of vector or virus-based systems," Weiss adds. "Two of these approaches, Base editing and Prime editing, have the ability to introduce small changes or corrections to the host genome without introducing foreign DNA. Base editing has the potential to specifically change single nucleotides

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

in a defined pattern (G–A, C–T, T–C, A–G) depending on the type of Cas9/deaminase complex being used. Prime editing, meanwhile, involves the use of a reverse transcriptase complexed to a Cas9 nickase in combination with an extended gRNA that contains an RNA template used to confer the desired edit," Weiss explains. This process allows for a defined change to be made to a small region of the host genome without significant off-target and unintended mutations, he adds.

"Each of these CRISPR-based technologies open up new avenues to make targeted changes to a host genome with less of a potential immunologic response. There are certainly drawbacks with these approaches as well, and there is more work that needs to be done in this area, but it is a truly exciting development," Weiss emphasizes.

"With gene therapies, both with episomal expression and gene integration, the main purpose is to have a long-lasting effect. At this moment viral vectors are the most promising technology. That being said, other technologies such mRNAs delivered to the target cells with, for example, mammalian cell-line produced engineered exosomes, start to show their capabilities," Touw interjects. "As with all new technologies in biopharma, there are several hurdles to take, but we follow the developments in the field closely and take along the lessons learned from other molecules."

"Plasmid vectors, meanwhile, are one of the most common non-viral gene delivery tools that are used to insert transgenes into target cells," Chaudhary states. "Plasmids can be easily modified to deliver a therapeutic gene and optimize its expression in a host cell. The incorporation of a multiple cloning site into a plasmid enables the insertion of a gene of interest with the help of restriction enzymes. The circular DNA is nicked at the multiple cloning site and, after the incorporation of the gene of interest, the nicks are annealed via a ligation step. The gene present in these vectors is generally flanked by a promoter sequence and a transcription terminator sequence to facilitate proper expression after it is incorporated into the host genome. Plasmids with inducible promoters are widely preferred as gene delivery tools, owing to the fact that these vectors offer the flexibility to activate/deactivate gene expression as required," Chaudhary explains.

Various modified versions of plasmid DNA vectors have been developed, including minicircles and minivectors, that are being used as vectors, Chaudhary further adds. "Apart from plasmids there are liposomes, lipoplexes, polyplexes, and oligonucleotides [that can be used] as non-viral modes of gene delivery."

Vicaire also notes that using non-viral vehicles offers a safety benefit in that there is less immunotoxicity compared to viral vectors and potentially lower cost as well as ease of production, such as for synthetic or mechanical approaches. Challenges to using non-viral approaches, however, include poorer efficacy, the potential of shorter duration of the gene therapy expression (i.e., repeated dosing may be required), and poor targeted delivery because there may be less tissue specificity.

REFERENCE

1. S. Lehrman, *Nature* 401, 517–518 (1999). ◆

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

More on gene therapy-related manufacturing and development

For more on gene therapy and gene therapy-related manufacturing and development, read these articles on *BioPharmInternational.com:*

- MilliporeSigma Adds Viral Vector and Gene Therapy Capacity
  www.BioPharmInternational.com/milliporesigma-adds-viral-vector-and-gene-therapy-capacity-0
- MicrofluidX Raises Funds to Develop Novel Cell Bioprocessing Technology
  www.BioPharmInternational.com/microfluidx-raises-funds-develop-novel-cell-bioprocessing-technology
- Orgenesis, ExcellaBio Announce Breakthrough Manufacturing Process for Bioxomes
  www.BioPharmInternational.com/orgenesis-excellabio-announce-breakthrough-manufacturing-process-bioxomes
- Univercells Launches CDMO to Support Cell and Gene Therapy Developers
  www.BioPharmInternational.com/univercells-launches-cdmo-support-cell-and-gene-therapy-developers
- Amicus Opens New Gene Therapy Center of Excellence
  www.BioPharmInternational.com/amicus-opens-new-gene-therapy-center-excellence
- Navigating GMPs for Gene Therapies
  www.BioPharmInternational.com/navigating-gmps-gene-therapies
- Sourcing Success in Cell and Gene Therapy Development
  www.BioPharmInternational.com/sourcing-success-cell-and-gene-therapy-development-1
- Using the "Cubic Effect" to Drive Cell and Gene Therapy Commercialization
  www.BioPharmInternational.com/using-cubic-effect-drive-cell-and-gene-therapy-commercialization-0

EAGLE STAINLESS

Highly-Polished Sanitary Stainless Steel Vessels

FOR THE PHARMACEUTICAL INDUSTRY

SPOTLIGHT ON THE STB SERIES

- > Type III DMF
- > Certifications Available
  - 316L MOC Lot Traceability
  - 10 Ra Surface Finish
  - USP Class VI PSIL Gaskets

STB SERIES (1mL TO 750mL)

CTL SERIES (10L TO 300L)

BTB SERIES (0.5L TO 30L)

CTH SERIES (4L TO 500L)

EagleStainless.com | 215.957.9333

ISO 9001 Certified

Challenges in Bulk Drug Substance Management

Closing the gap between downstream and fill–finish.

Final liquid drug substance production and fill–finish activities rarely occur at the same site. Nearly all pharmaceutical and biotechnology companies outsource a portion of their fill–finish needs to contract manufacturing organizations (CMOs) to help them address unexpected demand, satisfy second source policies, or better match scale with need (1). Thus, the shipping of liquid drug substances worldwide is often inevitable as well as filled with challenges and complexities.

COMMON SUPPLY CHAIN CHALLENGES

Proteins or monoclonal antibodies with highly complex structures require cold chain handling. Insufficient freezing of these substances not only renders them ineffective, but also harmful and possibly even life-threatening to patients (2).

Many sets of hands are involved in packaging, lifting, hauling, and delivering products thus creating great potential for human errors. In addition, numerous challenges and complexities must be overcome throughout the supply chain to comply with good distribution practices as well as governmental and other regulations (3, 4).

HOW TO STREAMLINE THE BIOPHARM SUPPLY CHAIN

BULK.STREAM® from Single Use Support closes the gap between downstream and fill–finish activities with a new, secure management process for liquids (**Figure 1**). Using single-use bags and plate-freezing, BULK.STREAM® guarantees fast and safe protection, filling, freezing, storing, shipping, and thawing of high-quality substances from one set of hands to another. The single-use bag agnostic technologies increase patient safety and minimize the risk of biocontamination and product loss toward 0%. BULK.STREAM® also enables the storage and shipment of high-quality substances in a faster and more secure manner, and at a lower loading volume than to date.

- **Protect:** A Robust Storage and Shipping (RoSS) shell protects every single-use bag available through BULK.STREAM. Its sophisticated construction offers many advantages (www.susupport.com/en/bulk-stream/ross).
- **Fill/Drain:** RoSS.FILL is a flexible, fully automated platform for the allocation and distribution of bulk drug substance in single-use bags in RoSS shells (www.susupport.com/en/bulk-stream/ross-fill).
- **Freeze/Thaw:** RoSS.pFTU is a plate-based freeze and thaw platform that offers safe and consistent freezing of high-quality substances in various scales (www.susupport.com/en/bulk-stream/freeze-thaw).
- **Store/Ship/Track & Trace:** RoSS.SHIP is a highly robust, stackable, and coolable device that holds up to 20 RoSS trays for storage and shipping of frozen substances in a protected manner (www.susupport.com/en/bulk-stream/ross-ship).

HOW TO OVERCOME CRYOCONCENTRATION

Freezing drug substance is essential for storing and shipping, but the process stresses the protein and can lead to denaturation. Cryoconcentration leads to factors that induce protein denaturation. For example, the high protein concentration itself causes

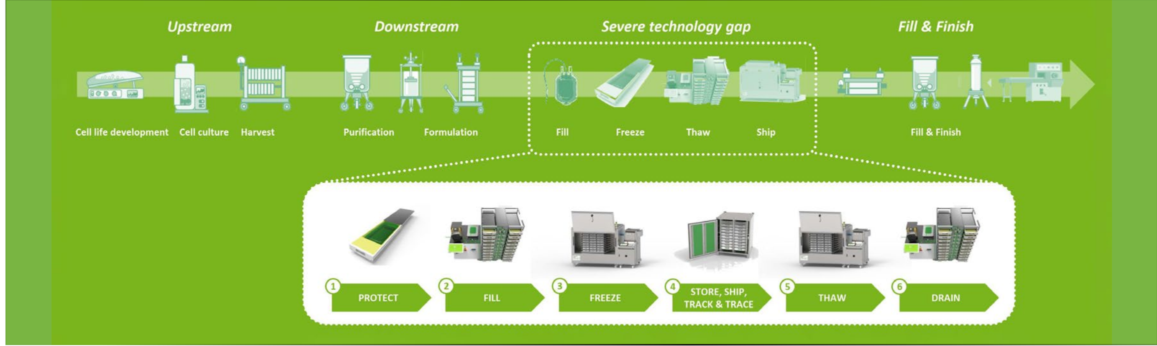


Figure 1. Single Use Support's BULK.STREAM closes the gap between downstream and fill–finish.

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

denaturation through protein aggregation (5, 6).

The solubility of the dissolved buffer salts decreases as temperature decreases. As freezing proceeds, ice formation excludes solutes (including protein) from the growing ice crystal. Solutes migrate in front of the growing ice that consist of small finger-like projections called *dendrites* (7, 8). These dendrites trap some solutes as they grow, so solutes are found in all regions of the block instead of only at the last point to freeze. As the solution trapped between dendrites freezes, however, it continues to undergo cryoconcentration as the water component is removed and as ice and the composition changes.

Cryoconcentration can never be eliminated, but with "controlled freezing," geometry and freezing rate are determining factors in practical systems of freezing (9). Using a slow freezing rate leads to a high cryoconcentration with a comparable low ice surface. The low freezing rate causes the ice to form slowly enough for the proteins to be pushed along the ice rather than being trapped by it. Exposure to concentrated solutes can result in a loss of a protein's thermodynamic stability, eventually causing aggregation.

Freezing rates that are too high lead to the opposite effect, namely a dendritic ice surface. The dendritic structure envelopes proteins in low cryoconcentrations, but compared with a plane ice front, the surface of the dendrites is larger.

Studies have shown that plate freezing technology achieves the best results because the fast freezing process hinders the accumulation of proteins and antibodies in the center of the bag. As demonstrated with blue color agent in **Figure 2**, slow freezing leads to cryoconcentration. When comparing the freezing results of static freezers vs. plate freezers, it is evident that fast freezing achieves more homogenous freezing and prevents excessive cryoconcentration.

Figure 2. Blast freezing versus plate freezing.

HOW TO COPE WITH SCALABLE MARKET DEMAND

Small volumes of drug substance are often produced for early-stage clinical phases. Traditionally, the use of single-use drug substance bulk freezing containers has been the norm for the 50 mL to 1 L range. While this size offers an acceptable operational fit for dispensing drug substance for smaller-volume pharmaceutical manufacturing processes, it is not ideal for larger-volume dispensing processes. It is typical for commercial programs to generate several hundred liters of drug substance per batch, for instance (10).

BULK.STREAM, an end-to-end solution for all scales, addresses these challenges by freezing large volumes of expensive drug substances in batches to facilitate the production of medicinal drugs based on real-time commercial or clinical requirements. Cleaning, sterilization, and repeating documentation are not needed with single-use systems. Moreover, the RoSS platforms represent flexible, fast, and scalable solutions that help decrease the risk of contamination and increase patient safety.

In general, awareness of the gap in bulk drug substance management (between downstream and fill–finish) is steadily increasing. Securing the highest possible standard is not only important in terms of efficiency, but also for safeguarding high-quality products for patients (11). ■

FOR REFERENCES, GO TO THE ONLINE ARTICLE AT BIOPHARMINTERNATIONAL.COM

Michael Eder
is marketing manager at Single Use Support.

Biopharma Insights—Thought Leadership from Marketers/ Paid Program

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Biopharmaceutical Manufacturing and the Power of Synthetic Biology

BioPharm

Synthetic biology has advanced the scope and scale with which biologically derived therapeutics can be developed.

FOR PERSONAL NON-COMMERCIAL USE

DAVID McELROY

Traditional small-molecule drug discovery—the mainstay of pharmaceutical R&D for more than a century and a half—has become increasingly challenging, as the "low hanging fruit" of universally effective therapeutics have been slowly exhausted. The only real potential areas for major growth in the small-molecule drug sector are now the discovery of new drug targets that come from furthering our understanding of disease and the development of precision medicines targeting specific subpopulations of patients.

Fortunately for the industry, the rise of biotherapeutics is making up for this gap in traditional discovery pipelines, providing greater specificity, new avenues of therapy, and the possibility of combating previously untreatable diseases. Somewhat less fortunately, the commercial development and large-scale production of biological agents are far more complex than classical chemical syntheses. This means that many novel biotherapies never make the transition from the lab bench to the clinic, simply because the requisite biological molecules can't be produced in the purity or quantity required.

PICK A HOST...

Biotherapeutic production generally relies on inserting genetic elements encoding the peptide, protein, or antibody of interest into a host organism capable of producing it in large quantities. There are a wide range of potential cellular hosts that can be employed for bioproduction—each with its own unique advantages and limitations. At present, *Escherichia coli* (*E. coli*) or Chinese hamster ovary (CHO) cells are the "go to" systems for the majority of labs looking to produce biological molecules that are ultimately intended for clinical applications. However, more often than not, this is because they are the host organisms researchers are most familiar with, rather than the optimal choice of expression system. This generally isn't an issue in an upstream R&D setting, where only small quantities of the biotherapeutic are required, but can lead to yield and solubility issues when

DAVID McELROY*, PHD, david.mcelroy@ingenza.com, is chief business officer at Ingenza Ltd, UK.

*To whom correspondence should be addressed.

ELNUR/STOCK.ADOBE.COM

attempting to scale up production for preclinical and clinical studies, or for manufacturing. This is a real stumbling block for many potential biotherapeutics, as the resources required to scale up production in an inefficient or unsuitable host can make it economically unviable.

... ANY HOST

Foreseeing and overcoming the various issues that can arise during the biomanufacture of novel drug products requires a broad understanding of the various host organisms available, their strengths, and, crucially, their weaknesses. While there are no set rules to determine which host may be the most effective for the production of a given biomolecule, a broad expertise and holistic understanding of each organism's metabolism can help to avoid problems downstream in product development. Experience is crucial here, as is having access to an extensive toolbox of synthetic biology (synbio) and metabolic engineering technologies (**Table I**) designed to optimize gene expression and production of the target molecule. The major challenge for drug discovery groups—or even biotech companies—attempting to develop and exploit these technologies in-house is that most simply don't have the breadth of knowledge necessary for success. They are, no doubt, experts in their specific technology or disease area, but the scale-up production of biotherapeutics requires a very different skill set, covering aspects of synthetic and molecular biology, fermentation, and chemistry as well as good manufacturing practice (GMP) manufacturing.

The true power of synbio for biomanufacturing comes from the ability to combine commercially validated synbio tools (**Figure 1**) with other techniques and technologies to enhance the overall production system. For example, transcriptomics and proteomics—and even more traditional bioengineering techniques, such as ultraviolet (UV) mutagenesis—can be used to identify further enhancements that improve the hosts' overall production capabilities. The expression capacity of the system can then be further increased through strategies such as ribosome engineering—to uplift overall gene expression—as well as the over-expression of regulatory genes, and the suppression or knockout of genes that may compete for resources, or could metabolize or destabilize the final product.

THE NEED FOR A HOLISTIC APPROACH

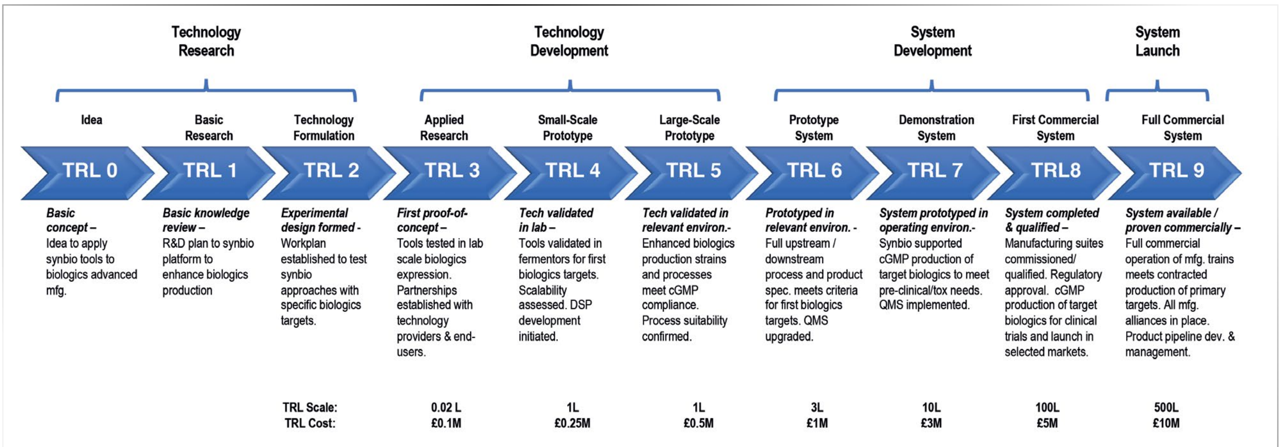
The main challenge in biomanufacturing is that scaling up production of therapeutics requires not just an understanding of the individual elements involved in the process—cell culture maintenance and fermentation, genetic manipulation, codon optimization, directed evolution, metabolic engineering, transcriptomics, and proteomics, etc.—but also how all of these components

Table I. Summary of synthetic biology approaches to enhance biologics production. PCR is polymerase chain reaction. KOs are knock-outs. DSP is downstream processing.

Bioprocess steps	Current limitations	Synthetic biology benefits
Gene design—cloning and construction	Restriction enzyme-based DNA cloning/ manipulation: • Slow and inefficient • No rapid iteration • Error prone due to PCR-derived DNA mutations	"One-pot" combinatorial assembly of DNA: • Cheaper optimization of gene-expression designs by re-using all parts • Better construct integrity by eliminating PCR errors
Gene design—expression elements	Limited range of naturally occurring expression elements: • Limits freedom to operate • Unstable • Negatively impacts host gene expression	Synthetic elements to control target gene expression: • Greater diversity of induction protocols available • Better stability from non-repeating/orthogonal expression elements
Expression optimization —protein stability	Trial and error empirical testing to optimize protein stability: • Highly unpredictable and costly • No systematic definition of criteria to enhance stability	Superior protein stabilization using chaperones and stability elements: • Better yield of target using co-expressed chaperone • Cheaper process from simplified target handling
Expression optimization —protein yield	Traditional mutation/screening: • Slow and unpredictable • Damage to host from mutations • Difficult to define and combine beneficial traits	Omics-driven exploitation of host factors impacting target production: • Predictable genome editing to optimize gene expression • Faster and cheaper host bioengineering precision (e.g., protease KOs)
	Trial and error testing: • Slow, unpredictable • Costly rebuilding of systems to be tested	Direct selection and/or screening of high producing clones: • Faster identification of high yielding clones • Cheaper process optimization • Adaptable to apply to new targets
Expression optimization —yield stability	Random gene integration into the host genome: • Unpredictable outcomes • Slow process to identify best expressing strains	Precise gene targeting into the host genome: • Predictable integration at most favored genomic locations • Better expression and stability by re-use of optimal locations
Analytical methods —expression optimization	Trial and error testing: • Slow and unpredictable process • Costly rebuilding of systems to be tested	Gene design "rules" and colorimetric reporters for best expressers: • Cheaper production by direct selection of well-expressed soluble product • Better definition and translation of performance criteria to new targets
Upstream processing —fermentation	Large (>10,000 L) steel fermentation systems: • Expensive capital investment • Slow turnaround between runs • High resource consumption—water, energy, chemicals	Disposable (1 L–200 L), single use fermenters: • Lower capital investment • Cheaper manufacturing due to simpler process • Reduced environmental impact from less cleaning/validation
Downstream processing —protein purification	Large-scale, multi-column chromatography: • Time-consuming and costly • Multiple cGMP process steps • Separate operations for target purification and maturation	Proprietary target protein capture, release/ maturation, and concentration: • Faster and cheaper purification with fewer DSP steps • Better purity through unique capture • Adaptable to new targets • Safer handling of potent targets through simultaneous recovery/maturation

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

Figure 1. Technology readiness level cascade for the commercial validation of synbio tools. DSP is downstream processing. cGMP is current good manufacturing practices. QMS is quality management system.



interact as part of a holistic biomanufacturing system. Worse still, optimizing these bioproduction systems often requires the ability to screen hundreds or thousands of individual strains or mutants to identify the highest yielding candidates. With no hard and fast rules on which vector, insertion locus, individual strain, or even host organism may be best suited to the production of a specific biological product, it is easy to see why so many biotherapeutics never make it to the clinic.

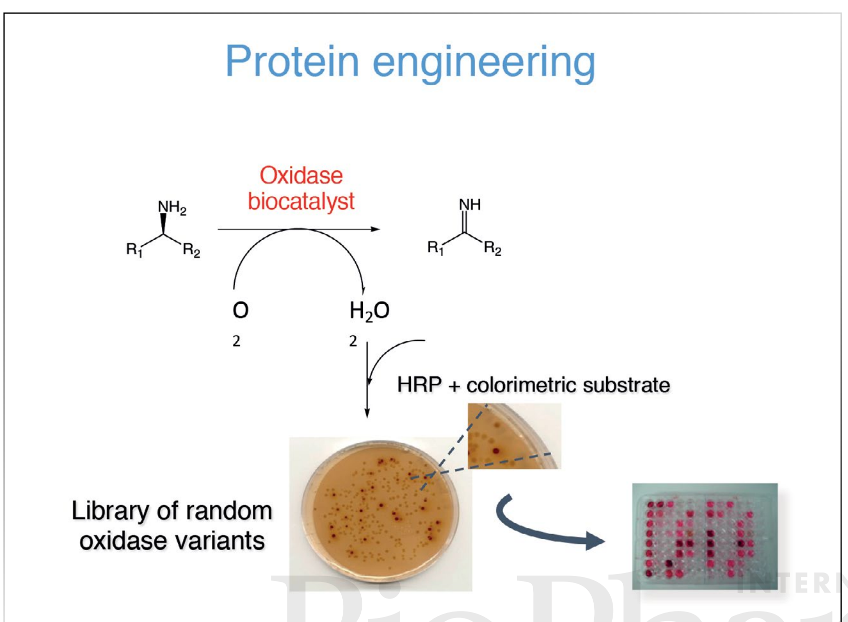
There are no shortcuts to overcoming this issue, but experience can mean the difference between success and failure. There are obviously numerous ways of tackling any given challenge that may arise during biomanufacturing scale-up, so working with an experienced partner

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

Changing the ART of analytical chromatography with µPAC™ capLC columns:
Unrivalled separation power
Micromachined reproducibility
Flow rate flexibility
1 - 15 µl/min
Plug-and-play connectivity
Designed for increased robustness and throughput without losing sensitivity.
PharmaFluidics
µPAC™ capLC
Discover our products on www.pharmafluidics.com

ALL FIGURES COURTESY OF THE AUTHOR.

Figure 2. A colorimetric screen for use in amine oxidase protein engineering and expression. This assay was developed and used by Ingenza to screen 105 individual *E. coli* transformants for target enzyme expression, with a one month per round of protein evolution. HRP is horseradish peroxidase.



is often the fastest way to get to an efficient and effective solution, without the cost and delays of following numerous dead ends. Outlined below are a number of issues commonly encountered during biotherapeutic development and scale up, with examples of various approaches to address them using different host organisms and synthetic biology approaches.

PROTEIN SOLUBILITY ISSUES

One of the most common challenges encountered when attempting to scale up bioproduction is the solubility of the target protein or peptide. The simplest, and often fastest, way to overcome this is to express your product in an alternative host organism. However, this requires an understanding of which hosts might be appropriate, the tools to design, build, and test a suitable expression vector, and the capacity to perform strain selection and growth optimization. While this may sound a daunting task for most labs, specialist synthetic biotechnology companies routinely perform this type of project, and have the knowledge and experience to rapidly transfer production of a target biomolecule into a new host. For example, switching production from *E. coli* to *Pichia pastoris* (*P. pastoris*) may eliminate solubility issues encountered in early scale-up studies, allowing the high yielding production and secretion of a high purity product for GMP manufacturing.

EXPRESSION SCREENING LIMITATIONS

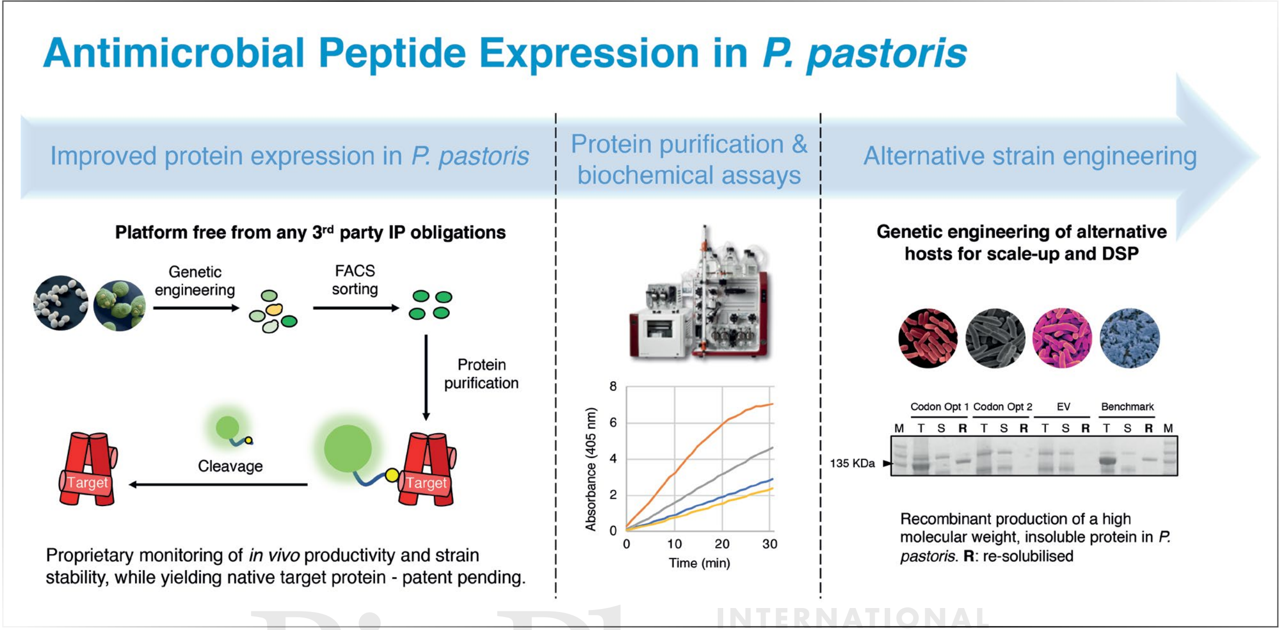
Protein engineering for improved therapeutic function is often a numbers game; the more gene variants that can be screened, the higher the chance of success. *E. coli* is often a good choice of host for this application—at least during the early development phase—due to the plethora of bioengineering tools available for this host. However, effective variant screening requires rapid, cost-effective assays that enable the qualitative or semi-quantitative assessment of target protein expression across large numbers of individual transformants. Unlike the functional or kinetic assays used in downstream characterization studies, these variant library screening assays must offer a quick and easy "yes/no" assessment of every transformant, and generally employ simple colorimetric or fluorimetric detection.

The challenge here is that the common approach of using a generic reporter gene-linked expression assay—combining the target of interest with the expression of a reporter protein of some sort—is often not appropriate when optimizing biotherapeutic production, and so a predictive assay tailored to the individual target protein must be developed. This is often the rate-limiting step in the identification and selection of improved gene variants, which can significantly delay development, or even halt progress entirely if not deployed successfully. Working with a partner that has extensive experience in designing and performing this type of screening assay can dramatically accelerate timelines, generating lead candidates much sooner. **Figure 2** shows an example of a screening assay designed to improve transcription/translation of a novel amine oxidase in *E. coli*. The assay, which was developed and validated in just a few weeks, uses a colorimetric substrate (added to the culture media) to assess the expression of an enzyme involved in the *in-vitro* biocatalysis of an advanced pharmaceutical intermediate. Combined with design of experiment (DoE) approaches and an automated workflow, this type of assay allows both the selection of high-yielding transformants and the determination of optimal expression conditions in a very short timeframe.

The development of efficient screening assays can also help to improve the product itself, by enabling larger studies to be performed than would otherwise be possible. High assay throughput provides the opportunity for further product optimization, potentially in combination with machine learning or artificial intelligence strategies, to identify potential enhancements or improvements in the design of the target biomolecule. For example, advanced screening has been successfully combined with

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Figure 3. Synthetic biology and high throughput screening can be combined with machine learning to aid in the development of new biotherapeutics. FACS is fluorescence activated cell sorting. IP is intellectual property. DSP is downstream processing.



BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

machine learning to further enhance the potency of epidermicins, a new class of antimicrobial peptides capable of rapidly killing potentially harmful bacteria, including drug-resistant species (Amprologix) (**Figure 3**) (1).

POST-TRANSLATIONAL EXPRESSION OPTIMIZATION

Looking beyond cell culture maintenance and the use of alternative transcription/translation elements of protein expression, there are a number of other factors to consider when attempting to scale up bioproduction. Even for a well-characterized host such as *Bacillus subtilis* (*B. subtilis*)—which is widely used in the production of enzymes—there are various opportunities to improve the overall biomanufacturing process in culture media composition, protein secretion, and endogenous protease gene knockout. Being able to extract the target protein from the bioreactor is obviously essential for efficient production, avoiding the need to destroy the culture to harvest the product. A number of secretion systems already exist for *B. subtilis*, so selecting a strain that excretes the heterologous target protein will help to simplify downstream purification.

It is also important to consider the stability of the target protein within the expression system. For example, *Bacillus sp.* have a number of protease enzymes that could cause the degradation of the target protein before it has even left the cell. Choosing knockout mutants that lack the major proteases can therefore increase overall yields by improving target protein stability. Another strategy to increase protein stability is to create a more favorable environment for your product. For example, co-expression of chaperonins has been successfully used in the production of recombinant factor VIII in CHO cells by allowing high levels of expression without leading to misfolding or aggregation.

Failure to consider these kinds of post-translational factors could significantly reduce the overall yield, and therefore performance, of a biomanufacturing system. Even beyond this, there are practical considerations when choosing the best expression system or host for a target biotherapeutic. For example, many *Bacillus* species are spore forming, which is an undesirable trait in a closely regulated GMP environment. The development of asporogenic strains has mitigated this issue, simplifying culture management and, consequently, reducing biomanufacturing costs.

USING THE WHOLE TOOLBOX

Advances in synthetic biology have made it possible to coordinate the expression of multiple genes to allow the creation of novel biochemical pathways. This allows the host organism to produce large quantities of a heterologous product that would normally be outside of its metabolic "repertoire". It is only by understanding each of these elements, while still considering the system as a whole, that biomanufacturing can reach its true potential, and working with a knowledgeable and experienced synbio partner is the best way to ensure successful scale up of novel biotherapeutics, on time and on budget.

REFERENCE

1. Ingenza, "Amprologix Secures Funding to Develop New Antibiotic with Ingenza," Press Release, Feb. 5, 2019. ◆

Fluid Handling Considerations

Pumps and other components meet the demands of single-use systems in biopharmaceutical downstream processing.

JENNIFER MARKARIAN

FOR PERSONAL NON-COMMERCIAL USE

The growing use of single-use systems and ongoing development of continuous processing place new demands on pumps and other fluid-handling components in downstream biopharmaceutical processing. Pumps used with single-use systems include peristaltic pumps, in which the fluid flows through single-use tubing, and diaphragm pumps, which can also be single-use and move the fluid using suction created by the diaphragm. *BioPharm International* spoke with Andreas Frerix, product manager for diaphragm pump manufacturer Quattroflow, a product brand of PSG, a Dover Company, and Gregg Johnson, global product manager for Masterflex and Masterflex Ismatec peristaltic pumps at Cole-Parmer, about trends and best practices in fluid management.

TRENDS

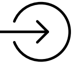
BioPharm: What trends do you see in downstream processing and how do these relate to the requirements for the fluid pumping system?

Frerix (Quattroflow): While by far most operations in downstream processing are in batch mode, there is a trend to continuous bioprocessing because the industry is seeking ways to advance their current technologies to improve efficiency, throughput, and product consistency while reducing manufacturing costs. A typical example is chromatography. In batch mode, a column filled with resin runs through the modes of equilibration, loading, washing, elution, and regeneration, one after the other. The continuous version is called simulated moving bed chromatography. In this process, several columns operate in parallel, with each of them in one of the aforementioned modes. Complex valve configurations are used to switch the column from one mode to the other. While this setup reduces the amount of expensive resin needed, it requires several pumps to run in parallel at high consistency over a long period of time. A high degree of pump reliability and flow stability is needed because these continuous processes can run for weeks rather than for only a day when in batch mode.

Johnson (Cole-Parmer): Both single-use and continuous processing are growing trends because both offer unique advantages depending on the application need. Single-use will be prominent in personalized cell/gene therapy. Continuous processing will grow in large production volume applications, such as virus or vaccine production, as operations search for opportunities to increase output and efficiency.

innluga/Stock.Adobe.com

Vaccines Illuminated: Biophysical Characterization, PAT, and Quality Control via Light-Scattering Techniques



Register for this free webcast at:
www.biopharminternational.com/bp_p/vaccines_illuminated

LIVE WEBCAST:
Tuesday, May 19, 2020
11am EDT | 8am PDT | 4pm BST | 5pm CEST

Event Overview

Accurate preclinical characterization of vaccine candidates enables timely product and process development, leading to effective manufacturing, and quality control procedures. This webcast will highlight the role of light scattering techniques for characterizing a range of modalities, including DNA and mRNA vaccines, viruses and virus-like particles (VLPs), and protein-based vaccines. The role of light scattering in downstream process analytics and quality control also will be discussed.

Key Learning Objectives

- How molar mass, size, titer, and composition of therapeutic and vaccine biomolecules are determined with size-exclusion chromatography coupled to multi-angle light scattering (SEC–MALS)
- How mRNA and lipid nanoparticle vaccines are characterized with field-flow fractionation coupled to MALS (FFF—MALS)
- How quality attributes of viruses and virus-like particles are quantified using MALS and dynamic light scattering (DLS)
- How high-throughput formulation and stability studies of are carried out with plate-based DLS
- How real-time MALS can monitor chromatographic purification of viruses and VLPs

Who Should Attend

- Researchers involved in the biophysical characterization of novel vaccines
- Downstream process development engineers responsible for implementing process analytical technology in vaccine production
- Vaccine quality control managers
- Regulators evaluating biophysical characterization for novel vaccines and therapeutics

Presenter

Sophia Kenrick, Ph.D.
Director of Analytical Services
Wyatt Technology

Moderator

Rita Peters
Editorial Director
BioPharm International

For questions or concerns, email mdevia@mjhlifesciences.com

Sponsored by

Presented by

BioPharm
FOR PERSONAL NON-COMMERCIAL USE

Peristaltic pumps are suitable in both the single-use environment and to provide, with a simple change of material, the long running life for continuous processes. These pump systems can address both areas of single-use and continuous processing. Continuous processes would potentially be looking for longer-lived tubing.

Continuous processes and single-use processes require pumps with modularity, easy maintenance, and dosing accuracy and precision. Metering pump technology featuring single-use wetted parts connected to a driving motor, such as the tubing in peristaltic pumps and the pumphead in diaphragm pumps, satisfy the modularity and easy maintenance requirements because the parts that come into contact with the fluid are simply replaced. The accuracy and precision of these systems is determined by the sophistication of the drive, and drive features such as precision RPM [revolutions-per-minute] control, programmable interfaces, and analog signal control have become baseline.

SINGLE-USE COMPONENTS

BioPharm: What trends do you see in single-use components for fluid handling?

Johnson (Cole-Parmer): Reliability and standardization of single-use components is growing in importance to mitigate risk and ease scalability. Single-use components are used in applications such as personalized medicines and cell and gene therapy. The ability to eliminate cross-contamination and decontamination processes drives the need for single-use components.

Peristaltic technology is low-shear and mechanically simple, consisting of a single piece of material. However, some concerns with single-use tubing in peristaltic pumps, which are mentioned with some frequency by pump users, are tubing life and spallation. Both concerns can be addressed by choosing the correct tubing material for the application. For long life with minimal failure risk, thermoplastic elastomer (TPE) formulations offer the capability of running for months at a time in a 'single-use' capacity. When low particulates are needed, selection of a smooth tubing surface significantly reduces spallation. The primary determinant when it comes to tubing selection is chemical compatibility, then any regulatory requirements, then the life of the tubing.

> "Single-use pumps have the most benefit when used in facilities with small to medium capacities and frequent product changes."
> —*Andreas Frerix, Quattroflow*

Frerix (Quattroflow): There are several trends affecting single-use components. While continuous manufacturing requires lower flow rates and longer operating times, we also see a need for handling larger flow rates as the volumes of single-use bioreactors are rising, which increases the demand for single-use equipment with larger diameters. Another trend where single-use components will play an important role is personalized medicine/cell and gene therapies.

Single-use pumps are used from process development up to commercial production. The main benefits of single-use pumps are:

- Eliminating cleaning requirements, as all wetted components are replaced with a new single-use set after every product run
- Reducing time needed to complete an installation and get the production facility up and running
- Reducing water and energy consumption for cleaning and sterilization
- Decreasing risk of cross-product contamination
- Allowing more operational flexibility with a modular approach
- Reducing capital investment in facilities and equipment.

Looking at these benefits, single-use pumps have the most benefit when used in facilities with small to medium capacities and frequent prod-

Predictive maintenance offers improved efficiency.

Preventive maintenance uses a time-based schedule to perform routine maintenance and prevent equipment breakdowns. Predictive maintenance, however, is need-based rather than only time-based. It uses data from equipment sensors so that, with insight into when a problem might occur, process operators and engineers can make better decisions about how to intervene with the least disruption to the process (1).

Predictive maintenance has been enabled by advances in artificial intelligence, such as machine-learning algorithms that learn patterns of how equipment should be operating and then can identify changes (e.g., in vibration or flow) that may indicate a problem will occur. Equipment can also be compared to similar equipment in the same plant or in facilities around the world, and these large datasets improve accuracy of the predictions (1).

Reference

1. J. Markarian, *Pharm. Tech.* 44 (1) 36-40 (2020).

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

uct changes, such as clinical-trial manufacturing or contract manufacturing organizations (CMOs).

Single-use diaphragm pumps have been used for many years. For critical flow and/or pressure-controlled operations like tangential-flow filtration, chromatography, virus filtration, inline dilution, and many more, the advantages of diaphragm pumps are their low pulsation when using multiple diaphragms (such as quaternary diaphragm pumps); the capability of delivering the needed pressure, especially for chromatography or tangential-flow filtration; the proportional flow characteristics; and the shear-sensitive handling of biological products.

"Reliability and standardization of single-use components is growing in importance."
—Gregg Johnson, Cole-Parmer

When it comes to processing large volumes (e.g., large-scale monoclonal antibodies [mAbs] or blood-plasma fractionation), single-use equipment has a defined limit in terms of its scalability and economics. Here, stainless-steel equipment is for some applications the preferred choice. In addition, single-use processes are widely adopted in the manufacture of mAbs from mammalian cell cultures; however, when it comes to microbial fermentation, stainless-steel equipment is mainly used.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

CELL AND GENE THERAPIES

BioPharm: How do fluid pumping requirements for the emerging areas of personalized medicine and cell and gene therapies differ from conventional biopharmaceutical processing?

Johnson (Cole-Parmer): Personalized medicine/cell and gene therapies require lower volumes of fluid, which requires the fluid handling system to have much lower flow rates, and the pump can be physically smaller. Pumps that offer independent multi-channel control and flows in the range of microliter per hour are needed to meet these new requirements.

Frerix (Quattroflow): One big difference is the batch size, especially because in autologous gene and cell therapy, the cells from individual patients have to be treated, modified within different processes, and then administered back to the patient. This technology involves one individual process per patient, along with relatively low flow rates, the need for disposable equipment, and the handling of low volumes. In these types of applications, particulate release from the pump cannot be tolerated, as these particulates can cause health risks once the modified cells are returned to the patient.

RELIABILITY AND MONITORING

BioPharm: Is remote monitoring/predictive maintenance being used for biopharma pumps?

Frerix (Quattroflow): While diaphragm pumps are reliable when used within the specified operating conditions, it is possible to use sensors to detect leak-causing diaphragm damage and stop the process. This detection is especially helpful when multiple pumps are used in parallel in a fully automated manufacturing process.

Johnson (Cole-Parmer): We have customers asking us now if we have solutions for remote monitoring/predictive maintenance. We have a tool for remote monitoring and control in place that provides operations the ability to 'see' what is happening with the pump system without actually being there. Coupling this insight with existing plant line knowledge gives operations greater flexibility on how they manage the line.

We are now seeing customers asking for pumps with more connectivity and automation options such as cloud-based monitoring and control. Cloud-based record keeping is also a growing request from our downstream customers, as manufacturing floors align with market trends to move business operations to the cloud while also meeting the growing demand from regulatory bodies for more rigor and accountability in operational data. ◆

Straight Talk on Closed Aseptic Systems

The industry's aversion to risk has led to its treating closed aseptic processing systems as miniature cleanrooms, resulting in redundant and expensive practices.

BioPharm International
FOR PERSONAL NON-COMMERCIAL USE

JAMES AGALLOCO

Commercial production of sterile pharmaceutical products has changed dramatically since the early 1900s (1), when most injectable products were filled manually, without the accoutrements that are now considered essential to protecting sterile products from contamination. Over time, the industry recognized that physically separating operators from the product led to improved patient safety. As a result, some installations began to use gloveboxes that were designed to protect sterile materials from operator-borne contaminants.

As sterile liquid-handling systems grew substantially in size, closed systems of varying complexity were also used in the aseptic manufacture of liposome formulations, suspensions, creams, and ointments. Because these systems can be reliably sterilized and maintained in a closed state, regulators did not formally require internal environmental monitoring of closed systems or of their background environments (2).

Nevertheless, a gap has developed between existing industry practices and the science behind closed systems, which are still restricted by use of design and control concepts that were developed for open systems (**Sidebar, p. 34**). This gap poses important questions for manufacturers and regulators. Until the 1970s, sterility testing and environmental monitoring provided the formal demonstration of performance capability for aseptic processing. The media-fill test, first introduced by the World Health Organization (WHO), eventually became a regulatory expectation for aseptic activities (1,2).

As manufacturers gained experience using this test, they sought adaptations that would accommodate the preparation of sterile APIs (3). This came in the form of industry documents, prepared jointly by the Parenteral Drug Association (PDA) and Pharmaceutical Manufacturers Association (now referred to as the Pharmaceutical Research Manufacturers Association [PhRMA]), the second of which formally defined the closed aseptic processing system (4,5). The 2006 revision to this document outlined the design and performance attributes that establish a system as 'closed'.

According to this document, a closed aseptic processing system is one that is designed to prevent the ingress of microorganisms by means of physical separation of materials and product from

JAMES AGALLOCO is principal of Agalloco & Associates. With nearly 50 years of industry experience with companies that include Merck, Pfizer, Squibb and Bristol-Myers Squibb, he has served as an officer or director of PDA and is a member of *Pharmaceutical Technology's* editorial board, jagalloco@aol.com.

prescott09/Stock.Adobe.com

the surrounding environment. In order to meet this definition, the system must be:

- Constructed, installed, and qualified in a way that shows that integrity is maintained throughout the full range of operating conditions and over a time period that includes periods of the longest expected usage (i.e., manufacturing campaign). This qualification must follow a formal protocol using generally accepted engineering principles and practices, and must be documented.
- Sterilized in place, or sterilized while closed, prior to use of a validated procedure.
- Capable of being used for its intended purpose without compromising the integrity of the system.
- Capable of being adapted for fluid transfers in and/or out while maintaining asepsis.
- Connectable to other closed systems while maintaining the integrity of all closed systems (e.g., via a rapid transfer port, steamed connection, or other comparable means).
- Safeguarded from any loss of integrity by scheduled preventive maintenance.
- Operated using sterilizing filters whose integrity has been tested, and which can be traced back to each product lot for sterilization of process streams (5).

ENVIRONMENTAL MONITORING

As first conceived, this definition embraced isolator systems, which had evolved from the earlier glove-box technology. These systems were mainly used in the later stages of bulk antibiotic production (e.g., during dry powder handling), operations that had previously been performed in operator-staffed cleanrooms. Even though regulators did not explicitly require environmental monitoring, manufacturers incorporated existing environmental monitoring requirements into routine practices.

The design concepts that were embodied in the 2006 document for closed aseptic processing systems represent two different approaches in closed system design (see **Table I**). This divergence has resulted in substantial differences in validation, monitoring, and operation.

Blind adherence to methods and practices developed for use with open systems makes no sense with closed systems, which, regardless of their configuration, separate process and operator, and are designed to be fully functional independent of the background environment. One question for the pharmaceutical industry to consider is whether the

Ca^{++}Pure-HA™ Media

Unique Multimodal Media for Bioprocessing Purification

A Best-In-Class Hydroxyapatite Media from Tosoh Bioscience LLC

✓ High dynamic binding capacity
✓ Increased durability
✓ High flow rate/pressure tolerance

Excellent for Purification of mAbs, DNA/RNA, Proteins, and Viruses

Want to learn more? www.tosohbioscience.com

TOSOH BIOSCIENCE LLC • Customer service: 866-527-3587 • Technical service: 800-366-4875, option #3

Tosoh Bioscience is a registered trademark of Tosoh Corporation.
Ca^{++}Pure-HA is a registered trademark of Tosoh Bioscience LLC in the USA, EU, India, and Japan.

TOSOH BIOSCIENCE
www.tosohbioscience.com

Table I. Divergence of design elements in closed systems. HEPA is high efficiency particulate air. ISO is International Organization for Standardization.

Core principle	Tank-based (wet)	Room-based (dry)
Primary use	Varied processes within liquid phase	Varied processes in air filled background environment
Major design elements	Tanks, valves, piping	Isolators, pass-throughs, chambers
Primary interior contents	Liquids	Air
Secondary interior contents	Headspace air	Liquids and solids
Filters used	Membrane filters	HEPA filters
Sterilization method	Steam	Hydrogen peroxide
Internal environment	Unclassified	ISO 5
External environment	Non-critical	ISO 8

2006 document is still applicable, given the many improvements that have been made to closed system designs since then (**Sidebar, p. 35**).

APPLICATIONS

Closed systems' utility has resulted in an increasingly diverse array of applications in biopharmaceutical manufacturing.

Sterile APIs and complex pharmaceutical formulations

Closed systems have proven useful in manufacturing sterile products in bulk containers, in situations where sterilizing final product by membrane filtration is not possible (e.g., for powders, suspensions, ointments, creams, emulsions, and liposomes). Their preparation requires multiple vessels and a unit operations that include particle sizing, ultra-filtration, subdivision, and bulk packaging (6). The equipment train is sterilized *in-situ*, and the overall system eliminates aseptic assembly and human intervention. The formulation-focused systems are contemporaneous with and similar in concept to the fixed-vessel configurations that were previously used for sterile bulk production.

Large, single-use disposable bioreactors

Enabling greater efficiency and process separation (7,8), these systems are often used in conjunction with support vessels for inoculums, media, buffer, and other fluids. Many of the designs incorporate means for including operations such as mixing, *in-situ* analysis, addition and/or removal of fluids, sampling, and filtration. As use of these bioreactors increases and their reliability is demonstrated, there is growing interest in locating them in less controlled environments (9–11).

Single-use disposable filling systems

Sterilizing filters, surge vessels, peristaltic pumps, and needles (12-15) have been shown to simplify the sterilization and aseptic assembly of the fill set. Use of disposables also eliminates the risk of cross-contamination. Because these systems are ordinarily used for aseptic filling into open containers with separate closure systems, they are typically installed in International Organization for Standardization (ISO) 5 environments.

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

Risk-aversion remains an obstacle to innovation

In the early 1980s, the first pharmaceutical isolators used in sterility testing were fitted with turbulent airflow systems and relied upon manual manipulation internally for test execution. High-efficiency particulate air (HEPA) filter integrity and particle counts were confirmed during system qualification, and internal microbial testing was performed. This mimicked established practices for the traditional operator-run sterility test suites that the isolators were intended to replace, for the simple reason that making fewer changes to procedures and manufacturing processes would make it easier to install and implement the new technologies in existing production facilities and, of course, secure regulatory approval for their use.

However, well before then, widespread practices had established that fixed vessels and piping systems in closed systems needn't be monitored internally by any means other than process simulation. Despite this, manufacturers continued to impose these requirements on their isolator operations, and eventually they found their way into regulatory guidance.

The reason that these practices have persisted reflects manufacturers' wish to avoid the regulatory headaches triggered by process change applications. In the heavily regulated world of current good manufacturing practices, change is anathema, and process improvements using new technologies require multiple layers of review, documentation, and approval before the changes can even be submitted to regulators for approval.

The result has been a situation in which procedures demonstrating the absence of human contamination are still required for systems that prevent any contact with operators in the first place. This is a bit like requiring training wheels on the bicycles used in the Tour de France.

Reflecting the current environment, some sterility test isolator vendors have even begun to offer unidirectional airflow designs, ignoring evidence based on 40 years of global experience with isolators and millions of successful sterility tests using turbulent flow systems.

Isolation technology

Today's most advanced closed isolators are vastly upgraded versions of those first used for sterility testing in the 1980s (16). Erroneous perspectives and aversion to change resulted in the wholesale adoption of cleanroom-like environmental monitoring programs for isolators without consideration of their utility (10). The integration of isolation technology with robotics has led to the gloveless isolators in which all aseptic manipulations are performed remotely and robotically. These systems can include autoclaves, support isolators, lyophilizers, and other operational elements that do more than simply fill and seal sterile containers.

Closed-vial technology

In this approach, closed sterile containers are penetrated, filled, and resealed using an open needle. Using this approach, closed systems have evolved to utilize a filling needle which is closed at all times except when dispensing inside closed containers. In an open needle configuration, an ISO 5 environment is required to protect the fill needles during setup and filling. The use of a closed needle obviates the need to have filling done in a classified environment.

Integrated systems

Many of the previously described closed systems include filtration, fluid and vent sampling, and connection components that facilitate their use. These features can be fully integrated with the closed system or incorporated as add-ons provided by others. These sub-systems are smaller and less complex, and provide unique means for sampling and connection that reduces or eliminates the potential for product contamination.

SEPARATING OPERATORS FROM THE PRODUCT

System performance can vary substantially, however, and not all of these technologies may be suitable for use in less well-controlled environments.

Is the 2006 Technical Document still relevant for contemporary closed systems?

Given the disparity between the principles of closed systems and some current practices, it is useful to ask: Is the 2006 definition of closed systems still applicable today, given improvements that have been made in the technology? Consider the following questions.

What is a closed system isolator? It is a decontaminated (internal sterilization of all surfaces is optional) air-filled enclosure, which is designed to provide and maintain a physical barrier between the interior and exterior at all times. A pressure differential is maintained during decontamination and use. This would include isolators that are equipped with gloves, but exclude those where materials and component ingress and egress are managed by air pressure differentials.

Is sterilization necessary for non-product contact surfaces of a closed system isolator? It's not required, but preferred when viable monitoring is no longer performed. When the entire interior is sterilized, then the closed isolator is identical in process capability to a closed stainless-steel vessel.

Are high efficiency particulate air (HEPA) filters sufficient to assure the integrity of a closed system isolator? HEPA filters can provide sterile air (or other internal gas [e.g., nitrogen]).Integral HEPA filters remove microorganisms quantitatively.

Does unidirectional air provide any benefit in a closed system isolator? No. Unidirectional air is a carryover from manned cleanrooms, where its purpose is to direct air flow from the supply past the cleanest environment (where sterile materials are exposed) past dirtier environments (where personnel are present) on its way to the air return.

In a closed system isolator, there is no reason to require use of unidirectional air flow because all internal surfaces can be sterilized, something that is not possible in a manned environment and the predominant contamination source, personnel, are excluded.

Is viable environmental monitoring inside a closed system beneficial? No. Properly designed and maintained closed systems do not require internal viable monitoring. Its inclusion only adds risk due to added system complexity and invasive manipulations, and thus should be avoided.

Is non-viable environmental monitoring inside a closed system useful? Depending on internal processes and equipment, it can be useful to confirm that these are not contributing particles to the materials. It can also serve as a more immediate indication that system integrity has been lost. It would only be appropriate, however, in systems that contain air (or other gases).

If the closed system is pressurized relative to the external environment, how important are leaks? Leaks from clean (i.e., inside the closed system) to less clean (i.e., external to the closed system) environments do not present a risk.

There is no evidence that microorganisms and particles can enter against an outward flow. This is a core premise in all manned aseptic processing operations. Periodic leak testing is a closed system requirement to assess integrity over time and identify performance decay.

Does the external environment in which a closed system is located have significant impact? No. If the system is compromised, it has failed and action is required regardless of the background environment.

Counterintuitively, fewer controls on the exterior environment can facilitate the detection of contamination because a breach may result in a greater impact on the interior, thereby shortening response time and facilitating corrective action.

BioPharm International FOR PERSONAL NON-COMMERCIAL USE

Table II. Contemporary monitoring for closed systems. ISO is International Organization for Standardization.

Closed system	Primary contact surface	Internal		External	
		Classification	Monitoring	Classification	Monitoring
Pre-2006					
Vessels for sterile API	Wet	N/A	None	N/A	None
Closed isolators	Dry	ISO 5	Non-viable	ISO 8 – CNC	Varies
Present Day					
API/complex liquid formulation systems	Wet	N/A	None	N/A	None
Bioreactors/biopharm processes	Wet	N/A	None	CNC	Varies
Single-use disposables	Wet	N/A	None	ISO 5 – CNC	Varies
Gloveless isolators	Dry	ISO 5	Non-viable	ISO 8 – CNC	Varies
Closed gloved isolators	Dry	ISO 5	Viable & non-viable	ISO 8 – CNC	Varies
Closed vial/open needle filling	Wet / Dry	ISO 5	Viable & non-viable	ISO 5	ISO 5
Closed system filling	Wet	N/A	None	ISO 8 - CNC	None

Collectively, these technologies represent the future of aseptic processing. Coupled with the benefit of achieving true separation between operator and product, it is difficult to imagine future pharmaceutical manufacturing operations that won't involve greater use of closed systems. However, increasing usage and diversity of closed-system technology is often in direct conflict with process-monitoring expectations derived from more open processes, **Table II** lists the suggested monitoring practices that are appropriate for these newer technologies.

WET VS. DRY SYSTEMS

Reviewing **Table II,** it's evident that 'wet' and 'dry' systems are considered differently with respect to internal monitoring. Systems that are used for liquids have never been subject to internal monitoring despite the direct contact between the equipment and the contained liquid.

In marked contrast, isolator systems (where the interior surface is not in contact with the sterile materials) rely heavily on viable and non-viable monitoring, adhering to practices derived from cleanroom monitoring. This is surprising, because 'wet' systems are more susceptible to leaks, as well as contamination from gaskets, seals, and other components.

Differences in the way the two types of systems are sterilized should not matter if they have been properly validated. Differences in filtration, membrane, or high efficiency particulate air are also irrelevant (10).

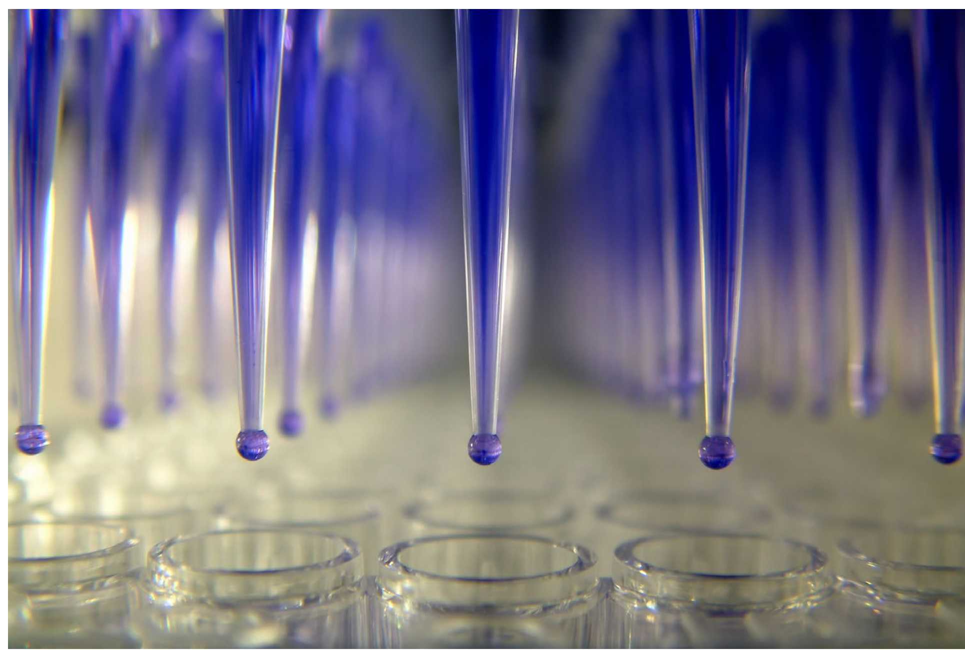
INTERNAL MONITORING CONFERS NO BENEFITS

Internal monitoring confers no benefits on closed isolators, especially those that can be operated without gloves. In fact, introducing sampling into these systems can increase the potential risk of contamination. In short, since closed aseptic processing systems were first introduced, they have found increasing use in the pharmaceutical industry. There is growing evidence as to their utility and reliability. If the industry is to remove obstacles to increased use of closed systems, regulators and manufacturers must address overly restrictive and redundant practices that impede use of these technologies.

REFERENCES

1. J. Agalloco, "Aseptic Processing: State of the Art and A Vision of the Future", presentation at AAPS Arden House conference, February, 2007.
2. J. Agalloco, *American Pharmaceutical Review*, 7(4), p. 26-29, 139, (2004).
3. World Health Organization, *Annex 4, General Requirements for the Sterility of Biological Substances*, Part A, Section 2,1973.
4. FDA, *Guideline on Sterile Drug Products Produced by Aseptic Processing*, 1987.
5. FDA, *Guide to Inspection of Sterile Drug Substance Manufacturers*, 1994.
6. PDA, TR #28, *PDA Journal of Pharmaceutical Science and Technology*, 52(4), Supplement (1998).
7. PDA, TR #28 revised, *PDA Journal of Pharmaceutical Science and Technology, Vol. 60, Supplement* S-2, (2006).
8. J. Akers, and J. Agalloco, *PDA Journal of Pharmaceutical Science and Technology*, 54(2), pp.110-111, 2000.
9. J. Agalloco, *Pharmaceutical Manufacturing*, 15(4), p. 34, (2016).
10. ISPE, *Baseline Guide, Biopharmaceutical Manufacturing Facilities*, Second Edition, November 2013.
11. S. Chalk, *BioPharm International*, 26 (11) pp. 49-50, (2013).
12. J. Agalloco and P. DeSantis, "*Sterile Bulk Pharmaceutical Chemical Production*", chapter in Bulk Pharmaceutical Chemical Production, 2nd edition, edited by S. Nusim, InformaUSA, New York, 2010.
13. S. Chalk, S., et al, *BioPharm International*, 24(8), pp. 44-59, (2011).
14. E. Bohn, *Pharmaceutical Technology*, 39(9), pp 54-55, (2015).
15. P. Estape, P., et al, *Pharmaceutical Engineering*, September-October 2017.
16. J. Agalloco, and L. Mestrandrea, *Pharmaceutical Technology* ebook, *Vaccine Development and Manufacturing* 2017, pp 31-38, November 2017. ◆

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE



Building Better Bioassays

Next-generation therapeutics and regulatory requirements create demand for complex, fit-for-purpose tests.

CYNTHIA A. CHALLENER

BioPharm
FOR PERSONAL NON COMMERCIAL USE

Bioassays for use in biologic drug development and final product release must be robust and meet an array of regulatory requirements. As the complexity of next-generation biopharmaceuticals increases, so does the complexity of the bioassays necessary for effective evaluation of their performance across a spectrum of activities. Collaboration is essential between drug developers, testing laboratories, and regulatory authorities to ensure that new bioassays consistently serve their intended purpose.

MOA, STABILITY, VALIDATABILITY, AND MORE

Regulators expect bioassays for any new biologic to reflect the mechanism of action (MOA) of the drug in question, have stability-indicating properties, and be validatable, as outlined in International Council for Harmonization (ICH) Q2(R1) (1) and *United States Pharmacopeia* (*USP*) General Chapter <1033> (2). "Assays need to be adequately robust, accurate, and precise to ensure consistency in batch manufacturing and to provide meaningful data to support stability claims," asserts Sharon Young, scientific manager, analytical and formulation sciences with Thermo Fisher Scientific.

MOA is important because it uncovers important clues—sometimes the most important clue—as to what is happening in the patient *in vivo*. "While the MOA rarely mimics exactly what is happening in the patient, in the best-case scenario the bioassay will mimic what is relevant for the patient (i.e., what makes the drug efficient)," explains Ulrike Herbrand, scientific director, global *in-vitro* bioassays at Charles River Laboratories.

Developing bioassays intended to measure the potency of biologics that truly reflect the relevant MOAs involves selecting appropriate indicator cell lines and relevant readouts that measure expected cell responses, adds Weihong Wang, technology development manager, cell and molecular biology services at Eurofins Lancaster Laboratories. "A master and/or working cell bank should be created for indicator cell lines and be well-characterized in order to assure meaningful interpretation of assay results and avoid artifacts unrelated to drug effects," she says.

In addition, a quantitative method is generally expected for potency measurement and results are usually expressed as percent relative potency when compared to a reference standard that is fully characterized, according to Wang. Sponsors should avoid using a surrogate assay unless they can provide sufficient justifications that address scientific rationale (MOA)

CYNTHIA A. CHALLENER, PhD, is a contributing editor to *BioPharm International.*

Caleb Foster/Stock.Adobe.com

and comparability to traditional assays that might be practically challenging/less robust.

A bioassay should also be able to determine the stability of a drug and for how long it will be stable. Stress tests are conducted over a prolonged time at moderate conditions, including the recommended storage conditions, and the results help determine how a drug should be stored (at what temperature and for how long), according to Herbrand. She also notes that accelerated stress testing at various stress conditions (e.g., temperature, oxidation, deamidation, shear-stress, repeated freeze-thaw cycles) is required to determine which types of alterations the chosen bioassay is capable of detecting by showing altered activity.

"Another key expectation for a bioassay is fulfillment of validation requirements by meeting the preset validation acceptance criteria, which is required for lot release and stability testing of the biologic at the end of clinical Phase II at the latest," Herbrand observes.

Young adds that bioassays should employ statistical monitoring to detect any shifts in performance over time and to detect any changes in the reference standard potency in real time. The use of more advanced statistical tools such as computational simulation can raise questions from regulators because they may seem unnecessarily complex, but such approaches can provide a much larger data set to determine assay acceptance criteria (3), she cites. The use of animal models is discouraged, Young comments, but is sometimes required and is acceptable if reasonably justified.

Ensuring the bioassay is suitable for transfer to other labs is not exactly a key requirement from a regulatory perspective, but nevertheless, Herbrand says it is an important point to consider. "A bioassay that is ridiculously difficult for other labs to use can delay programs since it takes too long to establish the method in a GMP-compliant [quality control] QC lab," she explains.

REPORTER-GENE ASSAYS SEE GROWING USE

Reporter-gene assays are a category of surrogate assays, notes Wang, that have been used more often in the past several years, especially in place of assays that require primary cells where assay performance can be quite variable and lack robustness desired as a QC testing method. They are particularly becoming common in early development and are increasingly being accepted by regulatory authorities, adds Young.

As demand for bioassays increases, laboratories look to simplify processes with ready-to-use cell banks and automated tools and processes.

As an example, Wang points to antibody-dependent cell-mediated cytotoxicity (ADCC) reporter-gene assays, which have been used to measure Fc receptor mediated response for monoclonal antibodies (mAbs) and in place of traditional peripheral blood mononuclear cell (PBMC)/primary natural killer (NK) cell-based cytotoxicity assays. "Instead of looking at direct cell killing (by primary NK cells), the assay measures activation of signal transduction associated with the cellular event," she explains. The resulting reporter gene assay is easy to perform with much shorter assay duration and in general delivers excellent assay performance.

The key to successful use of reporter-gene assays, adds Young, is to have additional bioassays that really reflect the biological mechanism(s) of action and then show that results using the reporter-gene assay are comparable, or better, to those bioassays that measure the cellular response(s) directly. "Bridging results need to include a large number of lot release samples as well as stability and force-degraded samples. Bridging studies should also include isolated impurities in these comparisons to directly demonstrate that all potential changes in the molecule can be detected in the reporter-gene assay comparably, or better, than in the direct assay(s)," she observes.

Wang highlights that the increasing use of reporter-gene assays is a good example that mechanism-of-action is the first and foremost important characteristic, and when this feature is proved, solid method performance including robustness is another key element of a successful bioassay.

SIMPLIFICATION TREND

The top trends in bioassay development observed at Thermo Fisher Scientific include the implementation of ready-to-use cell banks and automation. "Thaw-and-use cell banks can be thawed and plated directly in the bioassay, eliminating the need to spend a large amount of resources on maintaining actively growing cultures. It also simplifies monitoring of assay performance because impact from the age (passage number) of the culture need not be tracked and can reduce method variability attributable to variations in culture conditions between testing occasions," Young explains.

Laboratory/assay automation, from simple pipetting robots to end-to-end automation, is helping to increase throughput and reduce the risk of laboratory error, according to Herbrand. It also allows for miniaturization, which she says enables performance of six to seven tests simultaneously, affording higher throughput, the need for fewer reagents and lower cost.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

For instance, the use of automated liquid handlers is becoming increasingly common in bioassays because it significantly reduces assay variability attributable to pipetting, Young says. "Automation also mitigates variability attributable to positional bias by enabling randomization of plate layouts within and between plates without concern for analyst error, which significantly mitigates plate position and sequence (order of addition of reagents) sources of variability," she remarks.

CELL-THERAPY AND GENE-THERAPY CHALLENGES

With the fast growth of the gene- and cell-therapy pipeline, more potency assays for this category of product are being submitted to the regulatory agencies, according to Wang. Potency assays for cell- and gene-therapy products are generally more complicated than recombinant therapeutic proteins, as they need to demonstrate several characteristics of the product.

These products, according to Herbrand, require bioactivity testing, often in a multistep approach. For the first step, molecular methods such as a quantitative polymerase chain reaction (PCR) or droplet digital PCR are often the methods of choice to confirm the desired re-expression or suppression effect *in vitro* at the RNA level. In addition, she says the re-expression or suppression of a gene of interest can be shown on the protein level using enzyme-linked immunosorbent assay (ELISA) testing, or in the case of receptors, via flow cytometry. In some cases, a reporter-based method might be applicable as an MOA-reflecting, cell-based bioactivity assay to confirm the effect on the functional level.

"An assay for a gene therapy usually involves transfecting/infecting a cell line followed by measurement of the expression of the intended target gene at the nucleic acid or protein level. In addition, whenever possible, the function of the expressed protein should be confirmed, for example, by enzymatic assays, or receptor binding, etc., depending on the MOA. Therefore, often times several assays are needed to fully address all aspects of the product characteristics," explains Wang.

Potency assays for cell therapy products also have their unique challenges. "First of all," states Wang, "it may be more difficult to correlate *in-vitro* potency measurements to clinical efficacy compared to typical recombinant protein therapeutics." Second, depending on the product, she notes that it is sometimes difficult to truly establish a "reference standard" to which subsequent lots may be compared. Third, performance of functional assays, in general, tends to be less robust.

Because of these issues, setting specifications can also be challenging, according to Wang. "Last but not least," she observes, "some cell-therapy products require fast delivery to patients; therefore the balance between thorough characterization and short assay turn-around time may need to be carefully considered."

Because multiple assays may need to be deployed, from phenotypic (e.g., flow cytometry), to functional confirmation (e.g., cytokine release and/or cytotoxicity for chimeric antigen receptor T-cell therapies), these assays need to be developed as part of a 'matrix approach' in order to fully characterize the product and assure efficacy in order to gain regulatory acceptance," Wang says.

A recent approach noted by Young that has gained approval is flow cytometry detection of biomarkers that correlate with function, such as cell survival or differentiation. "Analysis of the selected biomarkers must be able to detect unacceptable behavior of the cells, and not simply rely on cell identity markers that are unlikely to do so under conditions that affect cell function," she notes. The rationale for selection of the biomarkers must be clear, particularly when a complex assay matrix is proposed, and the assay must be shown to be capable of rejecting a sub-par batch, she adds.

One of the main challenges, asserts Herbrand, is that there currently are no clear guidelines on how to evaluate bioactivity for cell-therapy and gene-therapy products. "For now," she says, "these therapies are being considered on a case-by-case basis by regulators. Many of the gene therapy companies are currently running tests that more or less reflect what would be required for the development phase, but they haven't nailed down what they need for QC release of a marketed drug."

What isn't in question is the complexity of cell and gene therapies. "What is understood is that the safest course to take is to make sure we reflect that the drug is doing its job on all relative levels. Twenty years ago, we pondered the same questions with mAbs and what was required for lot release. Today, at least we have imprecise guidelines," Herbrand comments.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

BISPECIFIC ISSUES

Many molecules have more than one mode of action, particularly bispecific antibodies, and regulators increasingly want a single assay that can reliably detect changes in potency through those multiple mechanisms, according to Young. "Combined approaches measuring the activities of both epitopes within the same assay rather than reporting two independent results for the activities of the individual arms of the bispecific have an excellent chance of being accepted by regulators," Herbrand agrees. The reason: it is important that bioassays for potency of bispecific molecules reflect any additive or synergistic effects of the bispecific mechanisms of action.

"This need," Young notes, "can create challenges in developing the method, particularly in cell-line selection, as well as in interpreting the results. Results for a bispecific with a synergistic effect will show changes

in EC50 as well as in asymptotes, for which tests of parallelism are not appropriate. Differences in asymptotes is traditionally unacceptable per *USP* guidance, as sample curves must be similar to reference curves for calculation of relative potency."

Currently, although the ideal assays from a regulatory and a sponsor's standpoint would be dual reporter systems, it is still more common to independently test the bioactivity using two different assays to measure each arm of the bispecific antibody, Herbrand remarks. "While easier to develop, they do not allow one to fully see the synergistic effects and combined activity of the antibodies," she notes.

Young observes that at the CASSS Bioassays 2016 conference, Bhavin Parekh presented an approach for calculating relative potency/efficacy for a bispecific product with synergistic activity that he developed based on the field of pharmacology (4). She thinks this simple approach to reach a discrete value to compare concomitant shifts in EC50 and asymptotes may be able to establish a precedent for regulatory acceptance.

3D CELL CULTURE IS GARNERING SOME INTEREST

Common in drug discovery, three-dimensional (3D) cell culture models have recently been attracting interest among biologics manufacturers for use in bioassay development as well. "The use of 3D cell cultures and 3D bioprinting has the potential to enable assays in more complex cellular environments, more like tissue structures," Young explains. "Such models," she adds, "are ideal for mimicking the complex cancer microenvironment as well as for culturing cell lines that rely extensively on the extracellular matrix environment for growth, differentiation, and function, such as skeletal muscle cells."

"These 3D systems are often a better reflection of the *in-vivo* situation than what is observed for traditional two-dimensional cell-culture-based assays and provide better information regarding what is occurring in the patient," agrees Herbrand. She does note, though, that it can be tedious to validate such tests, and they are often still time-consuming. Young also says that it can be challenging to control variability, find suitable methods of quantitation for potency results, and implement such complex techniques into a QC environment.

POTENTIAL FOR PROTEIN TAGGING AND HUMAN PRIMARY CELL ASSAYS

Protein tagging is based on peptide sequences that are attached to proteins to facilitate easy detection and purification of expressed proteins, according to Herbrand. They can also be used to identify potential binding partners for the protein of interest. The tags, which Herbrand notes are typically applied using CRISPR/Cas9 gene editing technology, are of particular interest because they are highly sensitive down to the endogenous level of the protein, often with over seven logs of dynamic range.

Because human primary cell assays are closer to the situation in patients, Herbrand suggests they may be more translatable than cell lines, which are immortalized/modified in their key characteristics and thus could exhibit modified cell behaviors. "However," she cautions, "human primary cell-based assays are not without risks." Human primary cells are subject to lot-to-lot variability and availability of suitable sourcing material. In addition, even if it was possible to identify the perfect lot, it wouldn't last forever. "There are no guarantees you won't have to adjust the assay. And even if you manage to get the assay running again, you have to revalidate it, at least partially, which requires a change in the regulatory filing and months of delays," Herbrand explains.

INCREASING BIOASSAY COMPLEXITY REQUIRES CONSIDERATION AND CAUTION

Caution is warranted in general when it comes to newer bioassays, according to Young. "As cell culturing techniques advance and increasingly complex multifunctional molecules are developed in the biopharmaceutical industry, bioassay development will become increasingly complex as well," she says.

One way the industry has responded, according to Wang, is to develop bioassays to support therapeutic biologics as early as possible, particularly when more complex products are involved. "While science continues to be the driving force for bioassay development, sponsors are encouraged to engage regulatory agencies early in order to obtain guidance on their assay development approach and avoid unnecessary surprises and delays in their product development programs," she says.

Young adds, "As an industry, we are constantly trying to find a better model to assess potency of our molecules, but we must be cautious about the complexity of bioassays. Bioassays are already complex, which results in increased variability and statistical uncertainty and can be difficult to control between labs. We must be careful to avoid unnecessary challenges in method execution from over-complicating bioassays."

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

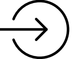
REFERENCES

1. ICH, Q2(R1) *Validation of Analytical Procedures: Text and Methodology*, Step 4 version (1995).
2. USP, *USP* General Chapter <1033>, "Validation of Biological Assays" (Rockville, MD, 2010).
3. M. Labant, *Gen. Eng. News*, 36(10), (2016).
4. B. Parekh, "Bioassay Development for Complex Molecules," presentation at CASSS Bioassays (Silver Spring, MD, 2016). ◆

An In-Depth Look at Intact Biotherapeutic Characterization and Quantitation in Biological Matrices

ON-DEMAND WEBCAST:

Aired: Thursday, April 23, 2020



Register for this free webcast at:
www.chromatographyonline.com/lcgc_p/biological_matrices

Event Overview

This webcast discusses a highly sensitive and automated workflow for intact biotherapeutic characterization and quantitation from biological matrices. We will also discuss native protein characterization and its challenges. Automated sample preparation, sample analysis and data interpretation will be presented as well as pitfalls encountered. Performance and robustness of the workflow will be discussed. Finally, future expansion of methods and techniques will be discussed.

In particular, we will discuss:

- An optimized workflow from sample preparation, separation, data acquisition to data analysis
- A high throughput and reproducible method with limited sample prep that enables various mixtures of mAbs can be quantitated simultaneously
- A robust workflow with excellent dynamic range of over 3 orders of magnitude and low picogram in sensitivity

Key Learning Objectives

- How to prepare samples using automated liquid-handling
- How to optimize HPLC methods with columns, for better MS sensitivity and reproducibility?
- How to achieve the best sensitivity using LC/Q-TOF for intact mAb analysis
- How to setup various data processing methods for accurate intact mAb quantitative analysis

Who Should Attend

BioPharma Lab Managers, Discovery and Development Scientists

Presenter

Christian Klein
LC/Q-TOF and IM-QTOF Product Manager
Agilent Technologies

Moderator

Laura Bush
Editorial Director
LCGC

Sponsored by

Presented by

For questions or concerns, email mdevia@mjhlifesciences.com

BioPharm INTERNATIONAL
PERSONAL NON-COMMERCIAL USE

molekuul.be - Stock.adobe.com

Shaping IR Spectroscopy into a Powerful Tool for Biopharma Characterizations

DIPANWITA BATABYAL, LIBO WANG, JEFFREY ZONDERMAN, AND MATS WIKSTRÖM

ABSTRACT

Microfluidic modulation spectroscopy (MMS) is a novel automated infrared spectroscopic technique with high sensitivity and repeatability. Here, the authors present a series of experimental studies showcasing the performance of MMS in the secondary structure characterization of biopharmaceutical products and compare the MMS results with the conventional Fourier transform infrared data.

BioPharm INTERNATIONAL

FOR PERSONAL NON-COMMERCIAL USE

The successful development of biopharmaceuticals involves the study of their higher order structure, a critical quality attribute, to ensure a therapeutically active molecule in appropriate formulation conditions (1–3). Robust structural characterization of the biopharmaceutical products is important throughout the development process. For instance, comparability studies are performed to ensure that a manufacturing process change during clinical and commercial development does not have an adverse effect on quality, safety, and efficacy (4–6).

Infrared (IR) spectroscopy is a powerful method for characterizing the secondary structure of proteins (7–14). However, the lack of automation of conventional Fourier transform IR (FTIR), along with relatively high sample concentration requirements, are major limitations with this technology. Far ultraviolet circular dichroism spectroscopy (far-UV CD) is an important alternative for the characterization of secondary structure, but it also has major drawbacks. Measurement is necessarily carried out at low concentrations, typically at 0.5 mg/mL but down as low as 0.1 mg/mL, which can undermine the relevance of the resulting data. The presence of certain excipients in the formulation buffer can also significantly interfere with the measurements. Furthermore, far-UV CD and conventional FTIR have been shown to lack sensitivity in the characterization of biopharmaceuticals proteins (e.g., immunoglobulin G1 [IgG1] and IgG2) (15).

Microfluidic modulation spectroscopy (MMS) represents a novel automated technique that directly addresses the current limitations with both conventional FTIR and far-UV CD by shaping IR spectroscopy into a far more effective analytical tool in biopharmaceutical product characterization (16). This article presents a series of experimental studies showcasing the performance of MMS applied to challenges in the characterization of the secondary structure of biopharmaceutical products including comparisons with conventional FTIR data.

MATERIALS AND METHODS

Conventional FTIR and MMS were used to determine the secondary structure of two biopharmaceutical samples: a monoclonal antibody (mAb) and a bispecific T cell engager (BiTE, a registered trademark of Amgen) (17) molecule (Amgen, Thousand Oaks, US). The BiTE molecule represents a fusion protein, created by linking the variable light and heavy chain corresponding to two antibodies. Polysorbate (PS) 80 was purchased from Fluka (Cat#: 59924-100G-F Lot: BCBC1232).

Conventional FTIR measurements were carried out using a Bruker Vertex 70 spectrometer equipped with an Aquaspec transmission cell that requires manual injection of the sample and reference buffer at room

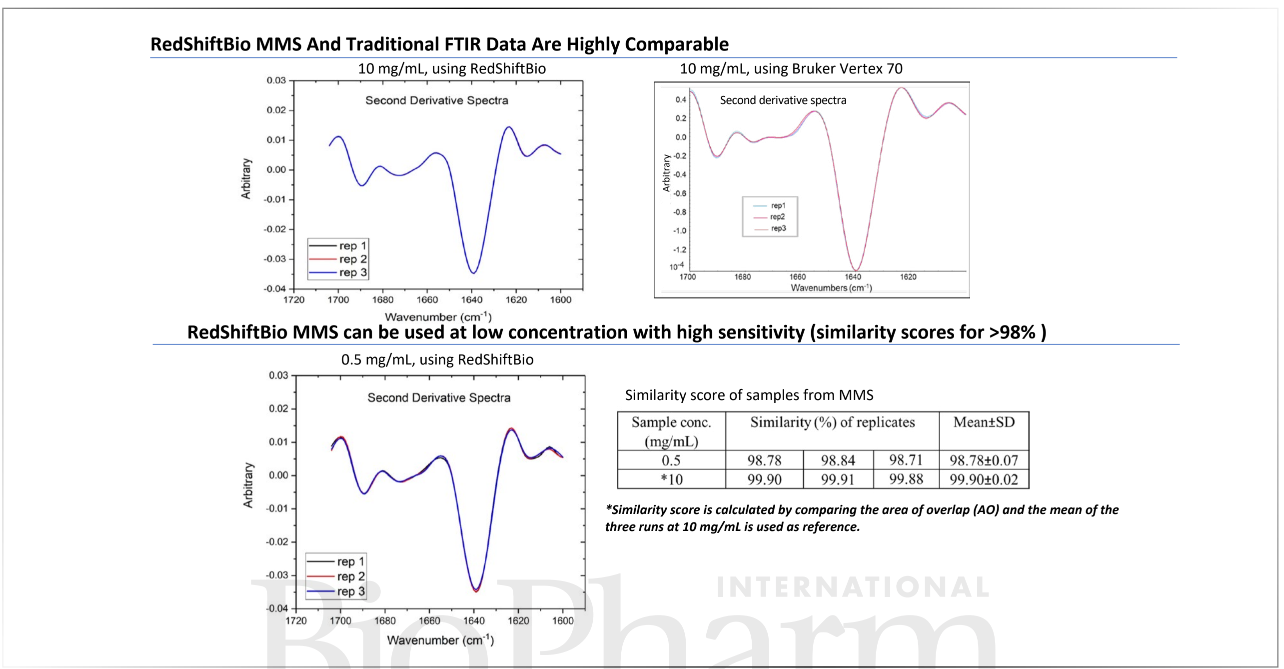
Dipanwita Batabyal*, dbatabya@amgen.com, is scientist at Attribute Sciences—Higher Order Structure Group, Amgen and **Mats Wikström***, matsw@amgen.com, is principal scientist at Higher Order Structure, BR&C, Attribute Sciences, Amgen. **Libo Wang** is principal application scientist and **Jeffrey Zonderman** is chief commercial officer at RedShift Bioanalytics.

To whom correspondence should be addressed.

PEER-REVIEWED

Submitted: Dec. 9, 2019
Accepted: March 9, 2020.

Figure 1. Conventional Fourier transform infrared (FTIR) and microfluidic modulation spectroscopy (MMS) measurements for a monoclonal antibody are highly comparable at a concentration of 10mg/mL. Unlike conventional FTIR, MMS can also measure with high sensitivity at a concentration of 0.5 mg/mL. Acceptable quality data (similarity score >95%) is not possible at 0.5 mg/mL using conventional FTIR.



Sample conc. (mg/mL)	Similarity (%) of replicates			Mean±SD
0.5	98.78	98.84	98.71	98.78±0.07
*10	99.90	99.91	99.88	99.90±0.02

Similarity score is calculated by comparing the area of overlap (AO) and the mean of the three runs at 10 mg/mL is used as reference.

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

ALL FIGURES COURTESY OF THE AUTHORS.

temperature. The reference spectra for buffer blank were subtracted from the protein spectra according to previously established criteria (18). Spectral similarity was quantitatively determined using the Thermo OMNIC software quality control (QC) compare function.

MMS measurements were conducted at ambient temperature using an AQS3pro system (RedShiftBio, Burlington MA, US) with multi-sample automation. A microfluidic transmission cell of approximately 24 μm pathlength was used. Streams of protein samples and reference buffers were introduced into the flow cell alternatively at a back pressure of 5 psi and a modulation rate of 1 Hz. Simultaneous modulation of the sample and an appropriate buffer enabled a real-time subtraction of the buffer background and allowed the differential absorbance measurement. Thirty-one discrete wavenumbers across the amide I band from 1590 cm^{-1} to 1714 cm^{-1} were scanned, and the differential absorbance spectra of samples were collected. Triplicate measurements were carried out for each sample. The data were analyzed using the software AQS^3 delta analytics package to produce the final spectra and analysis results. For each experiment, interpolate differential absorbance spectra, absolute absorbance spectra, and the second derivative spectra were obtained, and the similarity score was calculated using the area of overlap in the amide I band region (1700 cm^{-1}–1600 cm^{-1}).

RESULTS

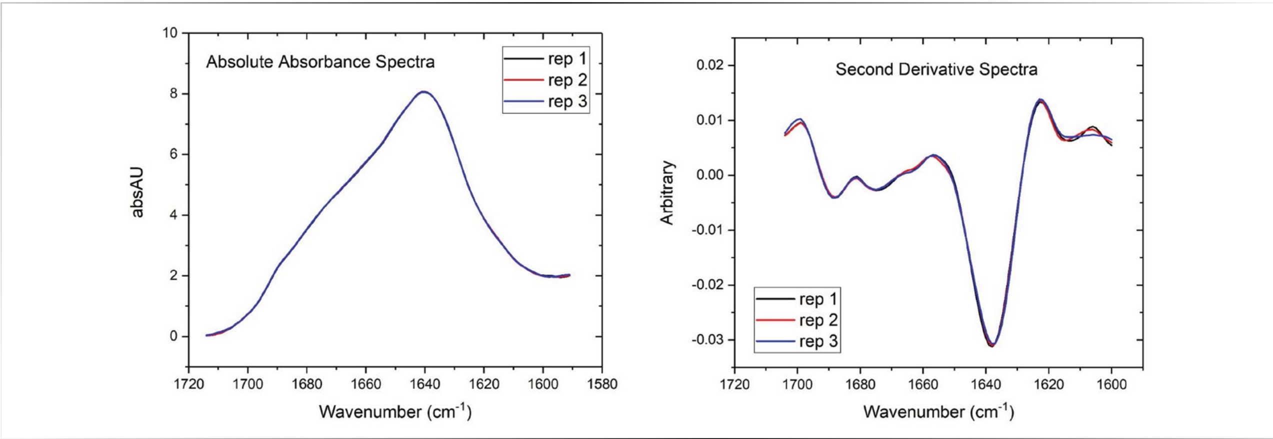
Case 1: testing instrument sensitivity

This section discusses testing instrument sensitivity at different protein concentrations and with different modalities.

To compare the relative sensitivity of MMS and conventional FTIR, mAb samples were analyzed using the two methods at concentrations of 0.5 mg/mL and 10 mg/mL in acetate buffer. At 10-mg/mL concentration, the data from MMS matched well with the conventional FTIR. Both techniques showed high repeatability quantified by high spectral similarity scores (> 99% for both, see **Figure 1**). The spectral similarity in the conventional FTIR was calculated using the QC compare function from the OMNIC software. The spectral similarity from MMS data was calculated by comparing the area of overlap of each sample replicate to the mean area of overlap of all three replicates. In general, there was very good agreement in similarity scores between the two methods. The MMS data were further analyzed using the QC compare function from OMNIC, and the results showed consistency between the similarity scores obtained by the two approaches.

At lower concentrations of the mAb (0.5 mg/mL), acceptable quality data (> 95% similarity score between the replicates) were not obtained by conventional FTIR, whereas the MMS data showed high repeatability at low concentrations, indicated by the high similarity scores (> 98%) between the three replicate runs. The similarity score in MMS is calculated by comparing the area of overlap (AO) of the replicates using mean AO of the three replicates as reference.

Figure 2. Microfluidic modulation spectroscopy data for the BiTE sample show excellent repeatability at low protein concentration (1mg/mL). Panel on the left shows overlaid absolute spectra, panel on the right shows overlaid second derivative spectra.



A BiTE molecule sample was analyzed at 1.0 mg/mL to further assess the sensitivity of MMS at concentrations lower than those accessible with conventional FTIR, with a different modality. As shown in **Figure 2** (left panel), the absolute absorbance spectra generated through triplicate measurements are almost indistinguishable, indicating high repeatability of the MMS measurements. The second derivative spectra of the three replicates (**Figure 2**, right panel) overlay very well, indicating the high consistency between measurements further quantified by comparing the similarity score. Overall the second derivative spectrum exhibits a strong β-sheet peak at around 1639 cm^{-1} together with a β-sheet peak at 1689 cm^{-1}. The similarity score of three replicates for the BiTE molecule are all > 99% (**Table I**), indicating high repeatability between the runs. The contributions from the different secondary structure elements (referred to as the higher order structure analysis, or HOS analysis) were further determined using Gaussian peak assignment from known correlations with absorption at specific wavenumbers within the amide I band (19). As shown in **Table II** the BiTE molecule antibody consists predominantly of β-sheets (58.67 ± 0.80%) along with some contributions from β-turns (31.38 ± 0.48%).

Case 2: buffer excipient

This section assesses the impact of the buffer excipient on secondary structure.

To investigate the impact of buffer composition and different excipients, an MMS test of a mAb sample (5 mg/mL) was carried out and analyzed in three different buffers—Buffer A, B, and C—with the same base composition but with different amounts of polysorbate (PS) 80 (0.01%, 0.05%, and 0.1% w/v, respectively).

As shown in **Figure 3**, the absolute absorbance spectra and the second derivative spectra of the three replicates are very closely matched, suggesting that different amounts of PS 80 have no effect on the secondary structure of the mAb. Similarity scores of the three replicates in all three buffers are > 99% (**Table III**), indicating high repeatability. In **Table IV,** the HOS analysis shows that the secondary structure of the mAb consists predominantly of β-sheets (approximately 61.5%), with β-turns at approximately 29%. When compared to the BiTE molecule HOS analysis (**Table II**), there is a relative higher proportion of β-sheet content and a lower proportion of β-turns in the secondary structure analysis of the mAb samples.

Table I. Similarity scores of the BiTE sample measured by microfluidic modulation spectroscopy. SD is standard deviation.

Sample conc. (mg/mL)	Similarity (%) of replicates			Mean±SD
1.0*	99.01	99.20	98.97	99.06±0.12

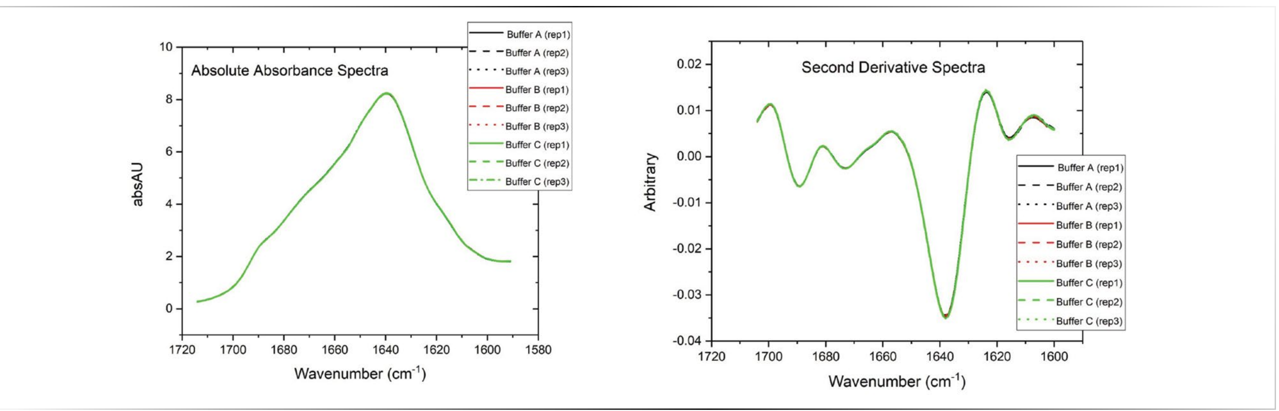
*Similarity score is calculated by comparing the area of overlap (AO), and the mean AO of the three replicates is used as the reference.

Table II. Higher order structure (HOS) contents (%) of the BiTE molecule sample determined by microfluidic modulation spectroscopy.

Sample conc. (mg/mL)	HOS% (mean±SD) of replicates			
	Beta	Turn	Unordered	Alpha
1.0	58.67 ± 0.80	31.38 ± 0.48	7.64 ± 0.78	2.32 ± 0.36

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Figure 3. Microfluidic modulation spectroscopy data of monoclonal antibody samples with different amounts of polysorbate 80 (PS 80) in buffer. Buffer A: 0.01% (w/v) PS 80, Buffer B: 0.05 % (w/v) PS 80 and Buffer C: 0.1 % (w/v) polysorbate 80. The left panel shows the overlaid absolute spectra and the right panel shows the overlaid second derivative spectra.



Case 3: higher protein concentrations

This section discusses test consistency, precision, and accuracy of MMS at higher protein concentrations.

In this experiment, the same mAb sample that was used in the case 2 study was analyzed by MMS at concentrations of 50 mg/mL and 100 mg/mL in the base buffer without any PS 80. Tests were performed on different days, and the resulting data were compared to check for consistency and precision of MMS measurements.

The similarity scores are shown in **Table V** and were calculated using mean AO of a 50 mg/mL sample as reference. Overall, > 99% similarity was observed at all protein concentrations (ranging from 5 mg/mL to 100 mg/mL) indicating high repeatability of the MMS measurements. The high consistency of measurement is retained even though measurements are made over multiple days. The data further confirmed that neither the protein concentration nor the buffer excipient PS 80 impacts the secondary structure of the mAb. Further HOS analysis giving the secondary structure component also reflects the high consistency that was observed in the similarity score data (data not shown).

DISCUSSION

The criticality of HOS makes measurement essential throughout biopharmaceutical development. Robust formulation and process development relies on measuring the impact of concentration—of different buffers and of processing conditions as a drug candidate proceeds toward commercialization—with the different types of therapeutic molecules that are increasingly part of the new drug pipeline. All analytical techniques have strengths and limitations when assessed against this informational need. For example, unlike conventional FTIR or near-UV CD, MMS is not a general-purpose platform at this time. MMS has been optimized

Table III. Similarity scores of the monoclonal antibody sample in different buffers measured by microfluidic modulation spectroscopy. SD is standard deviation.

Samples @ 5 mg/mL	Similarity (%) of replicates			Mean±SD
In Buffer A*	99.62	99.79	99.76	99.72 ± 0.09
In Buffer B	99.76	99.64	99.52	99.64 ± 0.12
In Buffer C	99.66	99.55	99.69	99.63 ± 0.07

*Similarity score is calculated by comparing the area of overlap (AO), and the mean AO of sample replicates in Buffer A is used as the reference. Buffers A, B, and C contain 0.01%, 0.05%, and 0.1% (w/v) polysorbate 80, respectively, in the same base buffer.

Table IV. Higher order structure (HOS) contents (%) of the monoclonal antibody sample in different buffers determined by microfluidic modulation spectroscopy. SD is standard deviation.

Samples in	HOS% (Mean±SD) of replicates			
	Beta-sheets	Beta-turn	Unordered	Alpha-helix
Buffer A	61.41±0.09	29.40 ± 0.11	6.90 ± 0.01	2.29 ± 0.03
Buffer B	61.67±0.15	29.22 ± 0.06	6.87 ± 0.10	2.24 ± 0.10
Buffer C	61.63±0.13	29.29 ± 0.07	6.80 ± 0.10	2.27 ± 0.15

Table V. Similarity scores of a monoclonal antibody sample analyzed in different buffers and at different concentrations. PS 80 is polysorbate 80. SD is standard deviation.

Sample Conc.	Buffers	Similarity (%) of replicates			Mean±SD
50 mg/mL*	Base Buffer (no PS 80)	99.45	99.75	99.60	99.60 ± 0.15
100 mg/mL	Base Buffer (no PS 80)	99.37	99.36	99.37	99.37 ± 0.01
5 mg/mL	Buffer A (0.01% PS 80)	99.30	99.31	99.17	99.26 ± 0.08
5 mg/mL	Buffer B (0.05% PS 80)	99.18	99.28	99.06	99.17 ± 0.11
5 mg/mL	Buffer C (0.1% PS 80)	99.36	99.26	99.23	99.28 ± 0.07

*Similarity score is calculated by comparing the area of overlap (AO) and the mean AO of 50 mg/mL sample replicates, which were used as the reference. Buffers differ in the amount of PS 80 in the same base buffer.

for all types of protein- and peptide-based secondary structural analysis, which is of interest in biopharmaceutical development and manufacture, but not other structural features (i.e., tertiary) or molecule types.

In contrast, the application of multiple techniques to characterize HOS, a necessity when combining conventional FTIR and far-UV CD to cover the range of conditions of interest, is inherently problematic. Such an approach can introduce uncertainty, where there is overlap between techniques and discrepancies in the results produced as well as complicating analytical workflows. Any requirement for sample preparation can also undermine data integrity because proteins are labile, changing in response to their local environment. The adoption of techniques that can be applied directly, to a broad range of sample types, is therefore technically advantageous.

The results from this study clearly demonstrate the performance of MMS. A direct comparison with conventional FTIR illustrates a number of ways in which MMS is a superior presentation of IR spectroscopy for this application, while the ability to measure with high sensitivity and precision at high concentrations and in the presence of different buffers highlights the potential of MMS relative to far-UV CD.

The data show that MMS allows the determination of secondary structure over a much wider concentration range than conventional FTIR, thus removing the requirement of either dilute or concentrated samples for measurement. In this study, MMS measurements were successfully made across a concentration range from 0.5 mg/mL to approximately 100 mg/mL. In contrast, conventional FTIR measurements require a minimum concentration of approximately 10 mg/mL to acquire data of acceptable quality.

The capability to measure at low concentrations means that MMS is not limited to studies of mAbs, but can also be applied to other protein therapeutic modalities, such as BiTE molecules, which are typically measured at product concentrations below the minimum required for conventional FTIR. For low-concentration measurements, far-UV CD would typically be the technique of choice, but it can be unreliable for formulations containing chromophores other than those associated with the drug entity, necessitating filtration or dilution of the sample prior to the measurement. The data showing the repeatability of mAb measurements in solutions with different buffer concentrations are helpful in demonstrating the ability of MMS to address this limitation.

Finally, the results show that MMS data are highly repeatable with high precision, unlike conventional FTIR, which routinely exhibits instrument drift. This characteristic is due to the way in which MMS generates differential data via a process of continuous auto-referencing that eliminates the issue of background drift. High repeatability contributes directly to the ability of a technique to detect difference and indicates that MMS will exhibit greater sensitivity to changes in protein structure.

CONCLUSION

MMS is a powerful new technique for the assessment of the secondary structure of proteins. The results presented here show how it enables accurate, highly repeatable characterization across a wider concentration range than conventional FTIR, and measures with high sensitivity with different buffers. These capabilities offer potential to streamline the routine analysis associated with biopharmaceutical development for various protein therapeutic modalities, including mAbs and BiTE molecules.

REFERENCES

1. J. D'Antonio, et al., *J. Pharm. Sci.* 101 (6) 2025–2033 (2012).
2. Y. Jiang, et al., *J. Pharm. Sci.* 100 (11) 4631–4641 (2011).
3. C.H. Li, et al., *J. Pharm. Sci.* 100 (11) 4642–4654 (2011).
4. FDA, *Guidance for Industry, Q5E Comparability of Biotechnological/Biological Products Subject to Changes in the Manufacturing Process*, (CDER, CBER, June 2005).
5. B.M. Teska, et al., *Anal. Biochem.* 434, 153–165 (2013).

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

More on biopharmaceutical characterization

For more on biopharmaceutical characterization, read the following articles on *BioPharmInternational.com:*

- How Advanced Mass Spectrometry Technologies and Workflows are Delivering Comprehensive Protein Characterization
  www.BioPharmInternational.com/how-advanced-mass-spectrometry-technologies-and-workflows-are-delivering-comprehensive-protein-chara
- Biopharma Analysis Benefits from New Technology and Methods
  www.BioPharmInternational.com/biopharma-analysis-benefits-new-technology-and-methods
- New FT–IR Spectrometer Enhances Protagen Protein Services' Analytical Capabilities
  www.BioPharmInternational.com/new-ft-ir-spectrometer-enhances-protagen-protein-services-analytical-capabilities-0
- Accepting the Challenge of Protein Characterization
  www.BioPharmInternational.com/accepting-challenge-protein-characterization
- Protein Analyzer Offers Ability to Measure Protein Titer Directly from Bioreactor
  www.BioPharmInternational.com/protein-analyzer-offers-ability-measure-protein-titer-directly-bioreactor

6. N.N. Ding, et al., *Anal. Biochem.* 464, 60–62 (2014).
7. W. Wang and C.J. Roberts, *Aggregation of Therapeutic Proteins* (Wiley, 2010).
8. A. Elliott and E.J. Ambrose, *Nature* 165, 921–922 (1950).
9. H. Susi and D.M. Byler, *Methods Enzymol.* 130, 290–311 (1986).
10. W. K. Surewicz and H.H. Mantsch, *Biochim. Biophys. Acta* 952, 115–130 (1988).
11. A. Dong, P. Huang, and W.S. Caughey, *Biochemistry* 29 (13) 3303–3308 (1990).
12. S.J. Prestrelski, A.L. Williams Jr., and M.N. Liebman, *Proteins Structure Function and Bioinformatics* 14 (4) 440–450 (1992).
13. W. K. Surewicz, H.H. Mantsch, and D. Chapman, *Biochemistry* 32 (2) 389–394 (1993).
14. J.L.R. Arrondo, et al., *Prog. Biophys. Mol. Biol.* 59 (1) 23–56 (1993).
15. J. Wen, et al., *J. Pharm. Sci.* 109 (1) 247–253 (2020).
16. E. Ma, L. Wang, and B. Kendrick, *Spectroscopy* 33 (7) 46–52 (2018).
17. P.A. Baeuerle and C. Reinhardt, *Cancer Res* 69 (12) 4941–4944 (2009).
18. A. Dong and W.S. Caughey, *Methods Enzymol.* 232, 139–175 (1994).
19. J. K. Koenig and D. L.Tabb, "Infrared Spectra of Globular Proteins in Aqueous Solution," in *Analytical Applications of FTIR to Molecular and Biological Systems*, J.R. Durig, Ed. (D. Reidel Publishing Company, Dordrecht, Netherlands, 1980), pp 241–255. ◆

BioPharm INTERNATIONAL

FOR PERSONAL NON-COMMERCIAL USE

Call for Peer-Review Papers

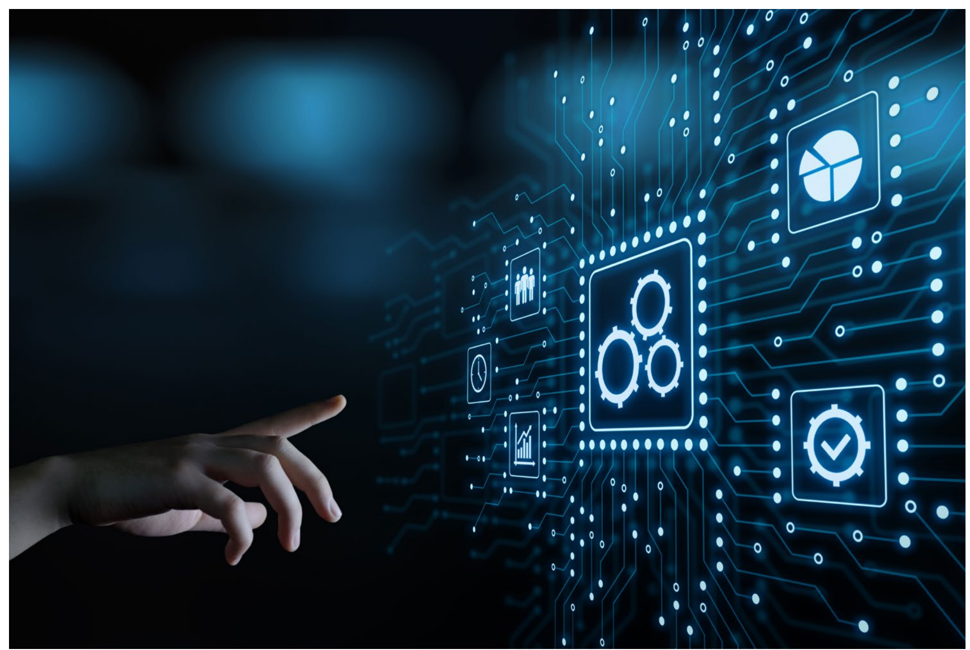
Peer-Review Research

Therapeutic Potential of Green, Synthesized Gold Nanoparticles

BioPharm International accepts four types of peer-review papers that are considered: standard data-driven, novel research; topical literature or patent review; technical case studies/technical application notes; and science-based opinion papers.

Manuscripts for peer-review papers are accepted on an ongoing basis. Publication priority is given to papers in the order they are accepted for publication. Submitted papers are initially screened by the editors, then submitted for formal review by a member of the editorial advisory board, who will review the article for technical interest and content in a double-blind review process. Article acceptance is conditioned on the reviewer's approval. Once accepted for publication, a paper typically is published within three to five months. Peer-review papers are published in the print and digital editions of *BioPharm International,* and on www.BioPharmInternational.com. Links to the online versions of peer-review papers also are featured in e-newsletters distributed to the publication's audience. Manuscripts may be sent to Editorial Director Rita Peters at RPeters@mjhlifesciences.com.

To learn more about the peer-review submission process, click the Submission Guidelines link on www.BioPharmInternational.com. We look forward to hearing from you.



Good Automation Practices for Remote Operations

Having remote operations in place is crucial to maintaining good automation practices.

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

LAUREN LAVELLE

BioPharm International spoke with Casey Snodgrass, market segment leader for Pharmaceutical Sciences at the Hamilton Company; Cynthia Pussinen, vice-president and general manager, Life Sciences and Specialty Chemicals for Honeywell Process Solutions; and Bruce Kane, industry technology consultant for Rockwell Automation, regarding the proper procedures for remote operations when complying with good automation practices.

PRACTICALITY AND SAFETY

BioPharm: How practical are remote operations in manufacturing facilities in terms of good automation practices?

Snodgrass (Hamilton): In practical terms, remote operations minimize the number of people that directly interface with the system. This is especially useful when working with hazardous materials or highly infectious pathogens, such as SARS-CoV-2, to limit the number of people with potential exposure to infectious agents spending valuable time with hands-on training, and reduces the amount of personal protective equipment (PPE) used at the facility. To balance this perspective, convenience and practicality often come at the price of complexity. Remote operations may require more extensive programming and project management. The stakeholders need to have a clear vision of the system's goals, along with an incredibly detailed map of the requirements, including interactions with other equipment and the laboratory information system. A remotely operated system is far different than a stand-alone automation platform for a dedicated workflow.

However, the term 'remote operations' can be a bit misleading, because a facility still needs people—let's call them super users—physically interfacing with a liquid handling system to load tips, consumables, and reagents onto a robotic system, and also providing periodic maintenance and troubleshooting, or error handling response. It is possible to configure an automated system with convenient remote access for users that are trained in creating a job, and at the end of the run, remotely retrieve the data or send samples downstream. Facilities can also link Hamilton's dissolved oxygen (DO), pH, cell viability, and conductivity sensors to a biocontrol system for remote monitoring and automatic event triggering.

Pussinen (Honeywell): Remote operations in manufacturing facilities are extremely pragmatic, concerning

Sikov/Stock.Adobe.com

automation practices. A good reference document for the life-sciences industry, authored by the International Society of Pharmaceutical Engineers (ISPE), *The Good Automated Manufacturing Practice (GAMP) Guide for Validation of Automated Systems in Pharmaceutical Manufacture* (1), describes a set of principles and procedures that help ensure that pharmaceutical products have the required quality.

The use of remote operations in life sciences settings will likely evolve rather quickly as the industry seeks to ensure it is able to deliver life-saving therapies to patients around the world, without interruption, in case of natural disasters, inclement weather, pandemics, and/or in other situations where colleagues are not able to be physically present at a manufacturing facility.

Kane (Rockwell): Good automation practices are not exclusive to remote operations, or vice versa. Instead, good automation practices are centered around building quality controls into the manufacturing process. Remote capabilities can serve to enhance this quality. Remote technologies that enable safer, more frequent, and more timely monitoring and intervention into process disturbances are of great value to ensuring product quality. The technologies that allow both interactive, remote, and full remote are well evolved and suited for a wide variety of remote-use cases. We believe that, as the technology continues to evolve and augmented visualization technologies become more robust, there will be an increase in their uses throughout a wide cross section of facilities.

BioPharm: Are remote operations in manufacturing facilities safeguarded?

Pussinen (Honeywell): Yes, absolutely. Customers around the world are also looking for Honeywell to provide remote support in light of restrictions on personnel traveling and entering a site. Honeywell has deployed multiple remote service options to continue providing expert support via remote connectivity to our equipment. These remote options also use the power of Honeywell Forge offerings to proactively detect issues and bring them to the attention of both the customer and a Honeywell expert.

With these three methods, we are able to keep customers safe while also maintaining business continuity. The industries we serve include:

- Life sciences
- Pulp and paper/flat sheet manufacturing
- Gas terminals
- Oil and gas.

In fact, we have employed these unique technologies within some of our own plants.

Kane (Rockwell): It depends on how the systems are designed, but, yes, modern systems employing current best practices in security, engineering, and safety controls are very safe and secure. There are several perspectives that should be considered when safeguarding remote operations. The most basic safeguard is that connections are secure and not open to attacks or hacks. Following good network design and remote access principles help ensure this safety of communication. Instrument Society of America/International Electrotechnical Commission 62443 also has recommendations for good and secure network design. One should also consider the nature of the remote operations and their impact on local personnel, for example, signaling that indicates equipment is under remote operation.

We recommend something similar to a process hazard and safety review be conducted on any remote operations before implementation to ensure safe operation. For example, some equipment may require a line of sight to visualize that equipment during start up and be able to immediately respond if there is a problem. In order to follow the same intention with a remote-start operation, additional equipment might need to be installed to provide the visual checks in the absence of local operators.

Snodgrass (Hamilton): For those interacting with Hamilton's workstations on-site, we offer safety features that prevent accidental access to a remotely operated system. Error handling is also a critical safeguard to enable frequent unattended use while preventing major disruptions in the form of unplanned downtime. Hamilton's systems can automatically recover from many error scenarios and send remote notifications to authorized personnel. For serious issues, some on-site intervention may still be necessary.

EQUIPMENT AND INSTRUMENTS

BioPharm: What equipment/instruments does your organization rely on when operating remotely?

Snodgrass (Hamilton): Hamilton Robotics has a number of automated liquid handling workstations that can be used in a remote operation, integrated with other devices into larger work cells, and linked to other operations via a laboratory information system. With different platform sizes, configurations and capabilities, it's easy for biopharma manufacturers to fine-tune a solution specific to their needs. Hamilton Storage offers automated storage systems along with automated cappers and decappers that can further enhance automated workflows in remote operations. In a remote environment, Hamilton's sensors monitor in real-time whereas many others only provide a before and after snapshot of the activity. By monitoring even tiny fluctuations in real time, users know exactly what's happening in the process, and make rapid determinations about next steps instead of having to fail an entire batch, which as you can image, is a huge risk for a multi-week process.

Pussinen (Honeywell): Honeywell provides solutions for our customers to support remote operations—cybersecurity is often a critical element, and a variety of solutions and services are available. One such offering, Managed Industrial Cybersecurity Services with security analytics, device management, and regionalized support services helps customers modernize industrial

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

control system (ICS) capabilities while minimizing operational issues caused by cybersecurity incidents.

Additionally available are real-time process supervisory control and data acquisition (SCADA) solutions delivered as a secure and scalable service. A member of Honeywell's suite of cloud-enabled solutions for operations technology and information technology (OT/IT), SCADA provides cybersecure access and collaboration anywhere, while ensuring regulatory compliance.

Kane (Rockwell): Rockwell Automation is an automation system provider, and in a sense our whole business is about providing remote control to process, whether it be sending a signal to a valve to open across the plant, or around the world. Our business has always been about removing the operator from having to be physically present at the valve to open it. We are using some of the newer and more exciting technologies available for remote operation. Those involve using highly flexible screen sharing capabilities with Thin Manager, a FaceTime-like conferencing with augmented reality and 3D persistent whiteboarding (Chalk). And for sharing work methods and training remotely, we have some unique augmented reality/virtual reality knowledge management systems (Knowledge Capture).

RECOMMENDED USE AND IMPLEMENTATION

BioPharm: Do you recommend use of remote operations in biopharma manufacturing?

Pussinen (Honeywell): Yes, taking advantage of remote operations is a great way in which to facilitate business continuity when it is not possible to have colleagues physically present at a manufacturing facility, and to allow visibility into one's operations at a contract manufacturing organization. Given the current day challenges posed with COVID-19, companies increasingly have fewer individuals working at an operating process location, in adherence to 'stay at home orders.' In some cases, quarantines of key individuals might impact the availability of skilled resources at the site. In response, Honeywell has issued new software that enables process operations to be monitored or even executed from remote locations outside of the plant facility—in other facilities or from home—depending on specific needs, this might present a very valuable option.

Kane (Rockwell): Remote operations are applicable for use in almost any manufacturing environment. I can't think of one where it would not be applicable. Provided good engineering and safety practices are followed, there is no reason why remote operations could not be employed. In fact, I can think of a few reasons why remote operations would be preferred: first, a reduction of personnel in an area reduces the sterile load on an area. Second, remote operations support centralized management and resource sharing. And lastly, remote operations allow more timely responses to disturbances.

BioPharm: How do you suggest other organizations implement remote operations into their biopharma manufacturing facilities (e.g., as applies to workflow, day-to-day operations, cleaning/sterilization, etc)?

Snodgrass (Hamilton): This goes along with the best practices, and we already touched on a few of these points. I can't stress enough just how important planning is, including trying to envision every possible case scenario. For example, it's not feasible for a user to program a liquid handling workstation in a vacuum, so to speak, and expect that others can begin to use it remotely. Next, make sure to implement error handling protocols and detailed personnel trainings as they are both critical to successful remote operations. Clearly define the workflow and support it through standard operating procedures (SOPs). Obviously, don't skimp on documentation. By their nature, biopharma companies already have a wealth of documentation to support their audit trails. Scheduling is important; dedicate time for preventative maintenance and for cleaning and reloading the system. Key steps like these can ensure smooth operations in the long run. Consider ways to further minimize risks, like redundant equipment or devices and spare parts, or training in-house service personnel.

Kane (Rockwell): Process hazard analysis, process workflow analysis, and engineering studies. By thinking about how to identify candidate operations for remote control, such as reviewing operations for opportunities to remove local actions and engineering new processes with remote operations. This is a very broad concept to consider, but focus should be on the question, 'What problem am I trying to solve with remote operations? Is it simply remote operations, or is it inspection or troubleshooting? And will an operator be local or not?' These are general questions to ask when implementing remote operations into facilities.

REFERENCE

1. K.Vaishnavi, *Good Automated Manufacturing Practice (GAMP)*, 225 (2012). ◆

More on automation

For more on automation, read the following articles on *BioPharmInternational.com*:

- **Automating Bioprocesses**
  www.biopharminternational.com/automating-bioprocesses
- **Automation in the Lab: What to Consider**
  www.biopharminternational.com/automation-lab-what-consider-0

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Biomanufacturing: Demand for Continuous Bioprocessing Increasing

BioPharm INTERNATIONAL

But are innovations sufficient to increase adoption? CMOs are demanding better continuous bioprocessing options.

FOR PERSONAL NON-COMMERCIAL USE

ERIC S. LANGER

In nearly all other manufacturing technologies, cost considerations dictate that continuous production will be the rule. But in bioprocessing, the normal evolution from batch to continuous operations has not moved as quickly as many had expected.

Continuous processing upstream has been around for decades as perfusion (e.g., fiber-based perfusion bioreactors for fused-cell hybridoma culture in the 1980s). But that's essentially the only continuous-adapted upstream unit process, with such things as culture media and additives preparation still done in batch processing. In some respects, perfusion has overall been a commercial failure. Sales of the leading alternating tangential flow (ATF) perfusion systems from leading suppliers, after more than 15 years, are under $20 million. And continuous processing downstream is still largely lacking and, where implemented, involves just a few of the many unit processes involved in downstream processing. Multi-column, countercurrent, and other variations of continuous chromatography units are just starting to enter the market. The classic and still predominant approach to bioprocessing, both upstream and downstream, remains batch processing, with manufacturing batch fluids essentially moving incrementally en-masse from one process step and set of equipment to the next.

Downstream processing continues to create bottlenecks in production, and improvements in batch processing are not really emerging. Therefore, the industry continues to seek solutions from innovators for better continuous processes that offer further process intensification and lower costs. In fact, 70.6% of bioprocessing professionals are either testing continuous bioprocessing downstream technologies or considering them. This is up from 68% based on data from our 2016 Annual Report (1).

According to BioPlan's *17th Annual Report and Survey on Biopharmaceutical Manufacturing Capacity and Production* (2), there has been a slow increase in assessment of the various continuous bioprocessing options over the past five years, and the

ERIC S. LANGER is president and managing partner at BioPlan Associates, Inc., a biotechnology and life sciences marketing research and publishing firm established in Rockville, MD in 1989; elanger@bioplanassociates.com, +1 301.921.5979.

valdis torms/Stock.Adobe.com

Figure 1: Facilities evaluating continuous bioprocessing (downstream) technologies in the next 12 months (2016–2020).

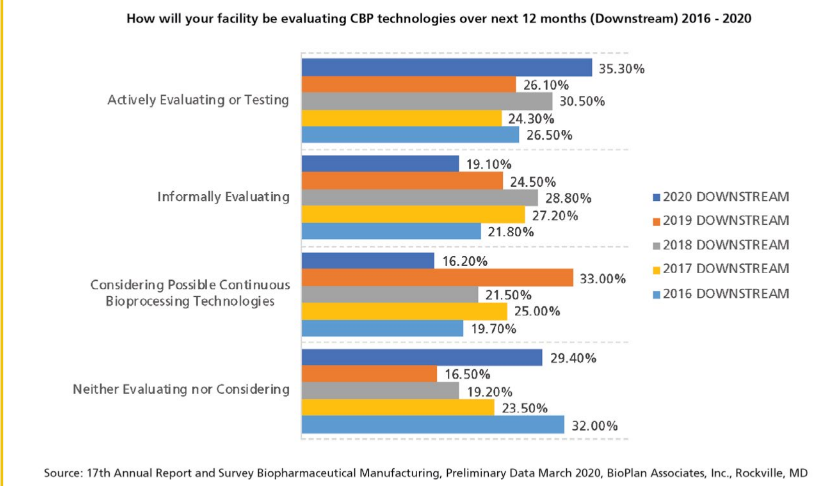
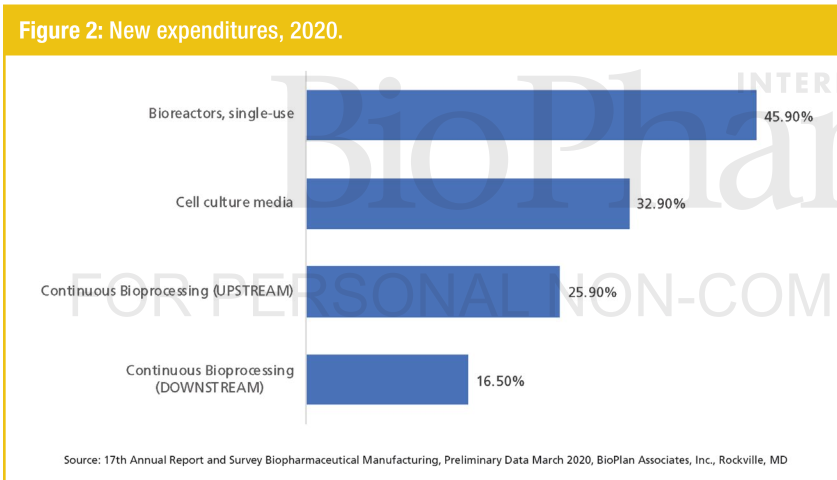


Figure 2: New expenditures, 2020.



BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

data support ongoing interest in the coming year (see **Figure 1**). Approximately 55% of facilities surveyed are actively or informally evaluating continuous processing technologies in the coming year.

Although there are a number of technologies providing process intensification and continuous purification steps, it appears that more robust continuous chromatography technologies, such as simulated moving bed (SMB) and periodic countercurrent chromatography, are generally not yet ready yet for commercial-scale adoption (other than adoptions performed using single-use upstream equipment generally limited to 2000-L scale).

OUTSOURCING AND CONTINUOUS BIOPROCESSING

Contract manufacturing organizations (CMOs) are often on the leading edge of new technology adoption. For continuous bioprocessing and process intensification, BioPlan's Annual Report shows that significantly more CMOs will be testing these technologies over the next 12 months (53% of CMOs will be evaluating downstream options, vs 38% of biomanufacturing facilities). On the upstream side, again it is the CMO outsourcing organizations that are seeking better products and more improvements. More CMOs than biomanufacturers (40% vs 28%) are indicating they want vendors to focus greater efforts on developing continuous upstream technologies (1).

BUDGETS FOR ADOPTION OF CONTINUOUS BIOPROCESSING

BioPlan's annual report for 2020 also evaluated adoption of bioprocessing technologies based on new technology purchases. When evaluating new expenditures, industry decision-makers were asked about new technologies they were budgeting for. Of the nearly 20 technologies identified, the top technologies this year included single-use bioreactors (noted by 45.9% of respondents), followed by cell culture media including optimization, and then continuous bioprocessing (upstream), and continuous bioprocessing (downstream), according to preliminary data.

BioPlan data in general indicate that the direction of the industry is more toward single-use novel devices, those that allow rapid transitioning from project to project, and options for continuous bioprocessing. Some of these technologies also support the increasing demand for biologics that may be called for in smaller quantities.

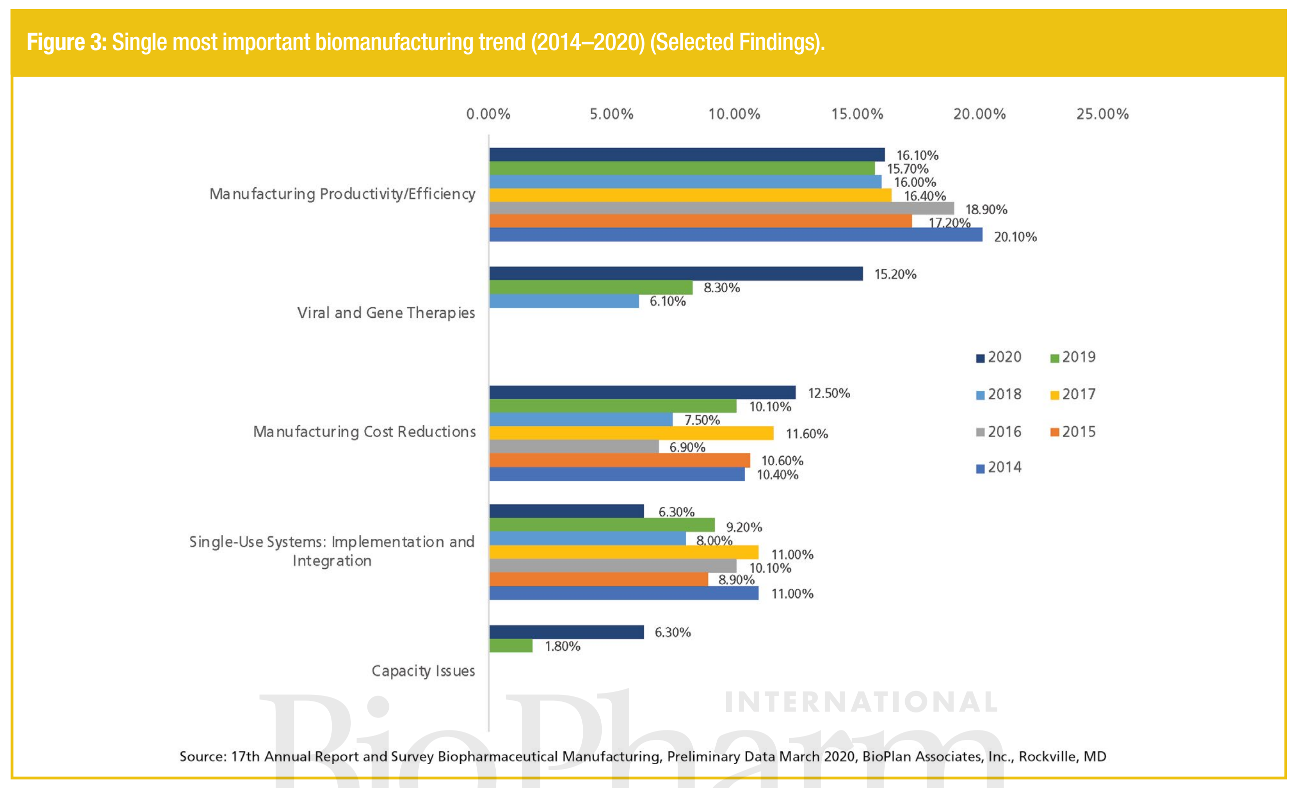
Figure 2 shows the economic commitment decision-makers are focusing on continuous bioprocessing, as evidenced by companies' top three new expenditures including both upstream and downstream continuous bioprocessing equipment, which was noted by a robust 25.9% and 16.5% response from decision-makers.

TRENDS MAKING CONTINUOUS BIOPROCESSING ATTRACTIVE

Several technological advances and related trends are making continuous bioprocessing attractive. Some established bioprocessing facilities are being retrofitted and upgraded for more continuous operations.

ALL FIGURES COURTESY OF THE AUTHOR.

Figure 3: Single most important biomanufacturing trend (2014–2020) (Selected Findings).



FOR PERSONAL NON-COMMERCIAL USE

There are many benefits to operating bioprocesses continuously rather than in batch mode, with many of these similar and complementing those of single-use and modular systems:

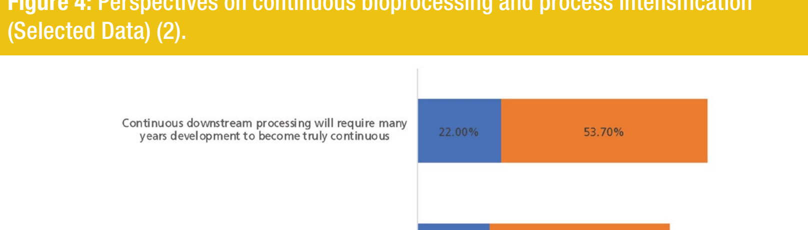
- Reduced costs: Operating continuously allows use of significantly smaller-scale equipment, with a smaller volume bioreactor.
- Increased productivity: Because much of the bioprocessing equipment is operated continuously, there is little need for large transfer/storage vessels and no halts between processes. Bioprocessing thus tends to move much more smoothly.
- Improved quality: Biological molecules are expressed continuously, and compared to batch culture, continuous culture tends to be more controllable, less intense and stressful, including less shear and media nutrient levels kept constant.
- Increased flexibility: Continuous manufacture enables more adaptability and efficient facility utilization, similar to the advantages of single-use devices. Bioprocessing also becomes much more portable, and facilities more cloneable.

Many upcoming continuous bioprocessing technologies are very novel. For example, a single 50-L bioreactor is expected to be able to manufacture the same quantity of product, often at better quality, comparable to a 5000-L bioreactor over the same time period. Case studies and other reports of such performance will further promote rapid adoption. There will be increasingly rapid adoption of single-use systems for new commercial manufacturing over the next five years; and continuous bioprocessing, particularly upstream processing, is expected to follow a similar trajectory. Use of continuous bioprocessing is likely to further increase with the arrival of more hybrid systems that use bolt-on-type technology, which retrofit components unit operations for existing systems. Other conventional downstream continuous adaptable technologies, such as centrifugation, will also see increasing adoption in coming years. Potentially revolutionary capillary fiber perfusion bioreactors and other new technologies, including those for downstream processing, will be likely coming online and be more widely adopted for commercial manufacture over the next 10 years.

CONTINUOUS PROCESSING TRENDS IN BIOPROCESSING

When respondents were asked about their 'single most' important biomanufacturing trend, or operational area on which the industry must focus its efforts, upstream and downstream continuous bioprocessing declined dramatically over the past six years, from 9.1% to 1.25% for upstream, and 10% to 4.7% for downstream continuous bioprocessing (**Figure 3**).

Figure 4: Perspectives on continuous bioprocessing and process intensification (Selected Data) (2).



While this might imply that interest in continuous bioprocessing is waning, combined with the increased expenditures in the area, it suggests that continuous bioprocessing is becoming a more mainstream bioprocessing area, and therefore, less trend-relevant, thus, the lower trend 'score.'

IMPLEMENTATION OF CONTINUOUS BIOPROCESSING

Although this is beginning to change, implementation of continuous bioprocessing is and has been slow (see **Figure 4**). At best, a few unit process/steps both up- and/or downstream have been implemented as continuous by a minority of facilities. Some commercial biopharmaceutical products that essentially require perfusion's generally milder/less intense processing conditions, including Factor VIII (the largest recombinant molecule biopharmaceutical) and coagulation factors, have been manufactured for decades using perfusion (other products use continuous centrifugation).

BioPlan studies have shown approximately 5% of bioreactors that are over desktop-size use perfusion, mostly for feeder, not production, bioreactors. There is more adoption of perfusion for early stage vs. large/commercial-scale manufacturing. BioPlan studies have shown that few processes are scaled-up, particularly for commercial good manufacturing practice (GMP) manufacture, using perfusion in continuous upstream bioprocessing CP USP. Perfusion adds considerable mechanical complexity and regulatory uncertainties (i.e., it is avoided for GMP manufacturing, expert staff are needed, etc.), as well has having limited equipment options and universal industry inertia restraining adoption.

Large-scale continuous downstream processing, particularly chromatography operations, remain rare. Even where continuous downstream processing has been implemented, it involves at best only one or few out of the usual multiple chromatography and other downstream processing unit processes/steps having been implement as continuous.

Survey data suggests that bioprocessing professionals may believe continuous processing is more ready for broad adoption for more unit processes than it currently is. Notably, continuous processing equipment manufacturers and users report that many of the problems long associated with perfusion and continuous bioprocessing have been resolved in recent years through the application of innovative technologies, including new developments in single-use equipment.

On the other hand, perfusion processing is now significantly less complex, less prone to contamination, and more readily scalable than previously. Negative assessments from within the industry of continuous perfusion fed-batch processing overall may reflect a lack of direct exposure or experience with continuous technology.

In BioPlan's annual report, for example, key areas where most respondents reported they perceive perfusion as presenting more concerns (vs. fed-batch) included:

- Process operational complexity (perfusion noted by 72% as more operationally complex vs. batch)
- Contamination risks
- Upstream development and characterization time
- Process development control challenges
- Process development general challenges
- Validation challenges
- Need for greater process control
- Cell line stability problems
- Ability to scale-up process.

Interestingly, while approximately 76% believe downstream continuous bioprocessing will be a long time in coming, 66% believes that perfusion systems will be adopted by most bioprocessing facilities. This shows the expectation that continuous bioprocessing is here for the long haul, but widespread adoption may not be in the near future.

REFERENCES

1. BioPlan Associates, *16th Annual Report and Survey on Biopharmaceutical Manufacturing Capacity and Production* (Rockville, MD, April 2019).
2. BioPlan Associates, *17th Annual Report and Survey on Biopharmaceutical Manufacturing Capacity and Production, Preliminary Data* (Rockville, MD, March 2020). ◆

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Implementing Attribute Monitoring Analyses for Biopharmaceutical Development and QC

ON-DEMAND WEBCAST: Aired Wednesday, April 29, 2020

Register for this free webcast at: http://www.chromatographyonline.com/lcgc_p/monitoring_analyses

All attendees will receive a free executive summary of the webcast!

Event Overview

There is an ongoing progression to extend high-performance LC-MS technologies beyond traditional characterization roles into product attribute monitoring assays deployable across development, manufacturing and quality control organizations. This webcast will cover the use of high-performance LC-MS for biopharmaceutical analysis, including transitioning product characterization knowledge into monitoring assays for product and process related quality attributes of biopharmaceuticals.

A case study presented from Merck Serono will:

- Detail their assessment of analytical technologies and workflows for multiple attribute monitoring (MAM) assay development
- Assess the benefits of these new assay formats
- Reveal plans for deploying these assays within development, manufacturing, and QC functions within their organization

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

Key Learning Objectives

During this webcast, you will learn about the following:

- Realize the capabilities of high-performance LC-MS, for biopharmaceutical analysis
- Recognize that LC-MS technologies have become more readily deployable and can now be operated by non-MS experts
- Learn how attribute-based analysis can supplement product knowledge gained through traditional assays, and potentially streamline testing for process monitoring and product

Who Should Attend

- Academics, R&D, Lab Directors
- Biopharmaceutical organizations, CXO, and research institutes focusing on large molecule drug development and manufacture
- Analytical scientists supporting biologics development, manufacturing and QC
- Lab managers, Directors, VPs in charge of characterization and monitoring strategies
- Biopharmaceutical regulatory authorities

Presenters

Scott J. Berger, Ph.D.
Senior Manager,
Biopharmaceutical Markets
Waters Corporation

Angelo Palmese, Ph.D.
Head of Characterization and
Innovative Analytics Unit
Merck Serono -
Guidonia Site, Italy

Moderator

Rita Peters
Editorial Director
BioPharm International

For questions or concerns, email KMoore@mjhlifesciences.com.

Sponsored by

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Presented by

BioPharm INTERNATIONAL

Pre-Packed Columns

Tosoh Bioscience introduced the SkillPak 1 mL and 5 mL pre-packed columns for fast method development and resin screening of monoclonal antibodies, antibody constructs, oligonucleotides, proteins, and viruses.

The columns come pre-packed with Toyopearl, TSKgel, or Ca^{++}Pure-HA process chromatography media and are ready for use immediately. Additionally, the high performance, scalable columns are available with short delivery times in affinity, anion and cation exchange, hydrophobic interaction, and size exclusion chromatographic modes. Peak performance is guaranteed, according to the company, and the columns can be operated directly with low- or medium-pressure liquid chromatography systems.

Tosoh Bioscience

www.tosohbioscience.com

Supercritical Fluid Chromatography System

The Nexera UC Prep, a preparative supercritical fluid chromatography (SFC) system from Shimadzu, works to provide maximum use of lab resources through flexible system configurations in a compact design that requires low installation space.

The system can be configured based on user specifications, which include chiral or achiral purifications, single injections, stacked injections, and fraction collections from microliters to liters. Additionally, the system comes equipped with LotusStream gas-liquid separator technology for high recovery and low carryover, and Prep Solution software for streamlining operations.

The SFC solution also reduces the need for solvents, shortens purification run time and dry down time, reduces the total size of the recovery system, and allows for easy rinsing.

Shimadzu

www.shimadzu.eu

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE

ReNu Single-Use Technology Cartridge Assemblies

ReNu SU (single-use) Technology cartridge assemblies from Watson-Marlow Fluid Technology Group are designed for the development and production of personalized medicines. Available with CPC AseptiQuik, Pall Kleenpak Presto, or GE Readymate aseptic connectors, the devices can be integrated directly into a customized fluid path, allowing for fast bioprocess integration while eliminating alignment errors.

The cartridge assemblies are equipped to work with the company's quantum peristaltic pump, which allows for a change in pump technology by providing higher accuracy with flow linearity without back pressure. Additionally, the cartridge assemblies offer lot traceability on every component, ISO Class 7 cleanroom manufactured and packaged assemblies, and product sterility of 10-6 SAL per ANSI/AAMI/ISO 11137 guidelines.

Watson-Marlow Fluid Technology Group

www.wmftg.com

Mass Flow Controllers

The SLA5800 Series Biotech and the SLAMf Series Biotech are two new mass flow controller (MFC) models from Brooks Instrument designed specifically for improved gas flow control in biotechnology applications. The new models allow biotech equipment manufacturers to reduce the number of MFCs they need for their equipment, which allows them to simplify their designs along with reducing system costs, purchasing complexity, and spare parts inventory requirements.

The MFCs have an enhanced control valve with a leak rate of 0.005 sccm for flow rates between 5 sccm and 150 slpm, and a rate of 15.6 sccm for higher flow rates. The low leak rate potentially eliminates the need for a separate shut-off valve in the gas delivery system, reducing component purchasing costs and saving engineering, testing, and validation time. The MFCs accommodate a wider range of gas flows and control up to four different gas flows including air, carbon dioxide, nitrogen, and oxygen.

Brooks Instrument

www.brooksinstrument.com

Ask the Expert — *Contin. from page 58*

a course with an approved training company. If however, you want to get a basic understanding of the freeze-drying process, you will easily find free tutorials online. Should you need hands-on experience, then training courses offered by universities or industry associations with in-house training centers will be the right choice.

SWITCHING CAREERS

What if you have been in the industry for a while, but would like to change positions and/or area of expertise? Very often this is less of a question of opportunity, but more of a question of an individual's preferences. There are equally excellent subject matter experts who never strayed from their vocation (say regulatory affairs, quality control, or manufacturing) and who are perfectly happy in their jobs, and there are those who worked in different departments to become more universal experts. Pharmaceutical companies probably look more for experts in a particular subject, whereas service providers, such as consultancies or contract research organizations, may have a need for experts with more varied backgrounds.

We may not always find the job we want, but we can always learn from what we do, and it will always be a beneficial personal and job experience. ◆

Soon to be unveiled....
a new control system designed
for upstream processing...

PendoTECH® Perfusion Controller
convert any bioreactor to a
perfusion bioreactor

Features Include:
• Vessel level control
• Perfusion pump control
• Filter feed pump control
• Pressure monitoring of external perfusion device, such as a hollow fiber filter (including filtrate pressure monitoring to indicate filter clogging) with PendoTECH Single Use Pressure Sensors™
• Optional monitoring of temperature in perfusion loop with PendoTECH Single Use Temperature Sensor
• Graphical User Interface with trending of process data and logging of data to a file
• Built-in data server to exchange data with OPC client software such as PI from OSIsoft®
• Filtrate scale to measure perfusion volume per day
• Additional inputs to log data from optional sensors

PendoTECH Single Use Sensors

Pressure

Temperature

PENDOTECH
Adding Value To Your Process
ISO 9001:2015 Certified • www.pendotech.com • Princeton, NJ USA

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Ad Index

Company	Page
ADVANTAPURE	31
AGILENT TECHNOLOGIES	41
CATALENT	60
CYGNUS TECHNOLOGIES LLC	11
EAGLE STAINLESS	19
MILLIPORE SIGMA	15
NYSE GROUP INC	2
PDA EUROPE	23
PENDOTECH	57
PHARMAFLUIDICS	25
ROCHE CUSTOM BIOTECH	13
SARTORIUS STEDIM BIOTECH	59
SINGLE USE SUPPORT GMBH	20–21
TOSOH BIOSCIENCE	33
USP	7
WATERS	55
WUXI BIOLOGICS	5
WYATT TECHNOLOGY	29

Ask the Expert

Starting a Career in the Bio/Pharmaceutical Industry

Having a better understanding about compliance will be of benefit when looking for a job or for furthering one's career.

Siegfried Schmitt, PhD, is vice-president, technical, Parexel Consulting.

Q: While working on a variety of projects in three different continents, I had the opportunity to meet and work with young, enthusiastic newcomers to the industry. They were from a variety of different professional backgrounds, including pharmacists, engineers, and chemists. During our conversations, most of them asked the following types of questions:

- The college or university I graduated from did not provide courses on compliance or industrial operations—how can I fill this gap?
- Though I applied for many positions, I have been unable to find a permanent position yet—how can I improve my chances?
- On the Internet, I found several courses on good manufacturing practices (GMP), for compliance experts, or similar. These are relatively expensive. Are they worth investing for someone like me (a beginner)?
- Should I work my way up within a particular department or would it be better if I gain experience in different departments?

A: The following are not exhaustive or the only answers to these questions, but they will give some insight.

It is true that few graduates have seen industrial operations by the time they graduate, but that doesn't mean that they don't come equipped with many of the basic skills needed in the industry, such as team working, presentation skills, analytical thinking, and the ability to self-study. Companies will provide training, as a minimum on the applicable and relevant internal processes and procedures, which will cover both the operations and the compliance side of the business. A lot will be learning on the job, from peers and often also from mentors.

Finding your first permanent job can be a frustrating experience, but persistence usually pays off. Gaining experience through temporary voluntary engagements, placements, or positions is what helps improve the chances for long-term or permanent employment. And don't forget to network

We may not always find the job we want, but we can always learn from what we do, and it will always be beneficial.

through portals such as LinkedIn, Bing, or similar sites that have a good reputation with industry and job agencies. Also, it's important to write a succinct and well-thought out *curriculum vitae*, and there is a lot of great advice available for free online on how to do this. The Internet is the place to research jobs, but often also for potential employers to find the right candidate.

Having a better understanding about compliance will surely be of benefit, whether looking for a job or for furthering one's career prospects. Whether you are lucky enough to have your employer pay for external courses, be subsidized (e.g., by a state job center), or have to pay yourself, in all cases you should scrutinize the courses offered:

- How relevant are they to your current or prospective work?
- Do they provide references? Is there feedback available?
- Are the certificates merely proof of attendance or are they widely recognized by the industry?
- Is it just classroom learning or is there also a practical element?
- Do they provide comprehensive documentation?

The answers to these questions will help you determine if the course is right for you. For example, if you want to become a certified auditor (be it for GMP or ISO 9001), you will have to pay for

Contin. on page 57

freshidea - Stock.adobe.com

BioPharm INTERNATIONAL FOR PERSONAL NON-COMMERCIAL USE

Biostat STR® Generation 3 and Biobrain

Simplifying Progress

Engineered for Ultimate Upstream Performance

The Biostat STR® single-use bioreactor system is designed for rapid process development and seamless scaling to commercial manufacturing. It is powered by the new Biobrain automation platform and BioPAT® analytical tools to deliver outstanding speed, quality, and productivity.

For more information, please visit www.sartorius.com/biostat-str

FOR PERSONAL NON-COMMERCIAL USE

Your one integrated biologics partner.

For Biologics and Gene Therapy, we have the passion to help you accelerate, simplify and de-risk from development and biomanufacturing, to fill/finish, analytical, clinical supply and commercial launch

25+ COMMERCIALLY APPROVED PRODUCTS THROUGH FILL/FINISH

600+ ANTIBODIES & 80+ RECOMBINANT PROTEINS DEVELOPED

5000+ CLINICAL TRIALS SUPPLIED

13 MARKETED PRODUCTS USING GPEx® TECHNOLOGY

60+ GENE THERAPY PROGRAMS

120+ CLINICAL TRIALS USING GPEx® CELL LINES

Biologics | Biosimilars | Gene Therapy | Oncolytic Virus | Next Gen Vaccines

Comprehensive solutions. Advanced technologies. Better biologics.™
US + 1 888 SOLUTION (765-8846) **EUR** 00800 8855 6178 biologics.catalent.com

© 2020 Catalent, Inc. All rights reserved.

BioPharm INTERNATIONAL
FOR PERSONAL NON-COMMERCIAL USE