Raw Material Control Strategies for Bioprocesses

by Gregory Beck, Mark Schenerman, John Dougherty, Ruth Cordoba-Rodriguez, Christopher Joneckis, Anthony Mire-Sluis, and Lorna D. McLeod

The 15th WCBP CMC Strategy Forum, "Raw Material Control Strategies for Bioprocesses," met on Sunday, 11 January 2009 in San Francisco, CA. This forum considered the design and implementation of control strategies for complex raw materials used in bioprocessing. Discussion focused on key approaches and application of risk assessment tools that can be used to identify and assist in mitigating potential safety and efficacy concerns that can affect the quality of biological products.

To fully explore the topic, the forum first focused on recognizing and assessing potential risks associated with raw materials (RMs) and then applied those rationales to actual case studies with audience participation. The morning session began with an introduction to the topic followed by five presentations. Areas of discussion included how expectations for raw material control are evolving within changing regulatory and business paradigms including quality by design (QbD), counterfeiting, complex supply chains, and sourcing changes. Presenters discussed risk assessment and mitigation strategies along with supplier risk management plans.

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During the afternoon, two case studies were presented, and forum participants were polled for their assessment of the level of risk pertaining to different scenarios. For the first time, the forum used an electronic audience response system, which made it possible to tally participant responses to a variety of questions and to compare and evaluate those responses in real time. (Responses are detailed in the "Audience Participation" box.)

After an introduction to the topic of raw materials by organizers John Dougherty (Eli Lilly) and Mark Schenerman (MedImmune), a series of presentations filled the morning schedule. They highlighted different aspects and issues involved in raw materials for use in biologic applications. Ruth Cordoba-Rodriguez of the FDA's Center for Drug Evaluation and Research (CDER in Bethesda, MD) spoke on "Raw Materials in the Manufacture of Biotechnology Products: Regulatory Considerations." A vendor perspective was presented by Kathy Carroll and Denise DeTommaso of SAFC Biosciences (Lenexa, KS) in "Raw Material: A Supplier's Perspective." "Implementing Risk Assessment Tools for Identifying Critical Raw Material Attributes" was presented by Gregory Beck of Eli Lilly (Indianapolis, IN) and followed by Eric Berg of Amgen (Thousand Oaks, CA) with "Supplier Lifecycle

PRODUCT FOCUS: ALL BIOLOGICS

PROCESS FOCUS: UPSTREAM AND DOWNSTREAM PROCESS DEVELOPMENT

WHO SHOULD READ: PRODUCT AND PROCESS DEVELOPMENT SCIENTISTS, ANALYTICAL, MANUFACTURING, QA/QC

KEYWORDS: INSULIN, PROTEIN A, QUALITY BY DESIGN, RISK MANAGEMENT

LEVEL: INTERMEDIATE

BPI EXTRA: CASE STUDY AUDIENCE PARTICIPATION

For the first time, the forum used an electronic audience response system, which made it possible to tally participant responses to a variety of questions and compare and evaluate those responses in real time. Questions were posed by the panel and displayed on screen. Participants had several seconds to respond to each using hand-held voting devices provided by Meridia Audience Response (www.meridiaars.com). Find the case study questions and responses online at www.bioprocessintl.com/bpiextra.

Management: Ensuring Raw Material Authenticity and Quality." Finally, Arifa Khan from the FDA's Center for Biologics Evaluation and Research (CBER, Bethesda, MD) presented "CBER's Perspective on Regulating Raw Materials in Biologics." The presenters were then joined by Keith Webber, CDER, as panel members, with Mark Schenerman facilitating subsequent discussion and development of a risk-assessment tool for raw materials.

Regulatory Considerations

The presenters pointed out the lack of a consistent definition of raw materials in regulations pertaining to the pharmaceutical industry. In its Q7 guideline, the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) defines raw materials as "starting materials, reagents, and solvents intended for use in the production of intermediates or APIs." However, the term as defined by different speakers could cover a wide range of materials including the following:

- starting or source materials (cell lines, viral or bacterial stocks, media components, chemicals, tissues, serum, water)
- in-process materials (resins, buffers, filters, column housings, tubing, reagents)
- excipients
- packaging components, both primary and secondary (syringes, vials, stoppers, plungers, crimps, boxes, trays, and labels)
- device/delivery components (pen/injector components, IV bags, filters).

Some regulations directly consider the control of raw materials, but they are not comprehensive and are scattered among the US *Code of Federal Regulations* (CFR), ICH, and other regulations/guidances. Although the regulations are not extensive, the need to control raw materials was clear from all presenters and is implicit in the sources cited below:

- 21 CFR 610.15 regarding constituents
- 21 CFR 211.80 regarding components and containers/closures
- 21 CFR 211.110 regarding control of in-process materials
- ICH Q5A/D for cell substrates and viral safety
- ICH Q7 discussing the need to control materials with appropriate specifications
- ICH Q10 stating that a biomanufacturer is responsible for the quality of purchased materials
- the US bill "Country-of-Origin Labeling for Pharmaceutical Ingredients," proposed in September 2008
- QbD principles requiring an understanding of the criticality of quality attributes for raw materials and their effect on processes and products.

Developing Control Strategies

Control of raw materials is essential to maintaining safety. Thorough knowledge of raw materials can mitigate the potential for contamination derived from such sources as microbes, chemicals, prions, and pyrogens. Raw material control for safety also includes identification — being able to verify that you have received the correct material — because the presence of an incorrectly identified material in a manufacturing process could compromise safety.

Control of raw materials is essential to ensure lot-to-lot consistency because variation in them can directly affect the variation of both product and process. So manufacturers must understand the critical material attributes (CMAs) of their raw materials and which of those affect variability — as well as how to control that variability. You must show that you are using appropriate analytical methods to characterize raw materials. Raw materials such as polyethylene glycol (PEG) isomers, trace materials in media and water, container and closure materials, and chromatography resins all have the potential to affect lot-to-lot consistency.

An effective raw material control program will also ensure consistent supplies. A single source for a vital raw material can be a significant financial and quality-assurance risk. If a supplier goes out of business or experiences quality problems, can that raw material be obtained elsewhere? Has a second source been qualified in case the primary source is no longer available? Does the second source have the capacity to meet your needs?

A QbD approach to raw material control requires that you understand the impact on your product's critical

The CMC Strategy Forum Series

The purpose of the CMC Strategy Forum series is to provide a venue for biotechnology/biological product discussion. These meetings focus on relevant chemistry, manufacturing, and controls (CMC) issues throughout the lifecycle of such products and thereby foster collaborative technical and regulatory interaction. The forum committee strives to share information with regulatory agencies to assist them in merging good scientific and regulatory practices. Outcomes of the forum meetings are published in this peer-reviewed journal with the hope that they will help assure that biopharmaceutical products manufactured in a regulated environment will continue to be safe and efficacious. The CMC Strategy Forum is organized by CASSS, an International Separation Science Society (formerly the California Separation Science Society), and is cosponsored by the US Food and Drug Administration (FDA).

Forum Cochairs: John Dougherty (Eli Lilly and Company), Mark Schenerman (MedImmune)

Program Cochairs: Gregory Beck (Eli Lilly and Company), Ruth Cordoba-Rodriguez (CDER, FDA), Christopher Joneckis (CBER, FDA)

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Elements of Raw Material Risk Assessments

Is the raw material biological, chemical, or physical (such as tubing or stoppers, materials that are not actual components of the end product)?

How likely is the raw material to introduce biological or chemical contamination?

Is the raw material or are its degradants able to directly affect the safety and/or efficacy of a drug substance (e.g., toxicity, chemical modifications)?

How complex is the raw material itself or its impurity profile?

How much prior knowledge (e.g., historical or published knowledge, current experience) do you have regarding the raw material?

What is the Intended use of the raw material (e.g., as a buffer, reagent, or excipient)?

Where in the manufacturing process will this raw material will be used (upstream/downstream)?

What is the extent of supply chain traceability (considering country of origin, supply chain complexity, and supply chain security)?

What is the extent of supplier quality assurance (from audits, monitoring, historical experience)?

How extensive is the characterization of the raw material (how well can the raw material be characterized; standard existing methods or novel techniques; the RM's impact on test methods)?

How stable is the raw material?

Is the raw material new to the process or a result of a change to an existing raw material (if a change, what studies have been executed to assure comparability)?

What is the depth of knowledge of the RM's own manufacturing process to assess the risk associated with its use (e.g., process contaminants)?

Does the use of the raw material in a manufacturing environment present safety and/or handling risks?

Does your process have the ability to clear the raw material?

Are there associated business risks (e.g., a solesource or multiple-source material, unique or not to the pharmaceutical industry, custom-made or not, and the supplier's ability to consistently meet specific requirements)?

What is your level of understanding of the raw material CMAs?

quality attributes. You will need to show that you understand the effect of raw material variability on your product as well as on your manufacturing process. Use of multiple lots during development can provide data on raw material lot-to-lot variability and its related effects on process and product. When that is not feasible, a manufacturer may consider including different lots of raw materials during bench-scale studies. In addition to the raw materials themselves, you should gain an understanding of whether and how raw material degradants might affect your process or product. A QbD approach can use relevant knowledge to help you define how to go about setting specifications, in-process controls (IPCs), and handling conditions.

Testing of Raw Materials

The forum discussed what levels of testing are important for specific raw materials. A supplier's certificate of analysis (CoA) is never sufficient for raw materials because good manufacturing practices (GMPs) require appropriate testing, and at a minimum, testing for identity. The material ordered may include additives, preservatives, degradation products, or contaminants. You must verify that the CoA is appropriate for control of the raw material, but you can't assume that at the outset. Similarly, CoA verification may be necessary only once a year once your experience with a given supplier has shown that quality is consistent.

Vendor qualification is an important factor in defining your testing needs. To ensure the quality of raw materials against adulteration, identity testing is essential. Currently, tests with fingerprint techniques — e.g., nuclear magnetic resonance (NMR) imaging and Raman, near-infrared (NIR), and Fourier-transform infrared (FTIR) spectroscopy — are used to assure the identity and quality of raw materials. Whatever techniques you use, it is important to retain samples for future investigations. Photographic libraries of materials and their packaging have also proven useful for identifying and preventing use of counterfeit products.

How often and in how much depth you need to verify a CoA through independent testing is an important consideration, especially for environments in which counterfeiting or contamination can occur. Once you understand the CMAs of your raw materials, you need to identify which tests are relevant for testing specific quality attributes (QAs) of those raw materials. Sampling plans need careful consideration and should be risk based, dependent on the nature and use of the RM, and any regulatory requirements. Such plans should always be justified in a report available for inspection and/or filing. It is important to consider RM stability and whether any special tests for degradants are needed for release of the material over time. A stability profile will dictate the purchasing program (storage of large quantities or buying as needed) as well as affect the associated testing strategy.

Supply Quality Management: Ensuring Quality and Availability

It is becoming increasingly evident in the current supply chain environment that management of suppliers and the "cold chain" is essential to assuring the quality of raw materials. How often and how thoroughly you perform vendor audits depends on your experience with a given vendor. A manufacturer's general experience with a vendor (prior knowledge) is an important criterion used to evaluate that vendor's suitability to supply raw materials. Items to consider when selecting a vendor include its quality systems and its solvency, as well as its length of time in business, its geographic area, and whether it supplies multiple industries or just one or two drug manufacturers. Those form part of a risk assessment relating to suppliers to be described in more detail below. Ensuring both the availability and qualification of secondary suppliers is important as well. Practices such as split purchasing may help ensure that you have good working relationships with multiple vendors.

Strict change control sections should be included in supplier agreements and should include details

requiring a vendor to notify you of changes in its product or suppliers. Such agreements should also provide for impact assessments from both supplier and manufacturer in the event that a supplier makes any changes.

Supply chain traceability is not as straightforward as it might seem. Although most manufacturers use country-of-origin (COO) questionnaires, those often prove less than ideal in revealing what you need to know. It is critical to craft questions that get the in-depth answers you need. For example, rather than asking "Do you purchase supplies from any high-risk countries?" you might ask "From what countries do you purchase supplies?" If the specified countries include any you consider to be high risk, you can follow up or choose another supplier. It is critical to use risk-assessment techniques for determining traceability to avoid a false sense of security that can lead to costly or even deadly errors.

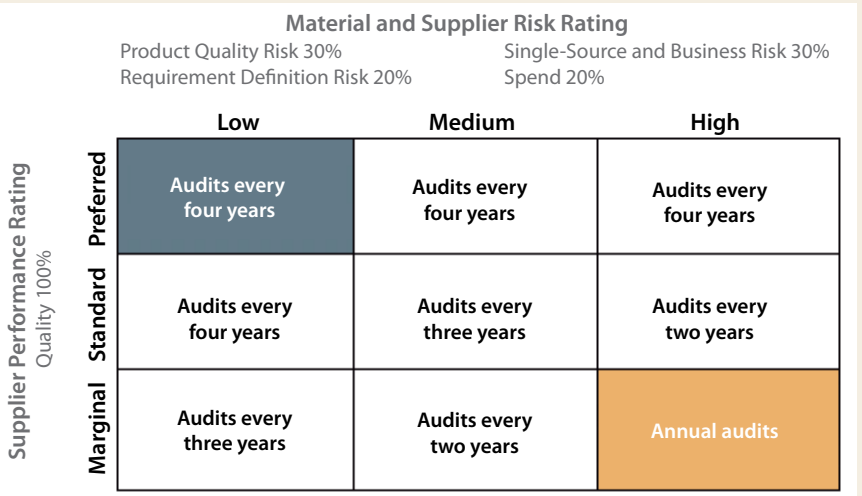
It is sometimes unclear exactly what roles are played by whom in a supply chain. Which companies are manufacturers, which are distributors, and which are intermediaries is not necessarily clear. A company that simply repackages a raw material from 55-gallon drums into smaller containers may consider itself a manufacturer. Due diligence will help ensure that you really know where your raw materials originated.

As part of assessing supply chain complexity, forum participants were informed of a proposed program whereby industry creates a system of cooperative audits in which vendors would be audited by a selected team representing all industry rather than multiple auditors from each company continuously auditing suppliers. The resulting audits would lead to certification that would assure all purchasers that each vendor meets certain defined criteria. Such a "360° Rx" program would enable increased depth of supplier audits and save manufacturers time and money (see box, right).

The Role of Compendial Standards: Compendia provide some assurance of minimum quality standards for specified materials. However, compendial standards may differ among the pharmacopoeias. Few of the complex raw materials (e.g., culture media, soy, yeastolates, most growth factors) used in biotechnology manufacturing are compendial, and those that are (e.g., insulin) may not have the appropriate compendial limits on specific quality attributes — or even test for quality attributes necessary to control pharmaceutical manufacturing.

Even for standard chemical raw materials (e.g., trace metals), compendial standards may not focus on quality attributes relevant for biotechnology process and product quality assurance. Those may be product- and/or process-specific. Furthermore, compendial standards do not necessarily help control for contamination, counterfeiting, or supply chain issues because a supplier can simply state it meets compendia — a statement that currently requires no certification.

Figure 1: Nine-block and example (risk factors and supplier performance drive audit frequency)
ERIC BERG, AMGEN INC.



Risk Management

Risk assessments are an important tool for ensuring the safety, efficacy, consistency, and supply of pharmaceutical products. Many companies in both the United States and the European Union are using ICH Q9 as a basis for risk assessment, control, communication, and future review.

Risk assessments should begin by identifying all raw materials and

Toward Global Quality and Regulatory Systems

On 5 June 2009, more than 125 global attendees representing some 70 organizations from the pharmaceutical, biotechnology, and generic drug manufacturing industries (along with their suppliers, professional trade associations, and regulatory agencies) came together in Washington, DC to officially launch Rx-360. This organization was incorporated in Pennsylvania as a nonprofit corporation under the name "Rx-360 International Pharmaceutical Supply Chain Consortium." Its mission is to develop and implement enhanced global quality systems and processes and help its members ensure product quality and authenticity throughout their supply chains.

Attendees explored the global impact of counterfeit drugs and the legal considerations and challenges of creating a consortium like Rx-360. In an overwhelming show of support, 100% of participants said they thought there was a need for such an organization. Most also indicated that of the four Rx-360 functions — shared supplier audits; adopting standards and best practices; political, macroeconomic monitoring and clearinghouse for suspicious events; and technology development — they were most interested in shared audits.

For more information on the consortium, details on the meeting presentations and polls conducted, and membership opportunities, go to www.Rx-360.org.

Case Study #1

Facilitator: John Dougherty (Eli Lilly and Company)

Panel: Sanjeev Ahuja (MedImmune), Gregory Beck (Eli Lilly and Company), Brigitte Brake (BfArM), Chana Fuchs (CDER, FDA), Kowid Ho (AFSSAPS), Christopher Joneckis (CBER, FDA), David Kolwyck (SAFC Biosciences), Mike Scott (MedImmune), Anthony Ridgway (Health Canada), Ron Taticek (Genentech, Inc.)

Summary by Anthony Mire-Sluis (Amgen, Inc.)

assessing their criticality to product safety, efficacy, and supply. RM risk assessments require cross-functional input from all departments including supply, product development, manufacturing, quality control, quality assurance, clinical, and any other contributors. It was clear from this forum's discussions that risk assessments are only as good as the people who carry them out. Having the right expertise over a spectrum of areas is vital if any risk assessment is to be meaningful.

Multiple risk assessment tools exist, but in general, a good risk assessment must address concepts such as impact/severity and likelihood/detectability. One tool discussed at the forum (Figure 1) used nine blocks to score a supplier's performance against material risk levels for audits, supplier qualification, supplier monitoring, change control, material specifications and testing, quality agreements, supplier certification, and sourcing, or other appropriate combinations of factors.

Risk assessment should also be performed in relation to country of origin. It is critical to be able to trace your raw materials to their source. Just as a biopharmaceutical manufacturer audits its suppliers, those suppliers must also know, audit, and qualify their own distributors. It is now well known that there are high-risk geographic areas where additional caution should be exercised to assure purity and identity of sourced materials. A potentially overlooked risk assessment issue is that manufacturers need to evaluate their raw materials and products in relation to opportunities for someone to make a profit through adulteration (e.g., by diluting a product to increase volume, and thus sales income). Any materials identified in such an evaluation should be managed with particular caution.

Risk assessments ensure that appropriate control strategies and raw materials (e.g., grade, origin) have been selected, which is relevant to a QbD approach. For regulatory filings, acceptable specifications, raw materials, and control strategies are tested with the necessary acceptance criteriia to ensure the performance of a process and the quality of its ultimate products.

A periodic risk review should include more than a mere cursory review of individual risk assessments. It should reevaluate the risk program itself based on experience and lessons learned. Your risk assessment should be phase-appropriate, and as such it will change as data become available throughout development. Early on, your raw materials risk assessment can be based on platform and previous knowledge, on the quality assurance of your suppliers, and adventitious agent introduction. As a manufacturing process develops, you will need to reevaluate that risk assessment including commercial considerations of scale and production frequency, high-risk raw materials control strategy, and handling and storage requirements.

During commercialization, design of experiments (DoEs) and collated knowledge will further define the CQAs of both product and RMs as well as potential and actual interactions among RMs, process, and product. At that point, you will be able to define and justify the raw materials for your commercial process and refine their specifications. By the time your product is ready for market launch, you will have updated the failure modes and effects analysis (FMEA), completed your raw materials specifications, set your sourcing strategy, put in place your supplier qualification program, defined your raw material control strategy, and made your risk assessment ready for filing.

The morning's session resulted in a list of elements to be included in a raw materials risk assessment (as listed in the "Risk Assessments" box).

Developing a Risk-Assessment Tool

One of the important lessons learned in the afternoon session of this 15th WCBP CMC Strategy Forum was the difficulty of coming up with the right questions to use in a risk assessment. Many questions had to be reworded (some several times) before panel and audience members could agree that they could elicit meaningful responses. Some questions in the "Audience Participation" boxes (in the online version of this article at www.bioprocessintl.com) were even asked twice, obtaining different results. In such cases, the meaning of a question had been discussed after the first results, and clarification caused the differing results. The amount of time spent in clarifying and reworking questions served to create a greater appreciation for the innate challenges presented in devising a risk-based assessment tool. For these reasons, we advise that companies use multidisciplinary teams led by experts to develop their own risk assessment tools.

Case Study One: A Yeast-Derived Recombinant Insulin

The first case study (see the "Case Study #1" box) focused on use and associated risks of yeast-derived recombinant insulin in cell culture. Insulin is involved in various cell culture functions such as stimulating cell growth, cell-cycle progression, regulation of glucose, and lipid metabolism. It has an impact on cell viability, cell density, and titer — all major considerations in a bioprocess.

Insulin production by yeast has certain advantages. Yeast is easy and cheap to grow at large scales and is well characterized. It uses relatively inexpensive fermentation media and has well understood genetics. It can provide some posttranslational modifications and has a short doubling time. High cell densities and yields are achievable with yeast. No endotoxins are released from the host-cell organisms. Yeast expression is

extracellular into low-viscosity cell culture media, with low secretion of host-cell proteins. No specialized bioreactor is required, and yeast production is safer than working with mammalian tissues or cell lines. It involves no viral contamination issues and uses no animal-derived raw materials (with the possible exception of some enzymes). However, using yeast does have the disadvantage of proteolysis, and it involves many single-sourced raw materials.

For the purposes of this case study, the manufacturer is obtaining insulin from a vendor that has provided a CoA and other information stating that the product is based on a native human sequence. There are no detectable yeast polypeptides downstream that need to be removed during purification. The manufacturer is producing this insulin at full scale; there will be no scale changes. The company's insulin has benn used in various manufacturing process for different biopharmaceuticals that have been approved by the FDA. The specifications are listed, and host-cell proteins and zinc content are all added.

The vendor in this example has shared its process with the manufacturer, so we know how the insulin is produced (Figure 2). Although the vendor has no history of contamination issues, the possibility of microbial contamination as a potential risk can never be ruled out. But there are relatively low levels of host cell proteins, nucleic acids, and endotoxins. This particular vendor has included a list of the raw materials; not all vendors may be so candid. The list includes glucose and salts (not specified), ammonium, and water. Several raw materials are from single sources; one is reprocessed by a third party. This kind of information is helpful in evaluating risk.

Discussion focused on evaluating the risk of using this insulin in a mammalian cell culture process. A risk-assessment tool was used to defines specific questions that assist in evaluating risk levels.

Can the use of yeast-derived recombinant human insulin affect the safety and/or efficacy of a drug substance? Recombinant insulin has less risk than animal-derived from a process and purity perspective — unless animal-derived enzymes are used in its manufacturing process. Proteolysis can still occur.

Rank the ability of yeast-derived recombinant human insulin to introduce bioburden, endotoxins, viruses, or other adventitious agents. Recombinant insulin has a lower risk of adventitious agents than does animal-derived insulin, depending on the source of its manufacturing raw materials. However, bacterial/fungal contamination can still occur.

What historical knowledge regarding yeast-derived recombinant human insulin can be used to assess the risk associated with its use in mammalian cell culture media? Insulin has been used safely for many years in the biotechnology industry, which reduces its risk.

Rank the complexity of yeast-derived recombinant human insulin (complex mixture, molecular complexity). As a protein, insulin is more complex than a chemical raw material, but it is less complex than most other proteins. It is better characterized than most proteins, as well.

Have we adequately defined the risks to product safety/efficacy for the use of human insulin in mammalian cell culture? The direct effects of insulin on people are well known. But its effects are mitigated because it is used upstream and cleared during the manufacturing process. There may be yeast host-cell proteins left over from the insulin manufacturing process, but generally they will also be cleared. The risk of contamination depends on whether the vendor uses animal-sourced or recombinant enzymes in its insulin manufacturing process.

Does the intended use of insulin affect its associated risk? Because insulin is used as a growth factor upstream in the manufacturing process, its risk to patients is low. However, its role in fermentation is critical to product yield and therefore is critical to ensuring the supply of the end product. Because the intended use is different from insulin's general application, the supplied CoA may not be appropriate in this case (for drug manufacture), although it may be appropriate for human use.

Does the point at which human insulin is introduced into a manufacturing process affect the risk associated with its use? Yes. That affects the ability of the process to clear insulin and any insulin-related impurities.

What is the impact of supply chain traceability? This is very important to preventing the use of counterfeit products and detecting unknown contaminants. In this case, the vendor has supplied a great deal of information, which allows the biomanufacturer to place less emphasis on aspects of supply chain traceability than it would otherwise. Lack of traceability information should increase the amount of RM vendor/manufacturer tracking required by a drug manufacturer.

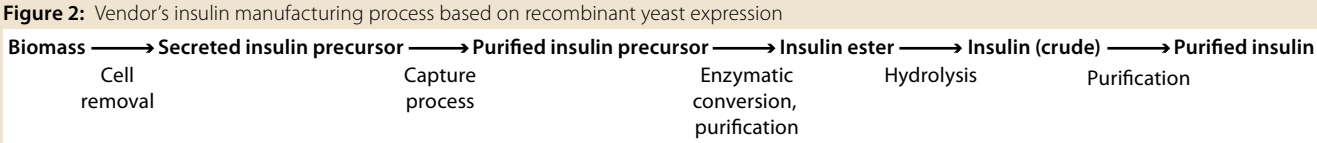
Case Study #2

Faciliator: Mark Schenerman (MedImmune)

Panel: Brigitte Brake (BfArM), Kowid Ho (AFSSAPS), Susan Hubbard (Eli Lilly and Company), Christopher Joneckis (CBER, FDA), Benedicte Lebreton (Genentech, Inc.), Paul Liu (Pall Life Sciences), Michael Mulkerrin (Oncomed Pharmaceuticlas, Inc.), Anthony Ridgway (Health Canada), Patrick Swann (CDER, FDA)

Summary by Anthony Mire-Sluis (Amgen, Inc.)

Figure 2: Vendor's insulin manufacturing process based on recombinant yeast expression



Walsh G. Therapeutic Insulins and their Large-Scale Manufacture. *Appl. Microbiol Biotechnol.* 67, 2005: 151–159

Rank the importance of vendor quality for the risk of using insulin. Vendor quality is vital for ensuring insulin quality through complete traceability of origin for the insulin manufacturer and suppliers of RMs used in its manufacturing process.

Can impurities from the insulin process affect risk? Trace contaminants could affect cells, or they may not be cleared during the manufacturing process. Insulin peptides could be immunogenic.

How easy is it to characterize insulin? Insulin is less complex than many proteins and can be characterized in detail. Available technologies (e.g., NMR) can screen for a wide range of contaminants, but such technologies are not yet routinely used in vendor companies. CoA assays may not be enough to fully characterize the insulin if there is a chance it could have come from sources other than yeast (e.g., porcine).

Does the stability of insulin pose a risk? Breakdown of insulin could affect its efficiency in a biomanufacturing process. Insulin degradants could be immunogenic, which makes their clearance very important.

How well can the manufacturing process clear insulin? Most cell culture downstream processes should be able to clear insulin-related contaminants from upstream, but you must assess your process to be certain.

Business risk: Is sole sourcing a risk to the process? From a supply perspective, it is. If your only source goes out of business or can't supply the product for any reason, your process essentially shuts down. It is often difficult to find suppliers of complex raw materials, making this a significant risk.

For GMP production, is it sufficient to assume that a CoA is OK and do only identity testing for insulin? The answer depends on the answers to all the above questions as well as your history with the vendor.

Although insulin is well characterized and commonly used in human applications, different considerations come into play for its use in cell culture. The overall risk of yeast-derived recombinant insulin is low, but a biomanufacturer will need to take precautions that include testing beyond what is listed by the vendor. Clearance of degradants will have to be verified, and it is advisable to find a back-up source for the product because of the risk associated with sole sourcing.

Case Study Two: A Chromatography Resin

Mike Mulkerin of Oncomed Pharmaceuticals, Inc. (Redwood City, CA) provided background for the second case study (see the "Case Study #2" box). Protein A chromatography resins capture monoclonal antibodies (MAbs) from large sample volumes. Protein A affinity chromatography is typically the first step in a MAb purification process, sometimes the second.

The resin used in this case study is manufactured by a microbial process using *Escherichia coli*. Columns contain an oriented coupling of recombinant protein A to a matrix with an epoxide linker and agarose. All three components — the protein A, the highly cross-linked agarose, and the epoxide linker — are themselves manufactured products that must be purified and pass a CoA before being sold, although end-users are unlikely to see those CoAs. The level of supply chain traceability therefore increases risk.

Recombinant protein A exhibits similar Fc region specificity to that of native protein A, but it shows enhanced binding capacity, providing ~95% purity in most MAb processes. Additionally, fewer regulatory concerns are associated with this form of recombinant protein A because human Ig is not used to purify the product.

Can use of recombinant protein A derived from *E. coli* affect the safety and/or efficacy of a drug substance? Because protein A is bound to a column, the greatest risk it poses is the potential for leaching off that column. Other column leachates also present a risk. Washing the column with microbicidal material should reduce these risks.

Rank the ability of this protein A to introduce bioburden, endotoxins, viruses, or other adventitious agents. Washing and storing the column with microbicidal material should reduce such risks. However, appropriate storage and prewashing must be validated.

What historical knowledge regarding protein A can be used to assess the risk associated with its use in purification? Protein A has been used for many years for MAb production, and its potential issues are well understood.

Rank the complexity of protein A (complex mixture, molecular complexity). Protein A linked to a resin is quite complex because of the presence of linkers and the resin backbone as an integral component. However, testing is usually limited through functionality.

Have we adequately defined the risks to product safety/efficacy for the use of protein A in purification? Protein A toxicology is known, and exposure data are available. Other leachates from column resins are not so well characterized, depending on the resin and linker. Agarose, which is used in this instance, is well known.

Does the intended use of protein A in purification affect its associated risk? Because protein A is a resin-bound material used in purification, its risk to patients is judged to be low, although product quality can be affected if it does not perform effectively.

Does the point at which protein A is introduced into a manufacturing process affect the risk associated with its use? Introduction of protein A as a first unit operation purification poses lower risk than a more downstream introduction would.

What is the impact of the supply chain traceability? Because you can wash or clean this resin, risk is reduced for risks such as contamination (both chemical and microbial). However, if you do not perform a for-use test, the ability of the material to function as intended may be reduced (more fermentation impurities or leachates may be present).

Rank the importance of vendor quality for the risk of using protein A. Poor-quality resin can severely affect

the purity profile and yield of a biological product. It can also have an impact on viral clearance.

Can impurities from the protein A manufacturing process affect risk? The risk here is low because although protein impurities in protein A might be linked to the column, all other impurities would most likely be washed off during prewashing procedures.

How easy is it to characterize protein A? For-use tests of Ig binding and leachables are easy to carry out. Sensitive protein A assay kits are commercially available.

Does the stability of protein A pose a risk? It does because it may affect column performance. But in general, the stability of protein A resins is part of validation, which defines the resin lifetime and thus significantly lowers the risk of a failure due to unstable protein A.

How well can the downstream process clear leached protein A? Most processes are able to clear protein A from such an early upstream part of the process. However, this needs assessment and often ends up as a specification until validated out.

Business risk: Is sole sourcing a risk to the process? It is from a business/supply perspective.

Is protein A custom manufactured for this particular application? In general, it is. Manufacturers make specific types of protein A resins that have different attributes. Changing to another manufacturer would take a good deal of comparability work.

For GMPs, is it enough to assume that the supplier CoA is correct and thorough, and use only identity testing? The answer depends on accumulated knowledge of the manufacturer and lot variability. For-use tests are usually included in the CoA.

Like insulin, protein A is well known and quite well understood. Certain risks involved in its use, however, must be addressed. Leachates are likely to be cleared in the process of washing the column to which it binds, but appropriate washing and storage of columns must be validated. An assessment should be made to ensure that protein A is cleared by the downstream process. For-use tests are recommended to ensure that the protein A is appropriate for its intended use. Again, a second source is important, although changing sources in this case would probably require a large amount of comparability work.

Not to Be Underestimated

In each of those case studies, the risk assessment process identified risks inherent in a given raw material. At that point in the process, the levels of each risk should be evaluated, after which mitigation strategies should be developed. Time considerations prevented going through all those steps during the forum, but the challenge of performing a risk assessment was effectively illustrated as panel and audience members struggled with defining potential risks and asking the appropriate questions in ways that resulted in meaningful answers.

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Disclaimer

The content of this manuscript reflects discussions that occurred during the CMC Forum workshop in addition to the personal viewpoints and experiences of the authors. This document does not represent officially sanctioned FDA policy or opinions and should not be used in lieu of published FDA guidance documents, points-to-consider documents, or direct discussions with the agency.

BPI Extra: Case Study Audience Participation

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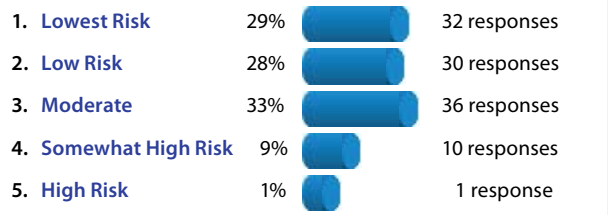
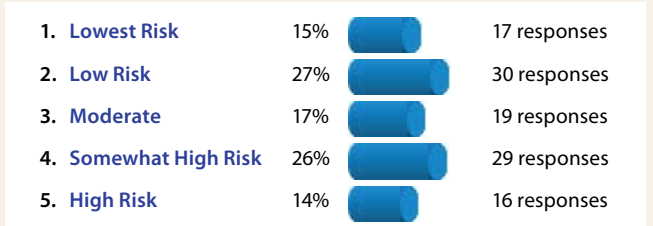
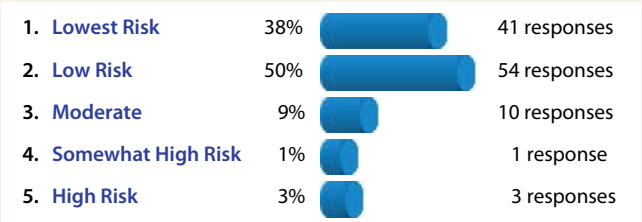
Question 1: Rank the ability of yeast-derived recombinant human insulin to introduce bioburden endotoxins, viruses, or other adventitious agents (109 total votes).



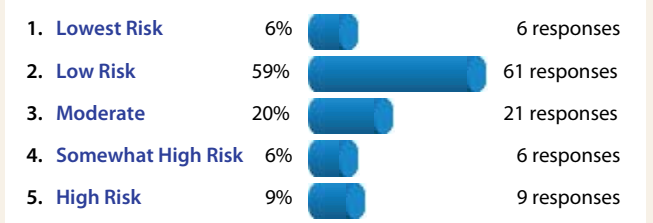
Question 2: Based on the extent of prior knowledge of insulin and its manufacture, rank the risk to patients (111 total votes).



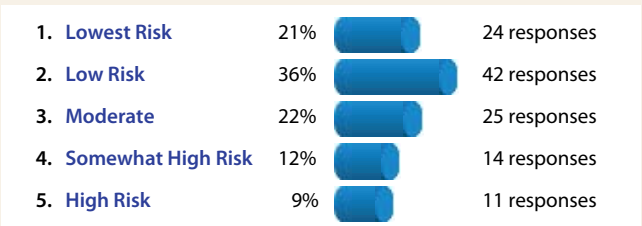
Question 3: Based on the extent of prior knowledge of insulin and its manufacture, rank the risk to patients (109 total votes).



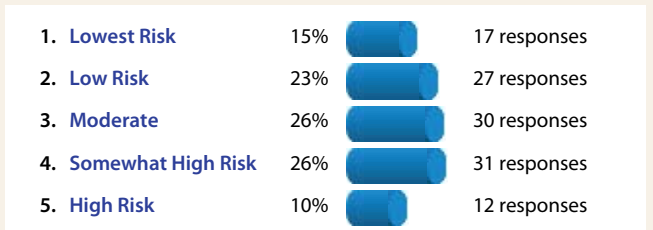
Question 4: Rank the complexity of yeast-derived recombinant human insulin (103 total votes).



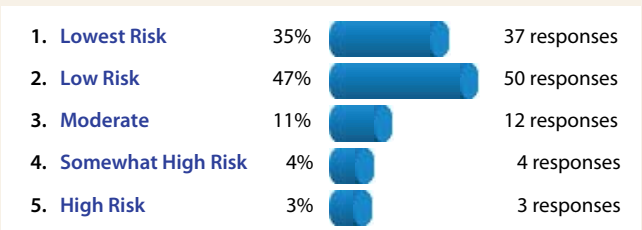
Question 5: Rank insulin's fitness for use and impact on product quality, safety, and/or efficacy (116 total votes).



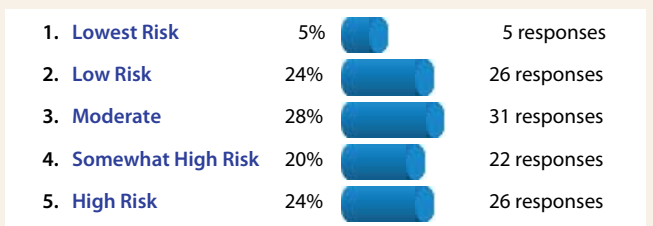
Question 6: Rank insulin's fitness for use regarding impurities and their impact on product quality, safety, and/or efficacy (117 total votes).



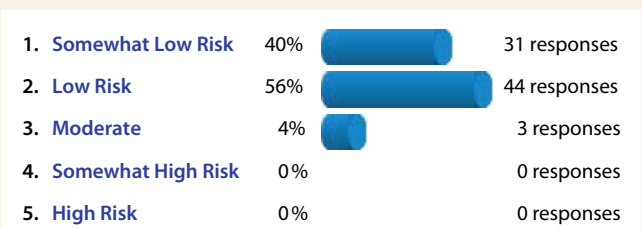
Question 7: Rank insulin supplier's information including questionnaire, audits, certificates of analysis, and country of intended use (106 total votes).



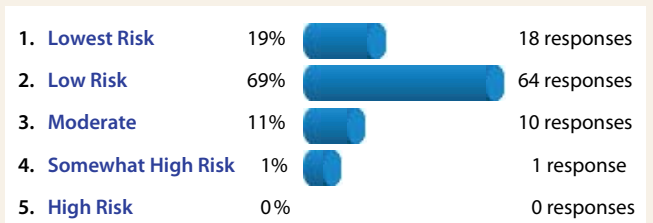
Question 8: Rank supplier information including questionnaire, audits, certificates of analysis, and country of origin's security (110 total votes).



Question 9: Does the fact that insulin is entirely derived from nonanimal sources affect risk to patients (78 total votes)?



Question 10: Rank the ability of yeast-derived recombinant insulin to introduce bioburden endotoxins, viruses, or other adventitious agents (93 total votes).



BPI Extra: Case Study #1 Audience Participation, continued

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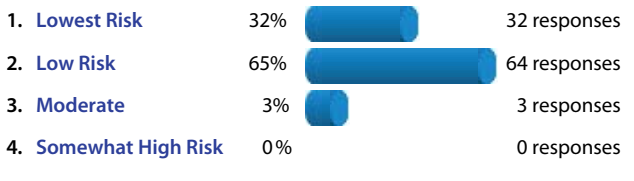
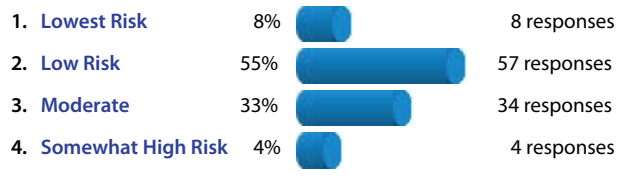
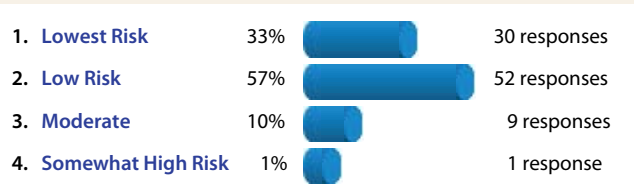
Question 11: Based on the extent of prior knowledge of insulin and its manufacture, rank the risk to patients (99 total votes).



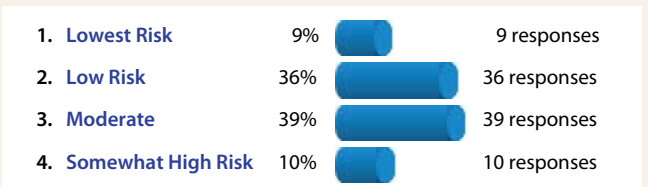
Question 12: Rank the complexity of insulin (103 total votes).



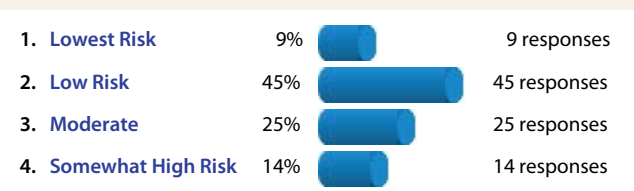
Question 13: Rank insulin's fitness for use and impact on product quality, safety, and/or efficacy (92 total votes).



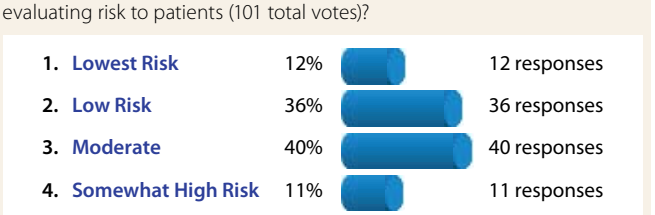
Question 14: Rank insulin's fitness for use regarding impurities and their impact on product quality, safety, and/or efficacy (99 total votes).



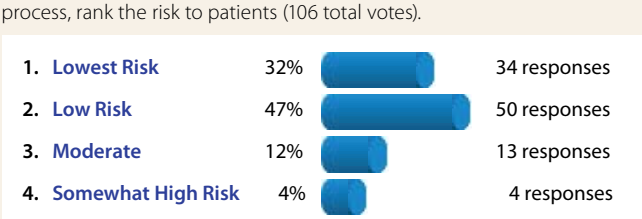
Question 15: Rank insulin supplier's information, questionnaire, audits, certificates of analysis, and country of origin's security (99 total votes).



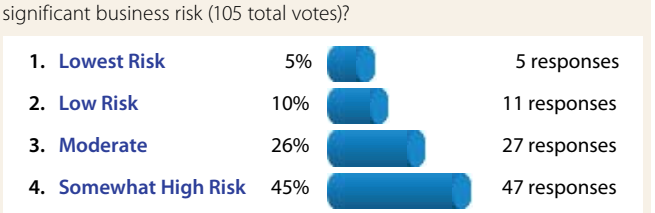
Question 16: Are current insulin analytical methods suitable for evaluating risk to patients (101 total votes)?



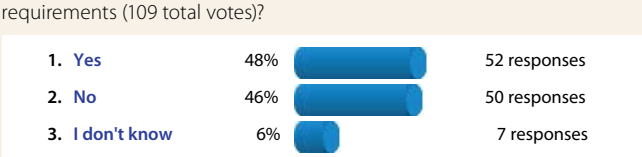
Question 17: Based on prior knowledge of insulin clearance in your process, rank the risk to patients (106 total votes).



Question 18: With a single vendor, does this raw material introduce significant business risk (105 total votes)?



Question 19: Is identity testing alone sufficient to meet GMP requirements (109 total votes)?



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Question 1: Does the fact that recombinant protein A is entirely derived from nonanimal sources affect the risk to patients (100 total votes)?

1. Somewhat Low Risk	36%		36 responses
2. Low Risk	55%		55 responses
3. Moderate	7%		7 responses
4. Somewhat High Risk	2%		2 responses
5. High Risk	0%		0 responses

Question 2: Rank the ability of recombinant protein A resin to introduce bioburden endotoxins, viruses, or other adventitious agents (102 total votes).

1. Somewhat Low Risk	23%		23 responses
2. Low Risk	55%		56 responses
3. Moderate	18%		18 responses
4. Somewhat High Risk	5%		5 responses
5. High Risk	0%		0 responses

Question 3: Based on the extent of prior knowledge of recombinant protein A resin and its manufacture, rank the risk to patients (107 total votes).

1. Somewhat Low Risk	32%		34 responses
2. Low Risk	60%		64 responses
3. Moderate	7%		8 responses
4. Somewhat High Risk	1%		1 responses
5. High Risk	0%		0 responses

Question 4: Rank the complexity of recombinant protein A resin (104 total votes).

1. Somewhat Low Risk	7%		7 responses
2. Low Risk	38%		40 responses
3. Moderate	40%		42 responses
4. Somewhat High Risk	14%		15 responses
5. High Risk	0%		0 responses

Question 5: Rank protein A's fitness for use and impact on product quality, safety, and efficacy (105 total votes).

1. Somewhat Low Risk	22%		23 responses
2. Low Risk	38%		40 responses
3. Moderate	26%		27 responses
4. Somewhat High Risk	12%		13 responses
5. High Risk	2%		2 responses

Question 6: Rank protein A's fitness for use regarding impurities and their effect on product quality, saftey, and efficacy (104 total votes).

1. Somewhat Low Risk	8%		8 responses
2. Low Risk	38%		39 responses
3. Moderate	39%		41 responses
4. Somewhat High Risk	11%		11 responses
5. High Risk	5%		5 responses

Question 7: Rank protein A supplier's information, questionnaire, audits, and country of origin security (105 total votes).

1. Somewhat Low Risk	8%		8 responses
2. Low Risk	50%		52 responses
3. Moderate	30%		32 responses
4. Somewhat High Risk	11%		12 responses
5. High Risk	1%		1 response

Question 8: Are current analytical methods for characterizing recombinant protein A resins suitable for evaluating the risk to patients (101 total votes)?

1. Somewhat Low Risk	22%		22 responses
2. Low Risk	50%		51 responses
3. Moderate	22%		22 responses
4. Somewhat High Risk	6%		6 responses
5. High Risk	0%		0 responses

Question 9: Based on prior knowledge of protein A clearance from your process, rank the risk to patients (103 total votes).

1. Somewhat Low Risk	36%		37 responses
2. Low Risk	50%		52 responses
3. Moderate	12%		12 responses
4. Somewhat High Risk	1%		1 response
5. High Risk	1%		1 response

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Question 10: Based on a single vendor, does this raw material introduce significant business risk (101 total votes)?

1. Somewhat Low Risk	1%	1 response
2. Low Risk	5%	5 response
3. Moderate	23%	23 responses
4. Somewhat High Risk	44%	44 responses
5. High Risk	28%	28 responses

Question 11: Is identity testing alone sufficient to meet GMP requirements (104 votes, then 94 votes)?

1. Yes	46%	48 responses
2. No	50%	52 responses
3. I don't know	4%	4 responses

1. Yes	82%	77 responses
2. No	16%	15 responses
3. I don't know	2%	2 responses

Question 12: Based on prior knowledge, rank the importance of literature, experience, and other for recombinant protein A (125 total votes).

1. Somewhat Less Important	0%	0 responses
2. Less Important	2%	2 responses
3. Important	42%	53 responses
4. Very Important	40%	50 responses
5. Critical	16%	20 responses

Question 13: Rank protein A's fitness for use in regards to its effect on product quality, safety, and efficacy; the product impurity profile; and related analytical methods (123 total votes).

1. Somewhat Less Important	0%	0 responses
2. Less Important	1%	1 response
3. Important	10%	12 responses
4. Very Important	27%	33 responses
5. Critical	63%	77 responses

Question 14: Rank the characterization of recombinant protein A resins in regard to analytical method suitability and new knowledge (125 total votes).

1. Somewhat Less Important	1%	1 response
2. Less Important	5%	6 responses
3. Important	24%	30 responses
4. Very Important	42%	53 responses
5. Critical	28%	35 responses

Question 15: Rank the clearance of protein A by your downstream process (126 total votes).

1. Somewhat Less Important	1%	1 response
2. Less Important	10%	12 responses
3. Important	23%	29 responses
4. Very Important	46%	58 responses
5. Critical	21%	26 responses

Question 16: Rank recombinant protein A's fitness for its intended use (105 total votes).

1. Somewhat Less Important	0%	0 responses
2. Less Important	6%	6 responses
3. Important	12%	13 responses
4. Very Important	50%	53 responses
5. Critical	31%	33 responses

Question 17: Rank the business risk of recombinant protein A resins (113 total votes).

1. Somewhat Less Important	4%	4 responses
2. Less Important	8%	9 responses
3. Important	30%	34 responses
4. Very Important	37%	42 responses
5. Critical	21%	24 responses