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CONFERENCE COVERAGE
Special Report on the
CMC Strategy Forum
How industry and regulators
view quality risk management  

FILTRATION
Essential Steps to
Ensure Accurate
Filter Scale-up
Why staining is crucial
in flow decay studies  

DOWNSTREAM PROCESSING
MAb Purification
Using Camelid
Antibody Ligands
A stable alternative to
Protein A chromatography  

MANUFACTURING ECONOMICS
RMMs: How Much
Do They Cost?
A case study in implementing
rapid microbiological methods  

REGULATORY BEAT
FDA voucher program supports
treatments for neglected diseases  

PERSPECTIVES ON OUTSOURCING
How to select the right partner
for media manufacturing  

FINAL WORD
New challenges in patenting
biotech inventions  

GLOBAL NEWS: FDA’s Plan to Step Up Enforcement  

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Contents

Volume 22 Number 9 September 2009

FEATURES

CONFERENCE COVERAGE
Quality Risk Management Demystified at CMC Strategy Forum
Laura Bush
A hands-on workshop tackled the questions and challenges involved in applying the quality risk management principles promoted in the ICH Q9 guideline.

FILTRATION
The Proper Use of 47-mm Flat Disc Filters in Filter Sizing Studies
Maik W. Jornitz, Theodore H. Meltzer, Wayne Garafola
The effective filter area of a 47-mm pilot filter is not always what it seems. Proper EFA calculations will prevent scale-up errors.

DOWNSTREAM PROCESSING
Comparison of Camelid Antibody Ligand to Protein A for Monoclonal Antibody Purification
Jia Liu, Aaron Cheung, John L. Hickey, Sanchayita Ghose
Camelid antibody ligands can provide binding affinity and dynamic binding capacity similar to those of Protein A resins.

MANUFACTURING ECONOMICS
Breaking the Rapid Microbiological Method Financial Barrier
Michael J. Miller
A case study in return on investment and economic justification.

COLUMNS AND DEPARTMENTS

10 From the Editor
A True Assessment of Risk
Laura Bush

12 Global News

14 Regulatory Beat
Biopharmaceutical Manufacturers Seize Opportunities in the Global Health Arena
Jill Wechsler

18 Perspectives on Outsourcing
Strategic Outsourcing of Media Design and Cell Culture Media Manufacturing
K. Fritchman, J. Kuchibhatla, Yann Pouliquen, J. Brooks, S. Holdread, A. Imam, C. Velnoskey, V. Weinknecht

20 FindPharma
A new vertical search tool designed just for pharma professionals. An easier way to find industry-relevant information, industry suppliers, and career assistance.

28 Filtration
The Proper Use of 47-mm Flat Disc Filters in Filter Sizing Studies
Maik W. Jornitz, Theodore H. Meltzer, Wayne Garafola

35 Manufacturing Economics
Breaking the Rapid Microbiological Method Financial Barrier
Michael J. Miller

54 New Technology Showcase

54 Spotlight on: Disposables

56 Classifieds

57 Advertisers in this Issue

57 Calendar

58 Final Word
Patenting Biotechnology Inventions Will Become More Challenging
Astrid R. Spain

Cover: BIOSTAT CultiBag, Sartorius Stedim Biotech’s single-use bioreactor.

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From the Editor

A True Assessment of Risk

Risk assessment exercises seem pretty simple. Make a list of everything that could go wrong. Quantify those risks, by assigning scores. Then decide if you need to do more to prevent the high-risk events from happening. Tedious, but not complicated.

The recent CMC Strategy Forum on quality risk management, however, revealed the challenges involved (see p. 20). For example, how do you deal with the subjectivity of scoring? And how do you score a potential harm based on risk to patients, especially when you’re analyzing upstream steps, where almost any risk would be mitigated before the product reached the patient? Group dynamics also can present challenges. You have to ensure that all voices are heard, and that the group really prioritizes risks, rather than just ranking everything high. That means finding a skilled facilitator, or training one.

None of the challenges is insurmountable, of course, and the discussion at the Forum revealed many ways to handle them. But then one must consider the substantial resources involved. The process requires many hours of meeting time from expert staff, and someone has to manage it all. Many large companies are creating new risk management positions and even departments.

Given the size of the task, I start to wonder whether it is truly necessary or worthwhile. Clearly, some sort of risk management is essential to ensure product safety and efficacy. But many would say that the industry has always done quality risk management, without calling it that. It was how one justified specifications. So if quality risk management is not really new, the question becomes, Is it necessary, or beneficial, to formalize the process?

That’s hard to say. Proponents cite various benefits. For example, the process documents institutional knowledge, as well as upper management’s awareness of risks. It gets staff from different departments to talk to each other. You also may be able to use the data in regulatory filings for site and process changes.

All that is nice, but the real value of formalizing risk management will be if it improves product quality. Yet most companies, I think, would assert that their drug product quality is already excellent; manufacturing processes are robust, and quality control measures ensure that work-in-process or final product that doesn’t meet specifications is rejected.

Assuming that’s true, “improving quality” really translates to two things: a) minimizing waste by reducing deviations, lot rejections, and investigations, and b) averting a major—but so far unforeseen—quality disaster that could harm patients. The latter, I have to assume, is the core goal of risk management.

To truly achieve the goal of preventing disasters, risk assessment exercises will have to identify risks that previously had not been considered or taken seriously. Because as several people pointed out during the Forum, if these discussions only identify “the usual suspects,” the process will not add much value.

If companies are going to take the time and effort to implement a new risk management program, they should focus on the dialogue—that conversation among experts and across departments that so many say doesn’t otherwise happen, or happen often enough. And they should make sure that in those discussions, people really question their assumptions, and don’t take a “been there, done that” attitude.

Otherwise, risk assessments will devolve into an exercise to please regulators, or just a paperwork drill, as many people say sometimes happened with process validation. In that case, we might as well continue doing things the old way.

Laura Bush is the editor in chief of BioPharm International, lbush@advanstar.com.
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Six Steps to Improve FDA Enforcement

FDA Acting Commissioner Margaret Hamburg outlined six steps to improve FDA enforcement in a speech given on August 6, eight weeks into her tenure.

Hamburg said that the current pathways for enforcement can be too long and arduous when the public’s health at risk. To improve the pathway, Hamburg outlined six key steps. In one key new measure, the FDA will set post-inspection deadlines, giving firms a deadline of 15 working days to respond to inspection findings. “This will help FDA issue warning letters in a timely basis and facilitate prompt corrective action,” she said. The FDA will also prioritize enforcement follow-up, either through an inspection or an investigation, to assess whether the necessary changes have been made.

Also, the agency will no longer issue multiple warning letters before taking enforcement action. And in some cases, she said, the FDA may take immediate action to protect public health without the issuance of a formal warning letter.

One additional step involves the development of a formal warning letter closeout process. Once a firm has fully corrected the violation raised in a warning letter, the agency will issue a “close-out” letter. The agency will also indicate on the web site that a close out has been issued.

Hamburg said that an effective enforcement strategy ultimately creates public confidence in FDA oversight, which in turn keeps trust in the safety of FDA-regulated products from eroding. “Such confidence is critical in the long-term interest of both consumers and industry,” she said.

—Haydia Haniff

SAFC Develops First Chemically Defined Hydrolysate Replacement

SAFC Biosciences (St. Louis, MO) has developed a chemically defined cell culture supplement as a replacement for undefined plant hydrolysates. The product, Ex-Cell CD Hydrolysate Fusion, is designed for biopharmaceutical cell culture processes using Chinese hamster ovary, NS0, and Sp2/0 cell lines.

Adding hydrolysates to some cell-culture processes can greatly enhance cell growth and productivity. Today, most hydrolysates used in biopharmaceutical production are derived from plants, such as soybeans and yeast. The difficulty in using them, however, lies in their variability.

“Unfortunately, hydrolysates by their nature are of variable composition, depending on the multitude of factors that influence the composition of biological materials like soybeans, compounded by variability in the hydrolysis process,” said Mike Ultee, PhD, vice president of process sciences for Laureate Pharma in Princeton, NJ.

They say that the new supplement provides cell growth and protein production capabilities equivalent to traditional undefined hydrolysate raw materials, but with greater reproducibility and lower risk.

“Raw material understanding and characterization is a leading concern for biopharmaceutical manufacturers interested in mitigating risk,” said Bruce Lehr, marketing director for SAFC Biosciences, in a statement.

Ultee noted, however, that the responsiveness of different cell lines to hydrolysates or new chemically defined substitutes varies with one cell line to the next. “So the effect of addition of these materials to a culture needs to be determined for each cell line,” he said.

—Laura Bush

Vaccine Report

New Vaccine Produced in Tobacco Plants

A new vaccine for norovirus has been produced in tobacco plants, a scientist reported on August 18 at the national meeting of the American Chemical Society (ACS) in Washington DC.

The new vaccine was “manufactured” in a tobacco plant using plant viruses engineered to produce high levels of virus-like particles (VLPs) in tobacco plants over a one to two week time frame. Such plant biotechnology opens the door to more efficient, inexpensive ways to bring vaccines quickly to the public, especially critical in times when viruses mutate into unpredictable new strains, said Charles Arntzen, PhD, of Arizona State University, who reported on the topic at the ACS meeting.

“The recent outbreak of H1N1 influenza virus has once again reminded us of the ability of disease-causing agents to mutate into new and dangerous forms,” Arntzen points out. “For a case like the H1N1 influenza virus, you want to be able to move very rapidly and introduce a commercial vaccine in the shortest possible time. We think we have a major advantage in using engineered plant viruses to scale-up vaccine manufacture within weeks instead of months.”

Other companies have been exploring the use of both VLPs and production based in tobacco plants for swift scale up in vaccine production. Novavax (Rockville, MD), for example, has already produced initial batches of a VLP vaccine candidate for H1N1 influenza. Companies developing vaccines produced in tobacco plants include Medicago (Quebec City, Canada) and Bayer (Leverkusen, Germany). Medicago is developing a pandemic influenza vaccine and Bayer is seeking to produce a patient-specific antibody vaccine for non-Hodgkin’s lymphoma.

Sometimes called the “cruise ship virus,” norovirus causes diarrhea and vomiting and may be the second most common viral infection in the US after the flu.

—Haydia Haniff
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The need to strengthen public health at home and abroad is expanding the development of new medicines and vaccines to prevent and treat infectious diseases around the world. Donor nations and organizations have increased funding over the last decade to bolster healthcare systems in less developed countries and to improve treatments for malaria, tuberculosis, and many neglected tropical diseases. Biopharmaceutical companies have joined public-private partnerships (PPPs) developing these therapies, attracted by the desire to expand sales globally as well as opportunities to improve third-world health. Many PPPs are poised to harvest rewards from over a decade of research efforts, but require billions in additional resources to finance costly research and clinical trials.

INVIGORATING VACCINES
A main thrust of these initiatives is to spur research and development (R&D) on new vaccines to prevent infections that kill millions of people every year. The PATH Malaria Vaccine Initiative is launching Phase 3 trials for a promising malaria vaccine developed by GlaxoSmithKline (GSK), and the Aeras Global TB Vaccine Foundation has several candidates in early clinical trials. Rotavirus vaccines recently received a strong push from the World Health Organization’s decision to add this preventive to national immunization programs for infants and children.

Vaccine development is strong at home as well, as new preventives for human papillomavirus, rotavirus, and shingles have hit the market. The US Food and Drug Administration approved three vaccines last year, and several important applications are in the queue. Meanwhile, the agency has its hands full reviewing filings for the new pandemic H1N1 vaccine, now being tested and produced as fast as possible. The US expects to spend some $8 billion for nearly 200 million doses of the flu vaccine from GSK, Novartis, Sanofi Pasteur, Astra-Zeneca’s MedImmune, and Australia-based CSL Limited. European and other nations have placed comparable orders.

The FDA and the industry face several challenges in meeting the demand for H1N1 vaccines, as discussed at a July meeting of FDA’s Vaccines and Related Biological Products Advisory Committee. The agency is allowing licensed manufacturers to file manufacturing supplements for a strain change, similar to what companies do each year for new seasonal flu vaccines. Because the H1N1 vaccine is a slightly different single-strain vaccine, companies have to conduct clinical trials to ensure safety, determine the necessary dose strength for effectiveness, and decide whether one or two doses are needed. Another question is whether the swine flu vaccine should be administered separately or with a seasonal flu shot.

The National Institutes of Health is conducting additional clinical trials to test dosing options for various age groups and the need to mix the new vaccine with an adjuvant to achieve the desired immune response. If an adjuvant is needed, the FDA plans to use its emergency authority to expedite access to what would then be a new vaccine. The agency took this route earlier in the year to permit treatment of infants with antivirals not yet labeled for that age group, but has not used this approach for a new vaccine.

Even if all the testing comes out well, it’s not clear how quickly the industry will produce the millions of needed doses. Companies experienced difficulties cultivating antigen from the available H1N1 strains, but yields began to rise last month, generating hopes for initial supplies in September.
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MedImmune reported great success in producing bulk quantities of its FluMist vaccine, which is made from a live virus, but doesn’t have enough nasal spray devices to deliver the inhaled product.

**BROADER IMPACT**

MedImmune’s excess supply, though, may lead to overseas sales, as vaccine makers capitalize on the opportunity created by the flu pandemic to expand production and distribution. Sanofi gained FDA approval for its new Swiftwater, PA, vaccine manufacturing facility earlier this year, while foreign manufacturers, such as Sinovac Biotech in China, have geared up to conduct clinical trials and expand production of an influenza vaccine at home. Struggling Connecticut-based Protein Sciences Corporation has received federal funding to develop new flu vaccine development methods, leading to testing for its experimental PanBlok vaccine. Inovio Biomedical of San Diego has gained attention from positive animal studies on its DNA-based flu vaccine, although it will take several years to bring such a product to market.

The demand for a pandemic flu vaccine also has spurred development of novel antigens and testing standards. Earlier this year, FDA scientists unveiled a new antigenic fingerprinting approach for testing the potential protective activity of vaccines under development. Vaccine makers are moving forward with the development of a cell-based manufacturing technology for influenza vaccine, the long-desired approach for accelerating seasonal flu vaccine production.

**MORE TREATMENTS**

While vaccines to prevent disease are the holy grail for tackling global health problems, a number of initiatives support development of new treatments for tuberculosis (TB), malaria, and multiple neglected diseases that cripple third-world countries. Efforts to combat multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB strains is high on the global research agenda and has attracted considerable attention at the FDA. Efforts to streamline clinical research approaches for TB drugs could expand under Commissioner Margaret Hamburg, who launched a major TB control campaign when head of New York City’s public health department in the 1990s.

An FDA advisory committee meeting in June discussed early trial endpoints for drugs to treat MDR TB, such as reduced bacterial count in sputum culture, followed by confirmatory trials to document low relapse rates, to accelerate the R&D process. The FDA followed up with a July workshop that examined noninferiority study designs, combination therapy regimens, and missing data issues for research involving drug-susceptible TB. Sequella Chief Medical Officer Gary Horwirth urged consideration of Phase 0 and adaptive clinical trials and surrogate endpoints, and Gail Cassell of Eli Lilly advocated “boldness in clinical trial design.”

Because there will always be limits on funds available to support costly clinical trials, the neglected disease community is testing the stimulus power of market-based “pull” mechanisms, such as advance market commitments (AMCs) that guarantee prices for new vaccines and drugs with low profit potential in the US or Europe. This approach is being used to fight malaria by subsidizing the purchase of artemisinin-based combination therapies (ACTs) that are effective, but too costly for most third-world health programs. By expanding the market for these more effective therapies, the program aims to encourage the production of quality ACTs and drive older, ineffective products out of the market.

Another AMC project supports the development of new pneumococcal vaccines that can protect against pneumonia and other diseases that claim the lives of millions of children each year. A broad coalition of public and private donors has committed $1.5 billion to guarantee a price for a vaccine suitable for third-world use, while also encouraging companies to invest more broadly in research and expanded manufacturing capacity for effective vaccines.

The FDA’s priority review voucher initiative offers another type of inducement for manufacturers to support R&D of neglected disease treatments. The program provides a voucher to sponsors seeking approval of a treatment for one of 16 designated tropical diseases. The voucher is good for a future priority, six-month review (instead of the usual 10 months or more), which should be highly lucrative to any company seeking market approval for a new medicine with a large sales potential.

The first voucher was recently awarded to Novartis in conjunction with approval of its antimalarial drug Coartem (artemether-lumefantrine) in the US. The drug already was available in most of the world, but the application got the program going while the FDA develops guidance and weighs whether to expand the list of tropical diseases that qualify for review vouchers. Several manufacturers appear poised to file applications, with the hope of gaining vouchers they can use or sell to support speedy drug approvals.
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In the biopharmaceutical industry, the drive to obtain higher therapeutic protein yields from a production process continues to be a key objective for controlling expenses and resource requirements. Although many factors contribute to protein yield, a key element is media optimization. A thorough understanding of a cell’s nutritional requirements in a given process is essential to developing a high performance medium and feed strategy. Large biopharmaceutical companies often develop and optimize their own in-house media formulations, the manufacture of which are then outsourced to a media supplier. A media optimization project, however, can be time consuming and labor intensive if the proper tools and resources are not available. Outsourcing media optimization to an experienced supplier, skilled in biopharmaceutical applications, can significantly decrease development time and costs.

COLLABORATE WITH THE OUTSOURCING PARTNER

To select the right partner for media design and optimization, several key factors must be considered, with the most important being the collaborative nature of the supplier. It is essential that the biopharmaceutical company and supplier work together in a partnership, with full transparency to all data. This helps ensure that proper decisions are made to increase the likelihood of success when the manufacturing process is implemented. Another key consideration is the supplier’s understanding of biopharmaceutical production process requirements and the media optimization strategy it uses. A methodical optimization strategy, specifically created for production cell lines and including Design of Experiments (DOE) is essential for an optimized formulation. The designed experiments must be coupled with a strong analysis of spent media to ensure a thorough understanding of the entire process.

In addition, significant consideration should be given to the facilities that will be used for the optimization of the media formulation and subsequent media manufacturing. The facility should not only be able to supply the resources needed to perform the media optimization and to scale up production of the cell culture media, but also must satisfy rigorous quality and regulatory requirements that meet and even exceed the high standards required by the biopharmaceutical industry.

PRE-AUDIT THE FACILITY

When outsourcing cell culture media manufacturing, one must consider the facility itself as a critical component of the overall process. To ensure product consistency and performance, the facility must be pre-audited before manufacturing begins. There are several key attributes of a cGMP cell culture media manufacturing facility that one should assess. A high level of control of the raw materials, such as animal-origin information and strategic sourcing for reliable supply, along with a highly segregated process and personnel flow, are paramount. These should include a stringent animal-free raw material pol-

Prepared by K. Fritchman, J. Kuchibhatla, and Yann Pouliquen are bioprocess applications and scientific affairs managers, J. Brooks is an R&D manager and S. Holdread is a project scientist, research and development, A. Imam is a senior manager, quality systems, C. Velnoskey is a bionutrients market development and marketing manager, and V. Weinknecht is an industrial regulatory affairs manager, quality management and regulatory compliance, all at BD Biosciences—Advanced Bioprocessing, Sparks, MD, 760.788.4577, Kathie_Fritchman@bd.com.
icy and complete segregation of all manufacturing areas, including personnel flow throughout the facility, as well as individual equipment suites with zoned HVAC systems to eliminate cross-contamination risk. The facility must include modular cleanroom systems with hardened PVC-coated walls and ceilings with cove transitions for ease of cleaning and full-viewing corridors that allow complete access to the facility without compromising room integrity. Automated clean-in-place (CIP) and steam-in-place (SIP) processes must be available as well as robust business continuity systems, including emergency utility back-up systems. The facility must have fully integrated cell culture media and supplement manufacturing capabilities with the ability to produce complete formulations for the full range of expression systems, including mammalian cell culture, microbial fermentation, and cell therapy.

The facility must be highly versatile with a fully scalable lot size capability including flexible packaging suites, that offer a wide range of filling capabilities and custom-packaging solutions. State-of-the-art milling technologies, such as pin mills, are preferable to ensure excellent particle size consistency, minimal heat generation, and easy cleaning using a CIP system with ultra pure water. Powder production must be highly versatile and fully scalable, offering a wide range of filling capabilities and custom-packaging solutions. In addition, the water used for liquids should be water for injection and USP-purified water to reduce bioburden and control endotoxin levels.

**SUPPLY CHAIN MANAGEMENT**

The foundation of a sound outsourcing strategy begins with due diligence in the selection of the raw materials and the packaging materials. Qualifying and maintaining the supply chain is best accomplished by designing a strategy for business continuity. This includes developing a supply chain that is aligned with one’s business needs and that can adapt to an ever-evolving regulatory environment.

Among the other challenges of sourcing cGMP-manufactured raw materials and components, the industry is faced with qualifying and securing animal-free materials. In 1996, following the appearance of variant Creutzfeldt-Jakob disease (vCJD), the FDA Center for Biologics Evaluation and Research recommended that manufacturers take necessary steps to ensure they are not using bovine materials from cattle born, raised, or slaughtered in regions affected with bovine spongiform encephalopathy. The FDA has issued various guidance documents for sourcing and processing such materials. However, with many materials still sourced from animals, particularly in cases where there is only one known supplier of a given material, it is critical to scrutinize the raw materials and other components of a manufacturing process and products.

In addition to vCJD, animal-derived materials used in cell culture media may contain infectious agents (e.g., viruses, mycoplasma, prions). To most effectively minimize the risk of these agents, it is optimal to select a supplier that does not permit any animal-derived materials in its cell culture media manufacturing facility. In addition, the presence of an antimicrobial in a cell culture medium can mask the presence of viable microorganisms that can pose a significant risk. The risk of antimicrobics in a cell culture medium can be reduced by sourcing from a supplier that does not use any antimicrobics in the facility.

The supplier’s process must ensure there are no places where traceability could break down, gaps in batch history could exist, or extraneous material or contaminants of any kind could enter the process between raw material receipt and delivery of the media product to the end-user. The supplier must be regulated under current GMPs and certified by the FDA or a similar regulator outside the US, or certified to meet applicable ISO standards.

**SUMMARY**

When outsourcing cell culture media design and manufacturing, biopharmaceutical companies need to ensure highest safety levels. To do so, one should seek a media supplier that can provide flexible cGMP manufacturing capabilities in a dedicated facility, optimization services with custom solutions, supply chain management with stringent qualification, control and traceability of raw materials and suppliers, and strong management of risks and regulatory exposure. The resulting benefits are listed in Table 1.

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### Table 1. Points to consider when selecting a media supplier for cell culture media design and manufacturing

<table>
<thead>
<tr>
<th>Features of the supplier</th>
<th>Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexible cGMP production and packaging capabilities</td>
<td>Ability to meet specific customer requirements</td>
</tr>
<tr>
<td>Dedicated pharmaceutical-like facility</td>
<td>A cGMP compliant and certified ISO 9001 facility which offers a high level of control and risk reduction and ensures product quality</td>
</tr>
<tr>
<td>Fully dedicated, standalone, animal-free cGMP manufacturing facility</td>
<td>Minimizes risk associated with infectious agents (e.g., viruses, mycoplasma, prions)</td>
</tr>
<tr>
<td>Good supply chain management</td>
<td>Control and traceability for each lot of raw materials up to the tertiary level</td>
</tr>
<tr>
<td>Optimization services</td>
<td>Custom base and supplement formulations that increase final product yield</td>
</tr>
</tbody>
</table>

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September 2009  www.biopharminternational.com  BioPharm International  19
ICH Q9 encourages companies to apply the concept of quality risk management. Easier said than done.

Quality Risk Management Demystified at CMC Strategy Forum

Laura Bush is the editor in chief of BioPharm International, Iselin, NJ, lbush@advanstar.com, 732.346.3020.

The prospect of conducting risk assessments, as part of the quality risk management (QRM) strategy promoted by the International Conference on Harmonization (ICH) Q9 guideline, tends to evoke blank stares and confusion. It also prompts many questions: Which of the many tools—PHA, HACCP, FMEA, etc.—should we use? How could we possibly conduct a line-by-line analysis of every risk known to bioprocessing? Is this really useful?

The CMC Strategy Forum on Practical Applications of Quality Risk Management, held in Bethesda, MD, on July 27 and 28, tackled these challenges and questions through a hands-on workshop and plenty of lively discussion.

In the workshop, small groups used the preliminary hazard analysis (PHA) method to assess the risks involved in operating a large-scale production bioreactor for a monoclonal antibody. As the groups considered inputs such as duration, media, and temperature, they identified the possible hazards involved and the harms those hazards could cause, and then assigned a severity score to each harm. They also assigned a separate risk score to each potential harm, based on the likelihood that...
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it would occur. Then they multiplied those numbers to reach an overall risk score, addressed the controls used to prevent or mitigate the event, and decided whether or not the risk would be acceptable (Table 1).

After the breakout sessions, everyone understood the concepts, terminology, and process much better, and had a good appreciation for the challenges involved in conducting risk assessments. They also had a lot of questions, which were discussed with the overall group.

**HOW TO ACCOUNT FOR CONTROLS**

One common question raised during the risk assessment exercise was how to account for the controls that mitigate risks.

Several speakers stressed that the effectiveness of controls should never affect how one ranks the severity of a potential harm. “Severity rankings are dependent on harm only,” said Joe Siemiatkoski of Biogen Idec. “You should never lower a severity score by taking credit for controls.”

**Table 1.** A partial risk assessment worksheet created during a fictitious exercise for the operation of a production bioreactor, using the preliminary hazard analysis (PHA) method. Each potential harm was assigned a severity score (on a scale of 1–9), and each potential hazardous situation was given an occurrence score, based on the likelihood that it would occur, taking into account the controls used to prevent or mitigate the event. The two scores were multiplied to reach an overall risk index.
For example, if a given harm, such as bioreactor contamination, is considered high, say 9 on a scale of 1 to 10, that severity ranking should not be lowered just because numerous measures are in place to prevent contamination. Instead, those controls would lower the ranking number assigned to the likelihood that such a harm would occur. Then, when the total risk is assessed by multiplying the severity and likelihood scores, its overall risk ranking would not be high, and thus additional controls may not be warranted.

Dan Weese of Amgen explained that this approach is important for documenting the potential risks to any given unit operation. “You may have a risk where you are confident that a later step will take care of it, but what if a later step is removed?” he said. “You need the risk to be flagged as severe in the earlier unit operation, so you don’t lose your record of it.”

### The Meaning of Severity Rankings: How to Assess Potential Patient Harm

Participants also struggled to understand the meaning of severity rankings. The ICH Q9 guideline instructs the industry to consider harm in terms of patients. For some examples, however, like the cell culture step discussed in the workshop, it is difficult to envision any problem—from contamination to aggregation—that wouldn’t be resolved before the product reaches the patient. Some cited improper glycosylation, but others pointed out that for many products, particularly monoclonal antibodies, even carbohydrate structures would not necessarily affect product safety or efficacy.

The solution, many speakers said, is to use in-process product quality as a surrogate for potential harm when assigning severity rankings.

“The first time we piloted a risk assessment during our implementation of ICH Q9, we attempted to use a severity scoring criteria that included harm to patients, and the highest severity score included serious injury or death,” said Weese. “That didn’t work for upstream assessments begun in the middle of Phase 2, because in many cases, you just don’t know how a potential failure in a unit operation might cause direct harm to a patient. But we can estimate the potential impact on product quality attributes.” As a result, he said, they decided it made more sense to use the potential impact on a product quality attribute as a surrogate for potential harm to patients.

The connection to patient risk would be clearer, some said, if clinical staff participated in risk assess-

<table>
<thead>
<tr>
<th>&quot;Occurrence (Probability of occurrence of hazardous situation or hazard)&quot;</th>
<th>Risk Index (S x O)</th>
<th>Is risk acceptable? (Risk index cutoff = 16)</th>
<th>Additional risk control actions required</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>25</td>
<td>N</td>
<td>Identify locations for alarms (action and alert limits). Reassess occurrence once operator training is complete. (Note: occurrence score of 5 assigned because facility and staff are assumed to be new).</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>N</td>
<td>Further analyze detection mechanisms and above actions</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>N</td>
<td>Reassess occurrence score once operator training is complete. Evaluate filter housing design.</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>N</td>
<td>Implement high throughput screening. Change vendor. Potentially change the process (i.e., from serum-containing to serum-free process). Purchase irradiated material.</td>
</tr>
</tbody>
</table>

In this example, the risk index cutoff was set at 16, meaning that any risk scoring 16 or higher was unacceptable (shown in red), and required additional controls.
ments. “We include clinical staff when evaluating the need for product recalls, so I would think companies could benefit from including clinical staff when assessing risks,” said Kevin O’Donnell of the Irish Medicines Board. “Clinical staff do see things that we don’t in terms of risk to patients.”

A few people responded that clinical staff participate in an earlier stage in the process. “We get input from clinicians in an earlier step, when we are determining critical quality attributes,” said Kathy Francissen of Genentech.

Others questioned whether any clinician could really be expected to predict the patient impact of a manufacturing change. “Unless you have a very strong event, you are not going to get a clear linkage, because of extreme number of variables involved,” said Wassim Nashabeh of Genentech.

Steven Kozlowski of the US Food and Drug Administration’s Center for Drug Evaluation and Research (CDER) asked if anyone has attempted to tackle this problem through data mining, using multivariate data analysis to compare clinical outcomes or adverse events to look at, perhaps by linking those data to lot numbers. “If we did this, would we find clusters of data that would be useful?” he asked.

Stefanie Pluschkell of Pfizer said her company is trying to do just that. “It’s complex, because the databases [of adverse events and product lots] are not always directly linked,” she said, adding that it also can be difficult to determine which specific adverse events to look at, because they can be diverse. “But I am reasonably hopeful that this effort will be useful,” she said.

SCIENTISTS AND SUBJECTIVITY
A number of participants also questioned the real value of risk-ranking scores, given their subjectivity. “Now may be the time to improve the science of our decision-making, to increase our confidence in the results of these risk assessment exercises,” said O’Donnell of the Irish Medicines Board. “There are other disciplines, such as experimental psychology, that we should look at. Much research work has been carried out and that research can be helpful to us when we are carrying out quality risk management exercises.”

“Subjectivity will not disappear, and we should not strive for that,” cautioned Rohin Mhatre of Biogen Idec. “The whole process is very subjective, from deciding how many experiments to run, to which parameters to study. We should try to build a good rationale for our risk assessments, but not aim for objectivity.”

Nancy Waites of the FDA’s Center for Biologics (CBER) agreed. “We’re all scientists and we don’t like ambiguity,” she said. “But you can’t think of everything. You need to be comfortable with not knowing everything.”

Terry Ocheltree of CDER also concurred. “I don’t think anyone at FDA expects you to remove all subjectivity,” he said. “What is important is to have the tools and use them correctly. But that’s not to say that everyone must run risk assessments in the same way.”

THE RIGHT TEAM AND THE RIGHT FACILITATOR
Various speakers stressed the importance of assembling the right team to conduct a risk assessment. “The scoring is as good as who is in the room,” said Amgen’s Weese, emphasizing the importance of including staff from all key departments. “Our rules say that if certain people are not present, we will not proceed,” he added.

But don’t underestimate the importance of a good facilitator, several people said. “It’s not just a question of involving the right subject matter experts, but also about the competence of the facilitator,” said Keith Webber of CDER.

In particular, managing the people and personalities involved can be tricky. “Often we will have one very outspoken person who dominates, especially if that person is an expert in the topic under discussion,” said Weese. “We need to ensure we hear from everyone else.”

Vince Hamner of Talecris agreed, adding that those who disagree don’t always voice their views. “Sometimes you get someone who sits back and doesn’t say anything, then suddenly speaks up at the end,” he said.

A good facilitator, Weese added, can help the group reach decisions, particularly when doubt leads a group to score everything conservatively. “If every risk number is extremely high, we are not achieving anything,” he said.

But many also pointed out that there are few people in the biotech industry trained in risk management. Some suggested turning to the medical devices industry, because medical device manufacturers have been conducting risk assessments for decades.

ARE RISK ASSESSMENT EXERCISES WORTH THE EFFORT?
Conducting risk assessments is a lot of work, however, as the group exercise made clear. Some raised the question of whether it is worth the effort, because it seems that in most cases, the risks identified are the usual suspects.

“The majority are the usual suspects,” acknowledged Richard O’Keeffe of Amgen. “But you are documenting what previously might only have been in people’s heads. One of the benefits comes from systematic documentation.”

“But it seems we are making it out to be more than it should be,” countered Mhatre of Biogen Idec. “I hope we are not taking it so far that it just becomes an exercise and we lose all the practicality of it, like
“We give out 483s for a lot of things that wouldn’t have occurred if you had done a risk assessment, such as lack of process validation.”

what happened years ago with process validation.”

Sally Seaver of Seaver Associates agreed that getting real value out of risk assessments requires doing them properly, and not letting them become a rote exercise. “I think if risk assessments are done well there will be surprises—we will identify some unexpected risks,” she said.

Furthermore, the work involved in formalizing risk management processes should get easier with time, said Genentech’s Nashabeh. “In the early stages, you layer these things onto what you are already doing,” he said. “If we get to a point where we integrate this into existing systems, we may save resources, but we are not there yet.”

Julia Edwards of Genentech agreed. “Conducting risk assessments needs to be integrated into your quality systems, not an additional activity,” she said.

Weese added that additional benefits are gained when risk assessment work is standardized throughout a company. “What we have done with Q9 is to establish a common framework across molecules, and across departments,” he said. “That’s the big benefit, in addition to reducing overall risk.”

The cross-functional dialogue that occurs naturally during risk assessments is another big gain, Weese said. He cited an example where staff from different groups found out important information about a process during a risk assessment exercise. “You are really doing that?” one group asked another. ‘You can’t do that!’ That was really important,” said Weese. “That exercise clearly paid for itself.”

Genentech’s Edwards gave a presentation in which she showed how her company is applying risk assessments to multi-use facilities, and then leveraging that work at other sites. The biggest gains, she said, can be achieved by using that information to file comparability protocols with the FDA. “By leveraging integrated quality risk management in regulatory submissions, we can effectively say, ‘if we make this change at one site without a problem, we should also be able to make it at a different site,’ since QRM allows us to account for site-specific considerations,” she said.

Risk assessment tools also can be used to get upper management to recognize and formally accept risks, several participants said. Nadine Ritter of Biologics Consulting Group cited the example of a project manager who used a form listing the risks of changing an analytical method. The form required a signature from senior management, stating that they accepted responsibility for the decision. “Then they would re-think it carefully,” she said. “That was brilliant.”

Yet making the business case for quality risk management work is not easy, said Krista Terry of Genentech, because many of the gains relate to preventing problems, and it is difficult to assign a dollar value to such benefits. “On the operations side, for example, we have seen a decrease in the number of discrepancies, perhaps because staff are more aware of the importance of following procedures,” she said. “It’s more about working toward cost avoidance.”

REGULATORY REQUIREMENTS

Applying quality risk management principles is not a regulatory requirement in the US, but it is in Europe, and may become one in other jurisdictions too.

“Under Chapter 1 of the EU GMPs, it is a requirement for companies to have a quality risk management program as part of the quality assurance system. This has been in place since July of last year,” said O’Donnell of the Irish Medicines Board. O’Donnell went on to explain that although additional guidance on quality risk management is available in Annex 20 to the EU GMP guide, that guidance is voluntary at this time, meaning that companies are not required to comply with it.

Anthony Ridgway of Health Canada also foresees that this requirement eventually could be adopted in Canada and internationally. “ICH Q9 clearly states that it is not intended to influence regulatory requirements, however, if doing risk assessments becomes a routine part of good manufacturing practices, it could potentially get included in a future version of GMP regulations,” he said.

And all the regulators at the meeting said they saw value in quality risk management.

“Conducting risk assessments is not a regulatory requirement [in the US], but companies that have risk management programs in place tend to have a good handle on their processes,” said Waites of CBER. “They communicate well among themselves and often catch problems earlier.”

Waites also noted that inspectors would look at the outcome of risk management efforts, not the process. “We won’t fault you for ranking a risk as a 3 rather than a 7, but the outcome of ranking something too low might be inadequate validation,” she said. “And we do give out
483s for a lot of things that wouldn’t have occurred if you had done a risk assessment,” CDER’s Kozlowski said that regulatory agencies should also use risk-based approaches. “One of the most common complaints we get from industry is that we pay too much attention to lower risks,” he said. “So if we apply the concept of risk management, it will benefit everyone.”

Some companies have also made effective use of quality risk management in regulatory filings. Examples mentioned included filings for process and site changes, and even expanded comparability protocols. “I’ve seen it used in cases where a legacy process needed to be updated, such as for introducing new equipment or operating principles,” said Patricia Hughes of CDER. “It’s useful for improving processes.”

Hughes also mentioned the example of changing a facility from single- to multi-product production. “This is especially useful in cases where you have very difficult cleaning validation issues for potent products, or different cell lines used in a multi-product facility,” she said. “Risk analysis helps you understand the risks involved, and determine how to handle them.”

SUMMARY

The discussions, presentations, and group workshop at the recent CMC Strategy Forum made it much easier to understand how to tackle the challenges involved in conducting quality risk assessments as part of a quality risk management program. The discussions clarified how to think about potential harms, how to account for controls that prevent or mitigate risks, and how to handle the subjectivity of scoring. Speakers also stressed the importance of skilled facilitation, and of assembling a crossfunctional team for risk assessment exercises to ensure that all critical groups involved in a given unit operation have input.

No one doubted that conducting quality risk assessments is time-consuming and a lot of work. But the benefits, most said, are worth it. Apart from the obvious gain of reducing risk, and avoiding future compliance failures and their related costs, some said that the results could be used to justify manufacturing changes and even to support expanded comparability protocols. In addition, many participants said the process itself was useful, by fostering crossfunctional dialogue and recording institutional knowledge. And although quality risk management is not yet a regulatory requirement in the US, it is already codified into European regulations, and that trend may expand.

For more on this topic, please visit www.biopharminternational.com/quality

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- An Opening General Session on Canadian Food and Drug Regulation (Wednesday, September 23, 2009)
- An Interactive General Session on Biomeasurement featuring scientists from the U.S., Canada, and several other countries. (Thursday, September 24, 2009)
- A Closing General Session on Nanotherapeutics and Measurement (Friday, September 25, 2009)

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The Proper Use of 47-mm Flat Disc Filters in Filter Sizing Studies

Maik W. Jornitz, Theodore H. Meltzer, Wayne Garafola

ABSTRACT
Small flat-disc filters, typically 47 mm in diameter, are often used as models in filter sizing exercises to quantify the effective filter area (EFA) needed to filter an entire batch. The proper implementation of the flow decline method of assessing EFA requirements, however, necessitates knowing the precise EFA of the model filter, which should be measured using staining techniques. Furthermore, such indicator studies should be followed by verification trials using pleated filters, to ensure greater accuracy.

Filters of small areas are useful as models for larger operations such as batch-scale processing. Flat disc 47-mm microporous filters are often used for this purpose, especially in flow decline (throughput) studies aimed at sizing the effective filter area (EFA) required for large-scale production.

In the flow decline method of filter sizing, also known as the flow decay or total throughput method, the quantity of effluent produced using a given small filter area is extrapolated to determine the filter area needed to process the drug volume of an entire production batch. It has customarily been considered convenient and economically useful, both in terms of effort and material costs, to conduct such sizing studies with 47-mm flat discs, because their small size minimizes the quantity of fluid involved and the operational time required for the assay.

If desired, however, discs of an even lower EFA may be used. The smaller the assay filter, the less product is consumed in the testing. This can be an important consideration if the fluid being tested is an expensive drug. Because pharmaceutical filtration is a technico-economic enterprise, the expenses involved are reflected in the drug’s cost of goods.

Regrettably, extrapolating from data obtained by using smaller filter areas or over shorter test intervals tends to give less dependable results. The smaller the filter area and liquid volume used, the less accurate the extrapolation. Consequently, the use of 47-mm flat disc filters in flow decay studies sometimes produces results that are so inexact that they must be used with an extremely high safety margin. Such allowances usually are set at 15–20%, but can be as high as 150%. Such over-sizing will result in high value losses because of unnecessary large hold-up volumes and unspecific adsorption. In such circumstances, the savings achieved from the flow decay studies are lost because of batch-to-batch running costs. For example, a 10-inch filter element could adsorb an average of 200 mg of drug (Figure 1), which commonly can be doubled to 400 mg for a 20-inch filter, which could be required if the filter were improperly scaled. The per-batch value of such waste can be calculated from the dosage and value of the drug product being filtered.1,2

The importance of proper filter sizing
also has received attention in the revised PDA Technical Report #26, which describes filter choice and trials in detail.³

THE EFFECT OF SUSPENDED PARTICLES

When dealing with a “clean” liquid (i.e., a liquid devoid of suspended particles), the flow rate and the resulting throughput, per unit of time, at the applied differential pressure is straightforward to measure, based on the selected EFA. (Temperature must be kept constant, particularly because the liquid’s viscosity is its reciprocal, and any increase in either factor will lead directly to a proportional rise in throughput.)

If the liquid contains suspended particulates, however, the filter’s porosity will be decreased by particle retention, so additional EFA may be required to compensate for the filter area that is blocked or clogged by the particulates.⁴ In such cases, it will be necessary to conduct experiments to assess the EFA needed for batch processing.

Alternatively, it may be possible to restore lost throughput by using higher inlet pressures rather than increasing EFA. If filtration is already in progress, that would provide a more manageable alternative. The effectiveness of such a measure would depend, however, on the total suspended solids (TSS) in the solution. Higher differential pressures could increase the pressure drop by compacting any filter cake that may have formed. In contrast, a larger EFA would be less likely to produce such compaction under the same conditions, as there would be little or no filter cake build-up. Those effects, in turn, can lead to losses in product yield or the need for larger EFA.

If the preparation presented for filtration is relatively free from suspended particles, flat discs of even smaller diameters may suffice for filtration sizing tests because the EFA available for liquid permeation would not be diminished by particle deposits. An example of such an application would be filter sizing for deionized waters. These liquids contain so little suspended matter that the flow decline data can be secured fairly quickly from tests using a small filter. The fewer the solids in the suspension, the less demanding the mathematical extrapolation.

Nevertheless, a sizeable inaccuracy inheres to the extrapolation of results from a 47-mm disc’s EFA of 1.49 in² to the EFA expected from a 6 ft² (or 3,864 in²) cartridge. At best, the results indicate only hypothetical, non-committal values; hence the large margins allowed for error. An assessment method that requires an EFA overdesign of as much as 1 or 1.5 times the extrapolated value does not merit endorsement.

It may well be that the extreme safety margins reported are exaggerations of the arithmetical uncertainties, reflecting the experimenter’s strong fear of having to interrupt the filtration mid-stream to install new filters to allow processing of the batch to be completed. Indeed, the aseptic replacement of a filter is a risk-prone operation, so its avoidance is strongly recommended.

RATIONALE OF THE FLOW DECLINE METHOD

The flow decline, or flow decay method, is used to determine the effective filtration area required to process an entire batch of any volume in an acceptable time frame under a given delta (∆) pressure. It is managed by a simple arithmetical proportioning. A small filter with a known effective area is used to measure the volume of a drug preparation that can be processed at a selected ∆P (differential pressure), given the rate of flow and the throughput it produces. The ratio of the filtration area to the sample volume is extrapolated to the filter area required to process an entire batch. The simplicity of the test ensures its easy mastery; its performance demands only a modest technical background.

In a flow decline experiment, a small ali-

![Figure 1. Variation in unspecific adsorption (mg per 10-inch cartridge) when different filter designs are used with the same filter polymer](image-url)
Filtration

The retained particulate load accumulates on the filter pores, successively diminishing filter porosity and the flow rate. At a certain point, the flow is judged to have ceased for all practical purposes; the effort and time that would be spent obtaining additional filtrate beyond that point is not economically feasible.

In these experiments, one measures the flow rate (volume over time), the throughput, and the total filtrate volume obtained over the duration of the filtration. The ambient temperature is also noted. The results identify the ratio between the EFA and the volume of filtrate that is produced before the filtration is terminated. The numerical value has a significance beyond that inherent in the specific filter. Rather, it is taken to quantify the capacity of filters of that type to retain the maximum amount of particulate matter present in that unique fluid preparation under the given filtration conditions. Its importance is in the nature of a validation exercise.

An arithmetical extrapolation of the experimentally obtained ratio can then be used to determine how large a filter area will be required to process a batch of product. The ratio that is established is:

\[
\frac{\text{Test volume filtered (mL)}}{\text{Test filter area (m}^2\text{)}} = \frac{\text{Batch size (mL)}}{X (m^2)}
\]

in which \(X (m^2)\) is the filter area required to process the batch.

The required filter area, thus calculated, can then be translated into the number and lengths of cartridges or other filtration devices one wishes to use for the batch operation, by using additional equations. The area of a flat disc filter is:

\[ A = \pi \times 4 \times d^2 \]

The 47-mm disc has an area of 17.4 cm\(^2\) (1.49 in\(^2\)). A 10-in cartridge composed of the same filter polymer will be assumed to contain an area of 6 ft\(^2\). (The areas of other filter cartridges may be calculated in the same way.) Therefore, the throughput volume of the cartridge relative to that of a 47-mm filter of the same polymeric composition is:

\[
\text{(Cartridge EFA ft}^2\times \text{conversion in}^2\text{ to ft}^2) \div \text{47 mm disc EFA in}^2 = \text{Multiplier}
\]

\[
(6 \text{ ft}^2 \times 144 \text{ in}^2/\text{ft}^2) \div 1.49 \text{ in}^2 = 580
\]

Thus, if the 47-mm disc yields a total throughput of 2,500 mL, then 2.5 L x 580 = 1,450 L (~383 gallons) will flow through the cartridge before it shuts down. The rate of flow measured on the 47-mm disc then indicates the number of 10-inch cartridges with an EFA of 6 ft\(^2\) that would be required to complete the batch filtration in a timely manner at the constant differential pressure, temperature being kept constant.

From the foregoing, it should be self-evident that the accuracy of the arithmetical extrapolation is highly dependent on the accuracy of the EFA attributed to the 47-mm disc. Nevertheless, it is in this very matter that many incorrect assumptions are made.

**ACCURATELY MEASURING THE EFFECTIVE FILTER AREA OF A 47 MM FLAT DISC FILTER**

In the classical application of the flow decline method, a 47-mm membrane is removed from its package and inserted into a stainless steel holder, where it is held in position by the compressive action of an O-ring. The area of a 47-mm filter disc is 17.4 cm\(^2\). In the holder, however, the O-ring pre-empts a certain quantity of the disc’s peripheral space by clamping down on the filter disc to prevent edge-leakage. This reduces the filtration area available for filtration. Thus, the EFA of the inserted 47-mm disc is reduced to less than 17.4 cm\(^2\).

The actual EFA of such an assembly was measured by filtering a staining solution of acridine yellow or Coomassie blue (Figure 2).
The stained area identified the sealed area in the confines of the O-ring. As can be seen, the staining solution did not extend beyond or under the O-ring. The stained area measured 41 mm in diameter, which has a total area of 13.2 cm$^2$. Thus, of the 7.4 cm$^2$ area of the 47-mm disc filter, 4.2 cm$^2$ were rendered unavailable for filtration by the O-ring’s preemptive sealing action.

**USING PREASSEMBLED 47-MM FLAT DISC FILTERS**

A pre-assembled, disposable unit containing a “47-mm” diameter flat disc filter is available. The pre-assembly offers the attractive advantage of disposability. It is likely that users assume that its EFA is that of a 47-mm disc. However, the membrane used for these devices is actually 50 mm in diameter, a common filter size. The staining technique using acridine yellow reveals its effective diameter to be 48 mm (Figure 3), which means it has an EFA of 18.4 cm$^2$. Its throughput, however, is being ascribed to that of a smaller EFA—the assumed EFA of the 47-mm disc ordinarily used, namely, 17.4 cm$^2$. The discrepancy in EFA is even larger if the stained 13.2 cm$^2$ area of an actual 47-mm diameter filter disc is used in the comparison (Figure 4).

The flow emanating from the 18.4 cm$^2$ EFA of the 48-mm diameter disc filter may mistakenly be ascribed to the actual 13.2 cm$^2$ EFA of an O-ring sealed 47-mm diameter filter. If so, the extrapolation exercise will lead to a numerical multiplier that will indicate that a smaller EFA is needed for processing than would result if the multiplier were based on the smaller (stained) value of 13.2 cm$^2$. The result will be a low EFA that is insufficient for batch processing needs. This may cause a mid-process filter change-out, which the use of the flat disc filter sizing aims to avoid.

**INDUSTRY USE OF STAINING TECHNOLOGIES**

Even though the use of 47-mm flat disc filters is widespread in flow decline work, the operational details of these studies may differ among users, because the method is not standardized. It is not known what EFA values the many users of 47-mm disc filters actually use in their sizing protocols, because these numbers are seldom reported. Measuring filter EFAs by acridine yellow or other staining does not seem to be a widely discussed or published procedure. Clearly, its application to the use of 47-mm discs in filter sizing studies is not universal.

It is recommended that the staining technique be used to determine the exact diameter of the disc being used, so that its actual EFA can be calculated from that diameter. This practice would minimize the risk of underestimating the EFA needed for processing a batch operation, and reduce the possibility of needing mid-process filter replacements.

**CONFIRMATORY ASSESSMENTS**

Even if staining techniques are used to improve the accuracy of filter sizing studies, the extrapolations from 47-mm flat disc filters to 10-inch cartridges are beset by the uncertainties derived from suspended matter. At best, flow decline assessments based on 47-mm flat disc filters constitute indicator trials.

Therefore, it is best to follow up these indicator trials with verification trials using larger-area pleated filter devices, (commonly 1.5 ft$^2$). Indeed, when costly drug preparations are involved and properly defined filtration area scaling is needed, the use of full-scale filters in assurance trials is recommended. Full-scale trials ensure that the filtration system will be large enough to filter the required batch volume without being oversized, thus minimizing product yield losses.

**USE OF PLEATED MODEL FILTERS**

The function of the 47-mm flat disc filter is to
Filtration serve as the model for the filter to be used in batch processing. Unfortunately, filterability trials that use 47-mm flat discs can only roughly indicate which filter combination might be optimal. To perform reliably in its pilot role, the model filter should be as similar as possible in all its structural details to the production filter. Moreover, it should be tested under the conditions and in a manner as similar as possible to those of the production operation.

Batch filtration will most likely involve pleated filter cartridges. The details of pleated cartridge construction, however, are substantially different from those of flat filters. The measurement of the effective filtration area of flat disc filters is straightforward, whereas that of pleated filters is appreciably more complex. As a result, flow and retention data will not extrapolate well from flat stock to pleated filter cartridges, and EFA forecasts based on flat stock may overestimate the EFA available from pleated filter constructions. This would lead to a need for mid-process change-outs.

The differences in the EFAs of flat disc and pleated filters can result from the pleat-pack constructions and density of pleated filters. These discrepancies may derive from the flow-attenuating influences of the cartridge’s support and drainage layers. In pleated filters, the entire filter area may not be available for the filtration function; a portion may serve to satisfy structural or mechanical requirements. How much of the remainder is available to the EFA function depends on how the pleating operation is conducted.

The details of the pleating operation are beyond the scope of this article.9 We can note, however, that the very nature of the pleat numbers, heights, and degrees of tightness affect the flow and retention properties of the cartridges composed of them.

The flow-attenuating influences of the cartridge’s support and drainage layers, and the retention-modifying effect of pleat construction features cannot dependably be assessed from pilot studies using flat stock. Therefore, if pleated filter cartridges will be used in batch processing, indicator trials using 47-mm flat disc filters must be followed up by verification trials using miniaturized pleated filter devices.7,8 Using pleated filters in these verification trials will mean that the test results
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Filtration

will be much more relevant. Pleated filter cartridges are available on the market in sizes as small as two inches in length.

When even more reliable EFA forecasts for batch processing are needed, assurance trials should be conducted using larger-scale cartridges. Conducting tests using filters with a larger EFA, even to the extent of using full 10-inch pleated cartridges, would proportionately increase the reliability of the extrapolated value.

ASSESSING THE EFA OF PLEATED FILTERS

As noted, the total area of filter material allocated to the pleating process is not necessarily converted to EFA usage. The uncertainty regarding the actual EFA of the pleated cartridge can, however, be resolved. A far more accurate prognostication based on the pleated model would then follow.

The exact amount of filter area used in constructing the pleated model should be known. Were it all converted to the EFA function, its extent could be calculated from flow rate studies as a function of applied differential pressures using Newtonian (clean) fluids at constant (ambient) temperature. Reverse osmosis product water could serve as the test fluid. The ideal flow rate would be the same as the flow rate if the filter matter in toto were used as EFA. That value could be obtained from the filter manufacturer, according to the filter type used, along with its porosity and thickness. The extent to which the expected flow is not realized would quantify the portion of the filter material not available as EFA as a result of pleating. The model filter’s EFA would then be known with certainty, and its extrapolation could then be made with confidence.

SUMMARY

The ultimate goal of the filter sizing exercise is to quantify the effective filter area (EFA) needed to filter an entire production batch over a set time frame. Such testing typically is done using a flat disc with a diameter of 47-mm. The proper implementation of the flow decline method of assessing EFA requirements necessitates knowing the precise EFA of the model filter, which can be measured using staining techniques.

A filter-sizing study using a 47-mm flat disc is intended as an indicator trial, which is preliminary to a more meaningful verification trial, or even to a more cogent assurance trial. The latter will require filter models characterized by a pleated membrane design. The EFA assessment of the pleated filter models can be performed with confidence. This will result in dependable projections of the EFA required for the batch processing filters.

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Comparison of Camelid Antibody Ligand to Protein A for Monoclonal Antibody Purification

Jia Liu, Aaron Cheung, John L. Hickey, Sanchayita Ghose

ABSTRACT
A novel Protein A alternative stationary phase based on the variable heavy chain fragment of immune camelid antibody was evaluated and compared to commonly used commercial Protein A resins. The parameters evaluated were elution pH, equilibrium isotherm, dynamic binding capacity, and host cell protein clearance using a set of Chinese hamster ovary-derived monoclonal antibodies and Fc-fusion proteins. Linear retention experiments were used to compare the specificity of these resins for both non-IgG model proteins as well as antibodies and Fc-fusion proteins. The experimental results showed that the new camelid antibody resin behaved very similarly to Protein A resins in terms of retention of non-IgG model proteins and IgG-based molecules. Dynamic binding capacity was found to be comparable for Fc-fusion proteins and slightly lower for antibodies. Host cell protein clearance profiles were also similar under preparative conditions using complex biological feeds. Finally, the binding mechanism was explored by using different mobile-phase modifiers in linear pH gradient retention experiments.

Protein A affinity chromatography has been widely used for antibody purification in the biopharmaceutical industry because of its excellent selectivity and product recovery.1-5 In recent years, it has been recognized as the industry standard for capture and purification of antibodies and Fc-fusion proteins. The use of this highly selective and robust capture step allows for faster process development and has enabled the use of a platform approach for monoclonal antibody (mAb) purification.6,7 Despite all of its advantages, Protein A chromatography suffers from the limitations of high cost, ligand leaching, and caustic instability.8,9

In the last decade, several mixed mode and Protein A mimetic ligands have been developed as alternatives to Protein A chromatography. One is hydrophobic charge induction chromatography (HCIC), which uses heterocyclic ligands at high ligand densities that can get positively charged at low pH values.10 Similar to Protein A, adsorption on these resins can occur by hydrophobic interactions without high salt concentration, while elution can be controlled by lowering the pH to induce charge repulsion between the ionizable ligand and the bound protein.10,11 Protein A mimetic ligands were also developed based on the IgG binding domain of Protein A using techniques such as molecular modeling, protein engineering, phage display, and directed evolution.12-14

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play, and synthetic chemistry. Although initial studies from the resin manufacturers had shown some promise for the above-mentioned alternatives, recent and more comprehensive studies have shown that none of these resins possess the selectivity offered by Protein A chromatography.

Recently, a novel technique was developed for the rapid identification of affinity ligands against a diverse set of targets using the variable heavy-chain (VHH) region of single-chain antibodies found in the Camelidae family. These molecules possess good specificity because of their enlarged hypervariable region and have very high physical and thermal stability because of their single-domain nature. Recombinantly expressed VHH fragments can be used as affinity ligands and have applications in laboratory-scale immunoaffinity and immunoperfusion chromatography. Their potential application at industrial scale became more promising after it was proven that these antibody fragments can be expressed efficiently in microorganisms such as the yeast Saccharomyces cerevisiae.

This technology has been commercialized by the Bio Affinity Company (Naarden, The Netherlands) to generate ligands (called CaptureSelect ligands) that can be customized for any purification challenge. Unlike other proteinaceous ligands (such as Protein A), these ligands have the distinct advantage of being stable in strongly alkaline solutions. One such ligand was generated against the Fc-region of human IgGs. This was shown to bind to all human IgG subclasses and no cross-reactivity was found with bovine or mouse IgG. This ligand has the additional advantage of being specific for human IgGs only and unlike Protein A, it can bind IgG3s as well. In 2007, the ligand was immobilized on a highly cross-linked agarose-based backbone through a long, hydrophilic spacer arm and marketed through GE Healthcare (Uppsala, Sweden) as IgSelect affinity medium. Although this resin can potentially be an attractive and manufacturing-friendly alternative to Protein A chromatography, very limited data exists so far on the performance of this new resin.

This article provides the first comprehensive evaluation of this new ligand for MAb purification. Using several industrial MABS and Fc-fusion proteins, the performance of this resin was compared to two of the most commonly used commercial Protein A resins: MabSelect from GE Healthcare and ProSep-vA High Capacity from Millipore (Billerica, MA). These two Protein A resins are on two different backbones (agarose versus controlled pore glass) and were specifically chosen to represent a wide spectrum of Protein A resins. The parameters evaluated were binding affinity, dynamic binding capacity, selectivity, and binding thermodynamics. Finally, the binding mechanism of IgSelect resin also was explored by linear gradient retention experiments with different mobile-phase modifiers.

MATERIALS AND METHODS

Materials

The three resins evaluated in this study (MabSelect, IgSelect, and Prosep-vA High Capacity) were purchased from their respective vendors. All resins were packed to 20 cm bed height in 1 cm I.D. columns made by GE Healthcare. The four test proteins—Fc-fusion proteins (A and B) and MABS (C and D)—were expressed in Chinese hamster ovary (CHO) cells and produced at Bristol-Myers Squibb (Syracuse, NY). Model proteins such as horse cytochrome c and human serum albumin (HSA) samples were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were purchased from Mallinckrodt Baker (Phillipsburg, NJ).

All chromatography experiments were carried out on an AKTAexplorer chromatographic system from GE Healthcare. High Performance Liquid Chromatography (HPLC) analysis was performed using Waters (Milford, MA) 2695 Separation Module and Waters 2996 Photodiode Array Detector. An Orbital Shaker 100 from ArmaLab (Bethesda, MD) was used for batch-adsorption experiments.

Methods

The elution pH of the various proteins was obtained by linear gradient experiments under analytical conditions using pulse injection of the samples. A gradient of pH was run from 6.5 to 2.5 over 10-column volumes in citrate buffer. The elution pH at peak maxima was calculated from the gradient and further verified from the effluent pH trace obtained from the online monitor pH/C-900 that is part of the AKTA system. In the binding mechanism exploration test,
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different mobile phase modifiers were added to citrate buffers at both pH values and an identical pH gradient was run for comparison with the original pH gradient.

Dynamic binding capacities were determined by performing breakthrough experiments at six minutes residence time. The column was equilibrated and regenerated using typical Protein A process conditions. Adsorption isotherms for the proteins on the various stationary phases were determined using batch experiments.

Selectivity of the three resins was compared under preparative condition using cell culture harvest material. Each resin was loaded to ~80% of its dynamic binding capacity for the respective protein. Typical Protein A equilibration, elution, and regeneration conditions were used for these experiments. Sample protein concentration was determined using an analytical Protein A assay. Host cell protein levels in the samples from the preparative experiments were determined using an in-house host cell protein ELISA assay.

**THEORY**

A Langmuir Isotherm, as defined by the following equation, was used to compare the binding thermodynamics of the three resins in this study:

\[
Q = \frac{Q_{\text{max}}KC}{1+KC}
\]

in which \(Q\) is the equilibrium concentration of the solute on the stationary phase (expressed in mg solute per mL column volume), \(C\) is the mobile phase solute concentration at equilibrium, \(Q_{\text{max}}\) is the maximum static binding capacity, and \(K\) is the affinity binding constant. The binding constant is thermodynamically representative of the protein’s affinity to the resin.

**RESULTS AND DISCUSSIONS**

**Comparison of Binding Affinity and Elution pH**

Both the ligands evaluated in this study (Protein A and the camelid antibody) use a similar operating scheme—the protein is bound at close to neutral pH and eluted with a lower pH. At lower pH values, the electrostatic repulsion between the ligand and the bound protein helps to overcome the attractive forces and hence cause elution. The pH of elution in a way can be used as a measure of the strength of interaction with the ligand and is a useful parameter to compare the affinity of various resins. Figure 1 plots the elution pH at peak maxima under linear gradient conditions using a wide variety of proteins. These proteins were carefully chosen to test a variety of possible protein–ligand interactions and included model proteins of varying hydrophobicity (Horse cytochrome c and HSA), MAbs (molecules C and D), as well as Fc-fusion proteins (molecules A and B). In an ideal case, a resin that is selective for IgG-based molecules should only bind those and not any other non-IgG–based model protein.

As shown in Figure 1, the model proteins cytochrome c and HSA had an elution pH of around 6.5 (binding condition as described in
the experimental section) on all three resins clearly indicating that they were not retained under these conditions. This is in contrast to results shown for other Protein A alternatives such as HCIC and mimetic ligands that bound model proteins as well as Ig-based molecules. The non-binding of the model protein to the IgSelect resin (similar to what is seen for Protein A resins) is indicative of its selectivity. Moreover, the elution pHs for all the IgG-based molecules (antibodies and Fc-fusion proteins) were somewhat comparable across the three resins and fell in the pH range of 3.5–3.9. Thus, it can be said that the new camelid antibody ligand had very similar affinity to IgG molecules as a conventional Protein A ligand under the conditions tested.

Comparison of Dynamic Binding Capacity
Dynamic binding capacity (DBC) is one of the most important performance parameters for chromatography resins. This is particularly true for Protein-A–based resins, which are very expensive, and are used as a capture step in antibody purification. DBC was calculated for all four antibodies and Fc-fusion proteins using the methodology described in the experimental section. Figure 2 plots the 1% breakthrough capacity on all three resins.

From Figure 2, it can be seen that the two Fc-fusion proteins (molecules A and B) had very similar capacities on all three resins. Furthermore, the Fc-fusion proteins had a lower binding capacity than the two antibodies (C and D), particularly on MabSelect. This has been noted in the literature and has been explained by the differences in the steric hindrance exerted by Fc-fusion proteins versus MAbs. On the other hand, a difference in DBC was seen amongst the three resins for the two antibodies, molecules C and D. Both antibodies showed the highest capacity on MabSelect. The capacity of the new resin IgSelect was less than MabSelect but comparable (or even slightly higher for D) to ProSep-vA. Controlled pore glass resins such as ProSep-vA have been shown to have lower binding capacities but improved mass transport properties. The performance of the new resin was in-between the two Protein A resins with respect to DBC. The dynamic binding capacity on all these resins were compared at 6 minutes residence time. These resins might have different responses with varying residence time based on the transport properties of their backbone. However, the response of MabSelect and IgSelect would be very similar because they are based on a similar cross-linked agarose backbone.

It has been conjectured in the literature that the monomeric VHH fragments might have a lower binding capacity because they do not have the capability to bind multiple IgGs like Protein A, which has multiple binding domains. This was found to be true by our experimental results with antibodies, particularly on MabSelect and IgSelect because their backbone and ligand densities are comparable. Interestingly, the single-binding domain did not seem to have any negative effect on Fc-fusion proteins because they have a larger steric hindrance and thereby cannot optimally access the multiple binding domains of the Protein A ligand.

Comparison of Adsorption Isotherm
Figure 3 shows the adsorption isotherms of Fc-fusion protein A at pH 7.0 for all the three resins. Table 1 lists the thermodynamic parameters ($Q_{max}$ and $K$). It shows that amongst the three resins, MabSelect had the highest $Q_{max}$ value, which was consistent with DBC results. On the other hand, $Q_{max}$ for ProSep-vA was slightly lower than IgSelect even though their relative trend for dynamic
binding capacity was opposite. This can be explained by improved flow properties for the glass-bead–based ProSep-vA resin, which causes its DBC to be closer to its equilibrium capacity. Furthermore, $K$ values for all three resins were very similar, indicating that the binding strength for Protein A and this new ligand was very similar.

**Comparison of Selectivity**
As mentioned before, it has been shown in the literature that none of the small-molecule synthetic ligands developed as Protein A alternatives were able to provide the lock-and-key induced fit as Protein A.$^{24,29}$ The non-specific binding of host cell proteins to these ligands have made their selectivity much lower than that of Protein A resins. On the other hand, the VHH fragment has the capability to recognize unique conformational epitope and hence has the promise of showing greater selectivity.

To compare the selectivity of the new resin to Protein A, host cell protein clearance was compared for the four antibodies and Fc-fusion proteins using complex cell-culture fluid. The details of these preparative experiments are outlined in the experimental section. Protein recoveries for all of these experiments were comparable and >90%. The elution pools from these experiments were collected and analyzed for host cell protein (Chinese hamster ovary protein, CHOP) levels. Figure 4 plots the CHOP log reduction values (LRVs) from these experiments. The higher the LRV, the more selective is the resin. Figure 4 shows that IgSelect demonstrated comparable host cell protein clearance to the Protein A resins. In fact, for molecules A, B, and D, the LRV values for IgSelect were slightly lower or comparable to MabSelect but higher than the other Protein A resin ProSep-vA. ProSep resins are known to give slightly lower CHOP clearance than the agarose-based Protein A resins because of non-specific interactions of CHOP with their silica backbone.$^{37}$ On the whole, the selectivity shown by this new camelid antibody resin appears to be very promising.

**Exploration of Binding Mechanism**
The binding mechanism of IgGs on Protein A ligand has been studied in detail by x-ray crystallography and it has been shown that the interactions consist of hydrophobic interactions along with some hydrogen bonding and two salt bridges.$^{15,38}$ To explore the differences in the binding mechanisms of Protein A ligand and the IgSelect ligand, linear gradient experiments were conducted with different modifiers in the mobile phase. Molecule A and D (one Fc-fusion protein and one antibody) were used as the test proteins. Only one of the representative Protein A resins (MabSelect) was used for comparison.

As summarized in Table 2, the results for both molecules with no additive in mobile-phase buffer showed similar elution pH on both MabSelect and IgSelect resins, which proved the reproducibility of the data previously included in Figure 1. Change in the elution pH on addition of mobile-phase modifiers was used as an indicator of the change in the strength of binding. An increase in the elution pH would suggest a decrease in binding strength and vice versa. Ethylene glycol (20%) was used in the mobile phase to test the role of hydrophobic interactions in IgG binding on IgSelect resin because this mobile-phase modifier can reduce the effect of hydrophobic interactions. Compared to the control (i.e., with no additives), both molecules A and D had higher elution pHs on MabSelect and IgSelect resins. This indicated that 20% ethylene glycol weakened bindings on both the ligands to a similar extent.

A suppressant of surface charge interactions (300 mM NaCl) was added to the mobile phase to investigate the role of electrostatic interactions.
interaction in IgG binding on the IgSelect resin. Compared to control, both proteins had lower elution pHs on IgSelect resin on adding salt to the mobile phase. In fact, molecule D did not elute from IgSelect even at pH 2.5 under this condition. This indicated that the binding between IgGs and the new ligand was significantly increased after the addition of a medium concentration of salt. In comparison, MabSelect actually showed slightly higher elution pH for both molecules under the same conditions. These results showed that a medium concentration of NaCl affected IgG binding on the two resins in different ways and it required further investigation to determine if the stronger binding on IgSelect was because of decreased electrostatic interactions or increased hydrophobic interactions or a combination of both.

NaCl used in the previous set of experiments could have contributed to increased hydrophobic interactions while decreasing electrostatic interactions. To explore this effect further, a stronger kosmotropic salt (100 mM sodium citrate) was tested as the third mobile-phase modifier to explore the effect of increased hydrophobic interactions with minimum surface charge shielding. Compared to the data with no additives, 100 mM sodium citrate caused a significant binding increase on IgSelect resin while the effect on MabSelect resin was very small. This proved that salt played a much bigger role in the binding on IgSelect resin than MabSelect resin. When the mobile phase contains medium concentration of Kosmotropic salt or even NaCl, the binding of IgG molecules with CaptureSelect ligand can be significantly increased. In comparison, the Protein A ligand did not seem to be significantly affected by the tested salt concentration.

Gaining a fundamental understanding of the interaction mechanism on this new ligand requires additional sophisticated analytical techniques (such as x-ray crystallography and docking calculations) and was beyond the scope of this study. The results shown here demonstrate a clear difference in the way Protein A and the new ligand interact with IgGs.

**CONCLUSIONS**

As the newest alternative to Protein A chromatography, the camelid antibody ligand provided very good selectivity for IgG-based molecules. This resin showed similar binding affinity and somewhat comparable dynamic binding capacity to the commonly used commercial Protein A resins. For antibodies, it did have a lower capacity than the leading agarose-based Protein A resin; however, this shortcoming might be compensated by the vendor by further optimizing the ligand density and appropriate resin pricing. This resin does offer the distinct advantage of being base stable. Different from previously reported mixed mode and Protein A mimetic ligands, this ligand showed as good host cell protein clearance as the Protein A resins used for comparison. Although the new resin has been primarily marketed for IgG3 purification, the results presented in this study demonstrate that this can potentially be used very effectively for industrial MAb purification. Parameters that remain to be evaluated comprehensively before its widespread acceptance and use are column lifetime and viral clearance. Finally, the binding mechanism exploration studies indicated a difference in ligand–IgG interactions between the two ligands. For IgSelect, medium to high concentration of kosmotropic salt should be avoided in the background buffer to maximize antibody recovery during purification.

**ACKNOWLEDGMENTS**

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Breaking the Rapid Microbiological Method Financial Barrier: A Case Study in Return on Investment and Economic Justification

ABSTRACT
The primary reason for the pharmaceutical industry’s hesitancy to adopt rapid microbiological methods (RMMs) for environmental monitoring is a lack of understanding of how to assess the cost of implementing such methods. This article explains how to apply a financial cost model to RMMs and presents a case study of three manufacturing facilities of different sizes. For the three facilities evaluated, the year 1 return on investment for implementing an RMM ranged from 183 to 365%, and the payback period ranged from 3.3 to 6.6 months.
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cient to substantiate the time and expense involved in qualifying and installing RMMs in their facilities.

There are obvious costs involved with the purchase, qualification, and implementation of RMMs. Depending on the capital expense and the process required to adequately validate a system for its intended use, the costs associated with implementing an RMM can be significant. However, it is important to fully understand all of the financial components that should go into an economic analysis before a decision is made about whether to proceed with a formal qualification program. These components may include the costs associated with the existing method, the costs associated with the initial capital investment of the new method, and the long-term financial benefits (cost savings and avoidances) that the RMM may provide. Dollar amounts for each of these components can then be used to develop a comprehensive economic analysis and business case for introducing the new method.

The three key steps for considering the implementation of an RMM are as follows:

1. Review existing conventional methods and recognize potential technology, quality, and business opportunities for implementing an RMM.
2. Understand the technical and business benefits of RMMs.
3. Develop a business case for RMMs.

Below, we explain these steps, and provide a case study to illustrate them.

| Table 1. Annual operating costs (in US dollars) for conducting environmental monitoring using a conventional method (CM) and a rapid microbial method (IMD-A) for three parenteral manufacturing facilities of different sizes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Facility 1      | Facility 2      | Facility 3      |
| CM              | IMD-A Year 1    | IMD-A Year 2+   | CM              | IMD-A Year 1    | IMD-A Year 2+   |
| Number of tests per year¹ | 40,000          | 8,000           | 8,000           | 70,000          | 14,000          | 14,000          |
| Cost per test (consumables, reagents, media) | 1               | 0               | 0               | 1               | 0               | 0               |
| Calculated annual cost for testing | 40,000          | 0               | 0               | 70,000          | 0               | 0               |
| Total sampling, testing, data handling, and documentation resource time per test (hours) | 1               | 0.1             | 0.1             | 1               | 0.1             | 0.1             |
| Cost of labor (local currency per hour) | 50              | 50              | 50              | 50              | 50              | 50              |
| Calculated annual labor | 2,000,000       | 40,000          | 40,000          | 3,500,000       | 70,000          | 70,000          |
| Cost to dispose of used media and reagents per test | 0.5             | 0               | 0               | 0.5             | 0               | 0               |
| Calculated annual disposal costs | 20,000          | 0               | 0               | 35,000          | 0               | 0               |
| Annual cost associated with laboratory equipment depreciation, calibration, qualification, space² | 50,000          | 135,000         | 135,000         | 50,000          | 180,000         | 180,000         |
| Annual maintenance and service contracts³ | 20,000          | 0               | 162,000         | 20,000          | 0               | 216,000         |
| Total annual costs | 2,130,000       | 175,000         | 337,000         | 3,675,000       | 250,000         | 466,000         |

¹ Because the IMD-A operates continuously, in this example we assume that the actual number of tests performed can be reduced by a factor of 5 compared with the CM.
² Depreciation for IMD-A equals 10% of the capital cost (which assuming unit cost of $90,000 each; pricing used is representative and is for calculation purposes only, as the supplier may vary the price based on configuration and quantities purchased). Assumes 15 units for facility 1; 20 units for facility 2; and 48 units for facility 3. ³ Annual maintenance and service contracts start in year 2 and are based on geographic region and services contracted. Pricing assumed equals 12% of capital cost (15 units for facility 1; 20 units for facility 2; and 48 units for facility 3; at $90,000 each).
**REVIEW CONVENTIONAL METHODS AND RECOGNIZE OPPORTUNITIES FOR IMPLEMENTING AN RMM**

Manual sampling and resource-intensive operating procedures are responsible for much of the cost associated with conventional, growth-based microbiology methods. In addition, the time to result when turbidity is visually detected (in liquid media) or colony-forming units are enumerated (on agar plates) can be quite long. Furthermore, microorganisms that are stressed, damaged, or in a viable but nonculturable (VBNC) state may or may not replicate when cultured on artificial media. In the event of a positive result or an out-of-specification finding, which could occur anywhere from a few days to more than two weeks after the original sample was analyzed, the opportunity to respond to the excursion in a timely manner has been lost. In addition, the effect of an environmental monitoring (EM) excursion on the manufacturing process could be significant, including holding product, rejecting a batch, or shutting down a manufacturing line. In contrast, obtaining these results faster, or even in real time, through RMM, would allow corrective action to be taken quickly, thus reducing the number of lot rejections and plant shutdown time for EM investigations. For these reasons, companies should explore the use of RMMs that may decrease the overall costs associated with conducting microbiology testing while at the same time continuously improving the manufacturing process, finished product quality, and operational efficiency.

**UNDERSTAND THE TECHNICAL AND BUSINESS BENEFITS OF RMMS**

When selecting an RMM, it is important to understand the technical and business benefits of implementing the new technology for its intended purpose. Technical benefits may include a significantly

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<table>
<thead>
<tr>
<th>Facility 1</th>
<th>Facility 2</th>
<th>Facility 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduction in lot rejection as a result of being able to segregate product during the detection of an actual EM excursion in real time</strong>¹</td>
<td>300,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td><strong>Reduction in the loss of additional product being made due to manufacturing down time during an EM investigation</strong>²</td>
<td>300,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td><strong>Reduction in investigation cycle time, laboratory resources, and testing during investigations of an EM excursion</strong></td>
<td>30,000</td>
<td>30,000</td>
</tr>
<tr>
<td><strong>Reduction in operator manufacturing downtime during investigations of an EM excursion</strong></td>
<td>20,000</td>
<td>20,000</td>
</tr>
<tr>
<td><strong>Reduction in deviations</strong></td>
<td>40,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td><strong>Increased product yield due to lengthening campaign time in isolators</strong></td>
<td>n/a</td>
<td>400,000</td>
</tr>
<tr>
<td><strong>Reduction in isolator glove integrity testing</strong></td>
<td>n/a</td>
<td>40,000</td>
</tr>
<tr>
<td><strong>Reduction in rework, reprocessing, and repeat testing</strong></td>
<td>50,000</td>
<td>50,000</td>
</tr>
<tr>
<td><strong>Total annual savings resulting from the use of the IMD-A</strong></td>
<td>740,000</td>
<td>3,540,000</td>
</tr>
</tbody>
</table>

¹ For facilities 1 and 2, the model assumes the loss of one batch per year, valued at $300K for facility 1, and $1M for facility 2. For facility 3, the model assumes the loss of three batches per year, valued at $500K each.

² For facilities 1 and 2, the model assumes one batch per year not being made, valued at $300K for facility 1, and $1M for facility 2. For facility 3, the model assumes three batches per year not being made, valued at $500K each.
The operating costs of the RMM are 5 to 8 times lower than the costs of the conventional method.

faster time to result (or even getting results in real-time); greater accuracy, precision, sensitivity, and reproducibility; single-cell detection; enhanced detection of stressed and VBNC organisms; increased sample throughput and automation; continuous sampling; and enhanced data handling and trend analysis. For RMMs that can provide results in real-time, the response time to an adverse trend or excursion can be immediate, which is not possible for conventional growth-based procedures. Implementing RMMs also may provide a company with numerous business benefits, including, but not limited to, reduced time and costs for product release testing; a reduction or elimination of off-line assays, laboratory overhead, resources, and equipment; a lower cost of product sold; decreased resampling, retests, and deviations; reductions in rework, reprocessing, and lot rejections; and a reduction in plant downtime.

DEVELOP A BUSINESS CASE FOR RMMs

Once a review of available RMM technologies has been completed, and when one or more technologies are identified that meet the technical and business benefits for the intended application, a business case for implementing the new method should be developed. This will include the use of financial models that can compare the overall costs associated with the current microbiology method with the costs and savings associated with the purchase, qualification, and implementation of the RMM. The goal is to obtain the necessary economic justification that manufacturing site heads will need to initiate the RMM project. Listed below are examples of the types of operating costs and potential savings that can be incorporated into a financial model.

Potential operating costs associated with a conventional growth-based method:
- cost of consumables, regents, and supplies
- total sampling, preparation, testing, data handling, and documentation resource time
- cost of labor (salary and benefits)
- media, reagents, and consumables disposal costs
- laboratory equipment depreciation, calibration, and qualification
- overhead for laboratory and storage space
- data management and record retention
- preventive maintenance and service contracts

Potential investment and operating costs associated with an RMM:
The same as for conventional methods, plus:
- capital costs during the initial investment
- technology and software training
- system qualification and method validation costs
- regulatory filing costs, if applicable (changes to in-process microbiological methods or methods that are not specified in an NDA or marketing authorization may not require a formal regulatory submission to implement the change)

Potential cost savings and cost avoidances associated with an RMM:
- reduced testing and finished product release cycle times
- reduction or elimination of laboratory equipment and overhead
- lower headcount
- reduced repeat testing and investigations,
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lot rejection, reprocessing, and rework because EM excursions can be detected and acted on more quickly
• reduction in plant downtime
• increased yields and lower cost of product sold
• reduced raw material, in-process, and finished goods inventory holdings

Each of the identified operating costs and cost savings should have an associated monetary value that can be added up and entered into an appropriate financial model. Two financial tools that can be used to calculate whether there is a financial advantage for implementing an RMM include the ROI and the payback period models. An overview of each model is provided below.

Return on Investment (ROI)
ROI is the ratio of money gained or lost on an investment relative to the amount of money invested. For RMMs, the cost of performing the conventional method (CM) is compared with the cost (and savings) of using the new method. The information is reported as a percentage and usually represents an annual or annualized rate of return. The ROI is calculated using the following formula:

\[
ROI = \frac{\left( \sum \text{Costs}_{\text{CM}} - \sum \text{Costs}_{\text{RMM}} - \sum \text{Savings}_{\text{RMM}} \right)}{\text{RMM Investment}}
\]

Payback Period (PP)
The PP is the time required for the return on an investment to “repay” the sum of the original investment. In the context of implementing an RMM, this would be the time (usually in years) required to realize sufficient cost savings to pay for the initial investment of the RMM capital equipment as well as for qualification and implementation activities. The formula used to calculate the PP is the inverse of the ROI formula:

\[
PP = \frac{\text{RMM Investment}}{\left( \sum \text{Costs}_{\text{CM}} - \sum \text{Costs}_{\text{RMM}} - \sum \text{Savings}_{\text{RMM}} \right)}
\]

A CASE STUDY IN THE USE OF ROI AND PP CALCULATIONS FOR JUSTIFYING AN RMM
A pharmaceutical company operates three parental manufacturing facilities using isolators and conventional cleanrooms. The company is exploring ways to implement an RMM that will provide real-time, in-process monitoring capabilities while realizing significant cost savings. After a review of current microbiology methods, the company identified active air monitoring as its most expensive and time-consuming activity, and identified the
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BioVigilant IMD-A (Tucson, AZ) as a potential replacement for its existing agar-based air sampling procedure. The company initially assumed that it could realize significant cost savings with this particular RMM because the system eliminates manual sampling and laboratory testing, has no consumables, reagents, media, or supplies, and can run continuously during the entire manufacturing campaign.

The company was also interested in the potential technical benefits of the system, because results are obtained in real-time for both viable and nonviable particles. The company gathered monetary values for each operating cost and cost savings component for use in its ROI and PP calculations, and applied these calculations to the three manufacturing facilities. A brief description of each facility and its EM program for active air samples is provided below, followed by a detailed overview of each financial component.

1. A small fill–finish facility that processes 40,000 active air samples per year. Manufacturing is performed in conventional cleanrooms. This site rejects one $300K product lot per year because of EM excursions on conventional media and shuts down the line to conduct an EM investigation.

2. A medium-size fill–finish facility that processes 70,000 active air samples per year. Manufacturing is performed in isolators. The site rejects one $1M product lot per year because of EM excursions on conventional media and shuts down the line to conduct an EM investigation.

3. A large fill–finish facility that processes

<table>
<thead>
<tr>
<th>Facility number</th>
<th>First year ROI (%)</th>
<th>First year cost savings ($)</th>
<th>Second and subsequent year ROI (%)</th>
<th>Second and subsequent year cost savings ($)</th>
<th>Total 5-year ROI (%)</th>
<th>Total 5-year cost savings ($)</th>
<th>Payback period (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>185</td>
<td>1,235,000</td>
<td>50,660%</td>
<td>$2,528,000</td>
<td>879%</td>
<td>$11,347,000</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>365</td>
<td>5,055,000</td>
<td>134,980%</td>
<td>$6,744,000</td>
<td>177%</td>
<td>$32,031,000</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>183</td>
<td>3,668,000</td>
<td>151,592%</td>
<td>$7,574,600</td>
<td>867%</td>
<td>$33,966,400</td>
<td>6.6</td>
</tr>
</tbody>
</table>
100,000 active air samples per year. Manufacturing is performed in conventional cleanrooms and the process is personnel intensive, resulting in the rejection of three $500K product lots per year because of EM excursions on conventional media. This site also shuts down a line three times per year to conduct EM investigations.

Financial components for operating costs, cost savings, and investments

For each of the three facilities, all of the financial components that needed to go into the ROI and PP models were identified and tabulated. Annual operating costs for both the conventional method and RMM are provided in Table 1. The first year operating costs for the RMM are 10 to 15 times lower than the operating costs for the conventional method because of the significantly greater amount of labor required for the conventional method. In the second and subsequent years, the difference in operating costs is slightly reduced because maintenance and service contracts will be required for the RMM. However, the overall operating costs for the RMM are still 5 to 8 times lower than the operating costs for the conventional method.

Table 2 outlines the annual cost savings when using the RMM instead of the conventional method for environmental monitoring. By reducing lot rejections and plant downtime resulting from EM excursions, annual savings amount to $740,000, $3.5 million, and $3.4 million for facilities 1, 2, and 3, respectively.

The annual RMM investment costs are detailed in Table 3. In the first year of implementation, the majority of these costs include capital equipment purchases and qualification, and range from $1.4 million to $4.4 million for the three facilities. In the second and subsequent years, the total cost is $5,000 for each facility, and this represents additional annual training for new operators.

Return on Investment and Payback Period Calculations

Using the formulas provided above, the company calculated the ROI and the cost savings for each of the three facilities for the first year, the second year, and subsequent years, and the total ROI and cost savings for five years. The payback period PP was also calculated. A summary of the results is provided in Table 4. As shown in the table, the first year ROI ranges from 183 to 365%, and the five-year ROI ranges from 867 to 1778%. The payback period ranges from 3.3 to 6.6 months.

SUMMARY

The ROI and PP data generated for each facility in the case study above clearly demonstrates sufficient economic justification for initiating a qualification and implementation plan using the chosen RMM as an alternative to conventional active air sampling. In addition, the payback period is relatively short because of substantial cost savings during the first year of implementation. From a business perspective, the use of this RMM would directly improve the company’s bottom line and should satisfy the financial expectations of site management. Furthermore, the models presented in this manuscript may be used to develop an economic assessment of any RMM implementation proposal. A robust financial assessment, coupled with a comprehensive validation plan, is the key to a successful RMM implementation strategy and the continuous improvement of manufacturing processes and efficiencies.

REFERENCES

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29: Downstream Technology Forum  
Location: Forrestal, NJ  

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1: Downstream Technology Forum  
Location: Berkeley, CA  

5–7: BioNetwork West  
Location: Laguna Nigel, CA  
[www.wbresearch.com/bionetworkwest](http://www.wbresearch.com/bionetworkwest)

13–15: BioPh: Bio-Solutions for Pharma  
Location: Madrid, Spain  
[www.bioph-online.com](http://www.bioph-online.com)

14: Downstream Technology Forum  
Location: Seattle, WA

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2–4: BIO-Europe 2009 15th Annual International Partnering Conference  
Location: Vienna, Austria  
[www.ebdgroup.com/bioeurope](http://www.ebdgroup.com/bioeurope)

8–12: AAPS Annual Meeting and Exposition  
Location: Los Angeles, CA  
[www.aapspharmaceutica.com](http://www.aapspharmaceutica.com)

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11–15: CHI PepTalk 2010  
Location: San Diego, CA  
[www.chi-peptalk.com](http://www.chi-peptalk.com)

31–February 4: IFPAC 2010  
Location: Baltimore, MD  
[www.ifpac2010.org](http://www.ifpac2010.org)

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The isolation and sequencing of a human gene that encodes a particular domain of a protein is the basis for many inventions in biotechnology. In a recent decision, however, the US Court of Appeals for the Federal Circuit held that claims to a specific DNA sequence were obvious and that it was “obvious to try” known methods to obtain the sequence.

In the case, In re Kubin, the invention focuses on DNA molecules encoding a protein known as the natural killer cell activation inducing ligand (NAIL). The patent specification discloses an amino acid sequence of a NAIL polypeptide, the isolation and sequencing of a polynucleotide that encodes a NAIL polypeptide, and the binding relationship between NAIL and a protein known as CD48.

The Board of Patent Appeals and Interferences rejected the claims over the combined prior art teachings of a patent and a laboratory manual. The prior art patent discloses a receptor protein called p38 that is the same protein as NAIL and further discloses, “[t]he DNA and protein sequences for the receptor p38 may be obtained by resort to conventional methodologies known to one of skill in the art.” The Board concluded that one of ordinary skill in the art would have been motivated by the prior art to make a cDNA encoding the protein at issue given the prior art’s teaching that the protein played a role in the immune response. The Court further sided with the Board that there would have been a reasonable expectation of success given the prior art’s teaching of how to use a monoclonal antibody specific to a protein to clone the corresponding gene.

The Court rejected Kubin’s argument that the prior art did not teach the claim’s requirement that the “polypeptide binds CD48.” The Federal Circuit effectively overruled, at least in part, its 1995 holding of In re Deuel, that claims to a genus of polynucleotide sequences coding for a known protein are not necessarily obvious. The Court concluded that “[i]nsofar as Deuel implies the obviousness inquiry cannot consider that the combination of the claim’s constituent elements was ‘obvious to try,’ the Supreme Court in KSR unambiguously discredited that holding.”

This decision, along with In re Gleave, rendered just a week earlier, represents an ongoing trend that makes patenting classic biotechnology inventions more challenging. In Gleave, the Court held that a comprehensive reference listing of every relevant sense oligonucleotide in a known nucleic acid sequence anticipates claims to specific antisense sequences. In the opinion’s dictum, the Court appears to suggest that the patentability of claims to specific antisense sequences, without more, may be precluded for any known gene. More than ever, successful patenting strategies require careful planning and taking into account nuanced legal standards to ensure creation of a strong specification and prosecution record.

Successful patenting strategies must take into account nuanced legal standards.
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