This article highlights key elements to consider in approaching the containment of pharmaceutical operations in terms of problem definition, common expectations, cost/benefit considerations, and finally a variety of additional resources for the containment engineer and project manager.

Reprinted from
PHARMACEUTICAL ENGINEERING®
The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

Containment Considerations

by James P. Wood

Common Expectations

As with all effective engineering solutions, the first step in addressing containment of emissions lies in accurate definition of the problem. The pragmatic engineer or containment specialist will take the time up front to probe for the actual criterion for success: what yardstick the containment project will be measured against once the project is finished. This will often involve driving a consensus among disparate and sometimes opposing viewpoints between the end users and the support organizations ultimately responsible for the process area's smooth operation. Frequently undervalued, this step remaining partially unresolved causes chronic communication problems throughout the life of the effort. Reaching a common set of expectations at the outset is just good business in general, and certainly applies specifically here.

A good starting point in such a conversation is to determine the issues driving the containment effort. Traditionally, "containment projects" have been strongly linked (and rightly so) to health and safety concerns, or *people* protection. However, there is an increasing awareness in the industry that effective containment, especially containment-at-source, also can significantly impact Good Manufacturing Practices or *product* protection; air/water pollution or outdoor *environmental* protection; and even an operation's financial profile or (if your will) *profit* protection.

A first step, then, should be to characterize, and if possible, quantify the nature of the design intent; that is to narrow down and translate the project issues into a specific target. In real-world containment, you'll often have several masters to serve (as discussed above) with various containment objectives to meet differing needs simultaneously. Keeping that discussion in focus can be a challenge. If need be, the customer can prioritize or rank these various criteria. Additionally, there needs to be agreement on the immediate vs. the longer-term needs that the containment systems will be expected to address. These items in themselves can precipitate the much-needed hammering out and debate of the project's true scope of work. The good news is that discussion along these lines is usually an indicator of problem definition having at least gotten past the surface level. From an engineering standpoint, your actual design target then becomes the most stringent among these various criteria, with double-checks that the remaining requirements are also met. The saving grace in such a multiple-criteria effort is that good source-containment design often benefits each of the other areas of concern as well. Financial reasons for tightening up your leaky milling step become more attractive as the unit value of product emitted goes from $10 per gram to $100 to $1000 and beyond. Tightening up the mill also might decrease potential worker exposure to increasingly potent compounds, possibly to the extent that respiratory equipment requirements can be downgraded, in turn further reducing costs. The ripple effects continue outward from there.

Metrics

Once a common understanding is reached regarding the issue to be addressed, i.e., what the *true* driver is for improving containment (product protection, health and safety, outdoor environmental, financial, etc.), the next task is to agree on how containment will be measured. The answer to that will vary according to the issue that your containment project is supposed to address. Don't fall into the trap of mixing the kind of verification data used for one type of issue with another. As an example, if *product quality* (product protection) is the issue, you may want to be wary of defining your success criteria in terms of achieving an Occupational Exposure Limit (OEL). Trying to measure how protected the *product is* by how protected the *people are supposed to be* is like checking your car's gas mileage by measuring the tread wear on your tires. You can wind up with a very precise answer to a question that's not being asked.

Gap Assessment

Once the nature of the issues has been determined and appropriate metrics for measuring the containment levels defined, an overall containment assessment or evaluation of the operations and major subsystems should be performed. Describing in any detail the overall approach or items to be considered in such an analysis is probably a good topic for a separate article in itself. Suffice here to say that a survey needs to occur which takes into account on a case-by-case basis how the operation is cur-

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rently being performed; not how it's supposed to be, but how it actually is. This is essentially a gap analysis, indicating where current emissions sources are, what can "go wrong," and what the implications are. The customer will often have good ideas of "what the problem is," but not always completely. Handling a high-potency API is in general a concern, but the dispensing step of the active ingredient will yield a different risk profile than packaging coated tablets of the same product. This evaluation stage of the effort is critical; it is essentially the foundational problem definition that the rest of the effort will be built on. Putting in quality effort here ensures that the right problem is being worked on from this point forward.

An overall systems-view to development of containment approaches is often needed to achieve effective results. A popular notion is that hanging a containment device around an open process will "take care of the problem." Often it does not. And even when it does improve emissions at a particular source, other emissions problems can be pushed further down the line. Other components besides hardware come into play, such as the overall facility layout philosophy and analysis (e.g., architecturally and mechanically self-sufficient operating cores vs. matrixed layouts), traffic flows of people, materials, and waste products, how inherently contained the process equipment itself is, procedures, monitoring, and early emissions detection capabilities. All these aspects work together in reducing emissions, and entire treatises can be written on each of these elements of the containment picture. As a general statement here; however, for an effective containment plan, design beyond the specific unit operation and plan beyond normal operating modes.

Design Beyond the Specific Unit Operation

Be wary of focusing solely on a single unit operation. Presently, there are many competing "containment designs" for individual operations. What is missing is often a holistic assessment of the process, facility, and procedures. Investigating along the entire process train often reveals unexpected features of the operation that affect the specific process step being focused on. Such unexpected features take the form of degraded equipment conditions, rearranged equipment or area configurations (beyond original design intent), ineffective operating procedures, or in adherence to effective operating procedures.

One example of this, just to make the point, is the spreading of contaminant via product containers' exterior surfaces brought in from upstream operations. A worker's outer clothing and shoes also may be overlooked as a vehicle of contamination, potentially tracking in residual compound from other areas that are technically "outside the scope" of the formal project (beyond the specific operation being focused on by the customer). At the potency levels being dealt with today, airborne or surface concentrations can be in the "significant" range while still remaining several orders of magnitude below visibility. Compound residual can be tracked in from other areas without ever being seen. Paraphrasing the old adage, what you can't see is more than ever likely to hurt you.

Plan Beyond Normal Operations

Normal operations, meaning, those times when the piece of equipment is functioning in a steady-state mode, is the most common scenario when analyzing a unit operation for improved containment. So much time and effort has gone into containing components of production that in some cases "normal operation" is no longer the main challenge in the overall containment chain; the neglected considerations of cleaning and maintenance become the hidden chinks in the armor.

Cleaning: If equipment cannot be completely decontaminated prior to opening it up, residual compound within the equipment must be kept contained until it has been *locked down* with water or other liquid coating so it can no longer become airborne when exposed to the open environment. After that occurs, opening the equipment for further cleaning can be undertaken without significant airborne exposure. Clean-in-place systems, representing a logical extension of this approach, can be a valuable remedy for residual compound if they are applicable and economically justifiable for the operation in question.

Maintenance: This can be a little thornier. The obvious ideal is for the equipment to be decontaminated prior to maintenance and repair. But there are instances when this is not practical. A tablet press has a compression chamber that is regularly cleaned, but it also has non-product contact chambers housing the mechanical and electrical components that may, over time, have built-up residual contamination. These cannot be easily or practically cleaned prior to maintenance access, and sometimes one needs to just acknowledge that reality, and protect the worker and environment by other means (personal protective equipment, etc.). Another example is the exhaust ventilation fan and filter system which by virtue of location can be more difficult to maintain in a contained fashion than the production equipment located within contained and controlled spaces. Mechanical equipment associated with ventilation is typically located in non-contained mechanical "plant" rooms. Such areas usually offer little in the way of secondary containment, and are architecturally uncontrolled spaces since they harbor rough finishes and nooks and crannies that are tough to clean with customary methods. The best strategy is to prevent contamination of such areas to begin with. In some cases, special "contained" areas within mechanical rooms can make sense.

A Quick Word on Costs

The "cost of containment" is typically seen through the lens of the obvious and immediately visible "what's all this going to add to my project cost?" This component is usually a capital cost, and is made up of extra equipment, extra space, "higher quality" space, etc. Beyond project cost, a second cost component is made up of owning and operating expenses. This component has potential to contribute extensively to an operation's financial burden, although less immediately visible during the more focused, and at times intense project delivery stage. Owning and operating costs aren't onetime; however, they are paid out daily throughout the life of the facility. Here, good engineering can save significant money. As one straightforward example, if local exhaust systems are going to be utilized for containment in operations that are periodic in nature, maintaining designated room pressurizations shouldn't necessarily depend on those exhausts running, throwing away conditioned air 24 hours a day, just to maintain designated room pressurizations during operational downtimes.

A Quick Word on Benefits

Solid and thorough identification of *benefits* is something that is overlooked frequently, yet is really the flip side of the same *cost*-coin mentioned previously. In other words, when proposing to incur the "additional containment costs," what "savings" can be expected from such implementation? This is yet another subtopic of containment that much more can be said on than there is space for this particular article. However, in general such

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items as yield increases, cycle time reductions due to less room cleanup between switchovers, the cost avoidance of regulatory non-compliance, and other factors these and other costs are "intangibles," as have been traditionally defined and accounted for in production operations. But they are real, and often they are significant. And, as more companies in the industry in their quest for leaner ledgers continue to refine their views and sharpen their pencils over where their hidden costs actually lie, these areas will be discovered more and more as harboring potential major operational savings in the long term.

Containment Resources

Resources are available to aid in inter-company and inter-industry benchmarking. Such resources come in many categories. The following four are listed below: Standards, Articles, Texts, and Vendors.

Standards

ANSI and AIHA

ANSI and AIHA are two organizations that develop standards that can pertain, directly or indirectly, to containment-related issues found in the pharmaceutical industry.

Examples: AIHA's Z9.2, *Fundamentals Governing the Design and Operation of Local Exhaust Systems* Z9.5. This standard sets forth requirements for design and operation of ventilation systems for certain types of laboratories. Its purpose is to establish minimum requirements and best practices for lab ventilation systems to protect personnel. It is in the process of being rewritten, and is close to final committee review and publication.

ASHRAE

ASHRAE Standard 110-1995; *Method of Testing Performance of Laboratory Fume Hoods*

American Glovebox Society (AGS)

The nuclear industry was the driving force behind this organization. More recently, AGS has expanded beyond that industry, addressing approaches such as glovebags and associated. See AGS-G002-1998; *Standards of Practice for the Design and Fabrication of Glovebags*.

ISO

ISO is working on a draft standard (DIS 14644-7), *Separative Enclosures (clean air hoods, gloveboxes, isolators, mini-environments).* This document is planned to specify minimum requirements for design, construction, installation, testing, and approval of separative enclosures. Note: the term "separative enclosure" is coined here by ISO to encompass the wide variety of configurations and designs found in industry.

ISPE

ISPE maintains a discussion on its Web site forum on a variety of pharmaceutical related topics with isolation, barrier, and containment issues periodically arising. This type of informal benchmarking can prove valuable in many circumstances.

NIOSH

This organization develops and periodically revises Recommended Exposure Limits (RELs) for hazardous substances or workplace conditions. They also recommend appropriate preventative measures for reduction or elimination of the adverse health effects of such hazards. They author various tions, including the *Pocket Guide to Chemical Hazards*.

Academia

Purdue University, West Lafayette has performed some basic research on actually determining fundamental parameters impacting a powder's propensity to remain airborne, i.e.; its dustiness. A result of that research was the defining of a Dustiness Index for compounds, which could become a starting point for truly characterizing a "containment challenge" standard. (For more information, see the book *Containment in the Pharmaceutical Industry*, referenced below.)

Articles

There are major trade journals and periodicals, both in the pharmaceutical industry and outside (nuclear, asbestos abatement, etc.), that highlight industrial and technology developments overall. On occasion, containment approaches are explored, and this can be a valuable source for benchmarking. Be aware of disparate underlying needs of publications outside the pharmaceutical industry however. For example, cleaning requirements in the nuclear industry are obviously different than in pharmaceuticals. A nuclear facility doesn't "switch over" on a weekly basis, requiring reusable equipment from one "product" to another. Instead, they may process a single product (uranium, plutonium) for 15 years and when finished, simply destroy the containment device. Also, that industry has a pretty reliable real-time "product-specific" detection technology in the Geiger counter. The same cannot be said for all the numerous compounds and intermediates being processed in a typical pharmaceutical manufacturing plant. These examples begin to point out some of the comparative constraints and latitudes between industries.

A sampling of trade journals relating to the pharmaceutical industry include such periodicals as:

- *American Pharmaceutical Review*
- *European Pharmaceutical Review*
- *Pharmaceutical Engineering*
- *Powder and Bulk Engineering*

While these magazines are not dedicated to containment per se, there are periodic articles carried in them pertaining to the control and containment of compound emissions.

Texts

There are a relative handful of books dedicated to the topic. Four are included here:

Industrial Ventilation; A Manual of Recommended Practice, published by the American Conference of Governmental Industrial Hygienists (AIGIH). This is an industrial hygiene standard reference of more traditional cross-industry applications. The book does a good job laying out many tried and true local exhaust ventilation hardware designs, as well as some useful mechanical engineering data (pressure drops, capture velocity drop off profiles, air cleaning devices, etc.). Some of these designs can be utilized in pharmaceutical industry settings with a bit of good engineering synthesis and reapplication. The actual emissions control designs put forth can be generally effective for low-to-medium containment applications, relative to some of today's more potent compounds.

Isolator Technology, Applications in the Pharmaceutical and Biotechnology Industries (Interpharm Press pub., 1995) is a book that primarily focuses on isolator applications for the

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publicacontrol of sterility. However, several chapters also are relevant to containment applications as pertains to pharmaceutical professionals, and others in the health, agricultural, and biotechnology industries.

Containment in the Pharmaceutical Industry (Marcel Dekker pub., 2000), takes a holistic view of containment in the pharmaceutical industry specifically, including some key topics that are associated with containment but not typically referenced under the same cover.

Institution of Chemical Engineers Guide to Containment is about to be published. This guide defines a number of containment strategy levels to meet increasing degrees of containment, and classifies various equipment types accordingly. Practical aspects of measuring and controlling exposures to hazardous substances are discussed. Also, the guide provides background information about the requirements that containment systems must meet, outlining the legal requirements for containment as detailed in legislation in the United Kingdom.

Vendors

When a process has not been adequately contained within the production equipment itself, the focus turns to remedial hardware, a solution that overlays containment hardware onto the process equipment. Remedial equipment can be grouped into two families, air-based or physical barrier-based. Some engineering designs incorporate both types of approaches.

Air-Based

Examples: An air-based solution might be a fume hood, biosafety cabinet, or filtered isolation booth within which ventilation carries away the problem.

Caveats: A sound design is needed, based on solid engineering principles of the science of compressible fluid flow mechanics. Air-based methods are highly sensitive to hardware configurations, the supply or exhaust air sources, small undesigned-for gaps between the terminal air flow device and the equipment; all these can make a critical performance difference, as does the precise orientation of the worker positioned around the equipment. A positive orientation marker or device should be incorporated into the design to cause operators to maintain the designed-for personnel and hardware orientation, assuring optimal effectiveness of ventilation devices.

Physical Barrier-Based

Examples: A physical barrier solution might be a glovebox, glovebag, or flexible barrier which imposes some type of material barrier between the worker and the emissions source.

Caveats: Ergonomics are important with physical barriers. If the operator interface is too demanding or restrictive, or if cycle times become too bogged down, the worker may choose not to use the equipment as it was designed to be used. Plan on multiple mockups of the design made of inexpensive materials to be evaluated by the operator as well as the engineer; this is a key to effective barrier containment. In fact, ownership of the design process by the operators is preferred, at least from an ergonomics standpoint. If an operational shortcut or timesaver to the containment approach is to be found, the operator will be the one to find it. Don't automatically assume this a bad thing. When it works, we call it innovation. When it doesn't, it's called human error. Either way, the engineer should be alert and receptive to looking into those "shortcuts," incorporating the best and designing out the rest. In the final analysis, the operator will make or break the hardware design. This person understands the real operation best, will be the one living with it, and should be well involved in its development.

Vendors know their own products best so learn from them. And then generalize from that information. But resist the urge to become the instant expert. Example: The vendor says an item "contains emissions down to 1µg." Before you start throwing that number around to production management, be sure you're advertising the right thing. What does "1 µg" mean? Total mass emitted over the operation's timeframe? Or is it on a per m3 of air basis? And, if so, measured where? The breathing zone? This is relevant if the issue being dealt with is people protection, but not necessarily if the concern is with, say, outdoor emissions or cost of production. Is this measurement calculated over an 8- or 12-hour weighted average? Or is it a raw-datapoint taken for just the duration of the activity? The vendor will know the answers to these types of questions best. However, they will *not* know the factors relevant to your needs. Remember that you know your problem definition best. So, actively question the vendor's product data. You'll be helping the vendor help you with a two-way partnership of information sharing. At the same time, be sensitive to the balance between utilizing a good vendor as a teacher vs. taking up his time by making him deal with issues that you, as the owner should have already addressed.

A potential pitfall of vendor-as-partner is the phenomenon of every carpenter seeing a problem as one more nail to drive, making every solution look like a hammer. The savvy vendor, in business for the long haul, also will be watching for this and will choose to let the client know that "other approaches" could be better in a particular case. These vendors are worth their weight in gold; their business cards will be the ones populating the card files on the desks of design engineers and production staff.

About the Author

James P. Wood is Team Leader of the Containment Engineering Technology group at Eli Lilly and Company. He is responsible for the design of several high-potency compound facilities, and advises on containment issues in Lilly's effort to standardize manufacturing processes globally. He also regularly travels to operations in England, Spain, Italy, Ireland, and the People's Republic of China, where he leads in the design, commissioning, and troubleshooting of high-containment areas. Wood has recently participated with the Health and Human Services Agency in Washington DC in an advisory capacity regarding defense against and preparation for potential future bioterrorism within US borders. The book *Containment in the Pharmaceutical Industry* of which Wood is the developer and editor was published October 2000. Wood is also the inventor of record for two patents, and is a speaker and course leader at organizations such as ISPE, as well as a guest lecturer to Purdue University. With a mechanical engineering degree from Purdue, he is a licensed Professional Engineer and member of the National Society of Professional Engineers and ISPE.

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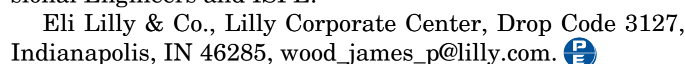

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This article highlights advancements in the use of silicone rubber, explores current approaches, and presents changes in silicone rubber connection.

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PHARMACEUTICAL ENGINEERING®
The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

Silicone Use: The Next Generation

by Stephen Warburton-Pitt

All engineers using silicone rubber need to understand the impact of current FDA expectations. The deceptively simple task of connecting different sources and vessels with flexible silicone tubing has developed over the past five years into a sophisticated product area. Silicone rubber has been an integral part of the equipment used in the manufacture of a significant amount of pharmaceutical products over the past 50 years. Improved standards of purity continue to be a major requisite within the pharmaceutical industry. With the advent of the FDA group "Team Biologics," numerous production procedures have come under full "process validation" scrutiny. The original use of natural rubber, silicone rubber, peristaltic pumps, and the methods of connection increasingly have become the focus during some FDA inspections or audits. By looking at where we have developed from, we can better understand the benefits that these advancements can bring to the industry. The physical properties of silicone have made it an integral part of processing systems. With these new developments, it is now possible to overcome some of the previous limitations.

Initially, the use of latex was the only option for medical grade flexible tubing; however, the development of silicone during WWII introduced an alternative, allowing in the 1950s, the use of the new peroxide cured silicone to develop as the standard. The emergence of peristaltic pumps with their capability of pumping fluids without product contact has long been a major factor in the use of rubber tubing. The pumps operated by compressing the tubing with a roller moving along its axis to push the fluid in the tube forward. Another roller followed, performing the same compression forward movement which, with constant cycles, created a pumping action. In early applications, these pumps used rubber tubing which could vary significantly in diameter. As flow volumes and accuracy of the output were required to increase, tighter controls were put in place both on the pumps and tubing. However, the silicone rubber could still vary significantly in its pump performance even with tighter controls on the dimensions. As the frictional forces became better understood both on the inside and outside diameters, the raw material formulations were developed to help meet the higher expectations industry demanded.

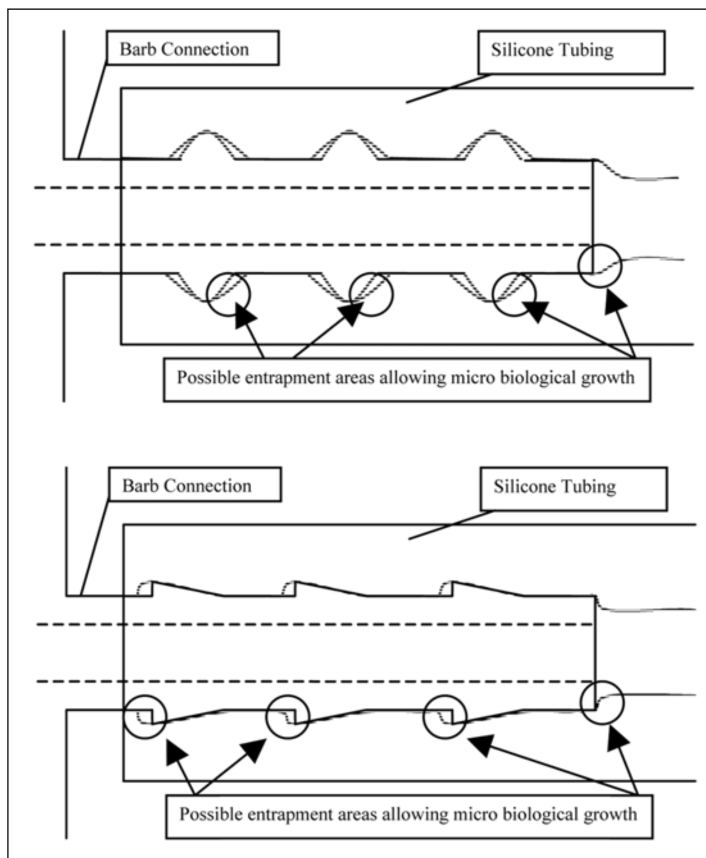

Figure 1. Diagram showing possible areas of micro biological growth.

The improved peroxide silicone rubbers were required to perform with reduced spallation (a breakdown of the wall surface resulting from frictional forces) on the inner surface that can introduce particles of the tube into the product. Concern regarding the possibility of spallation

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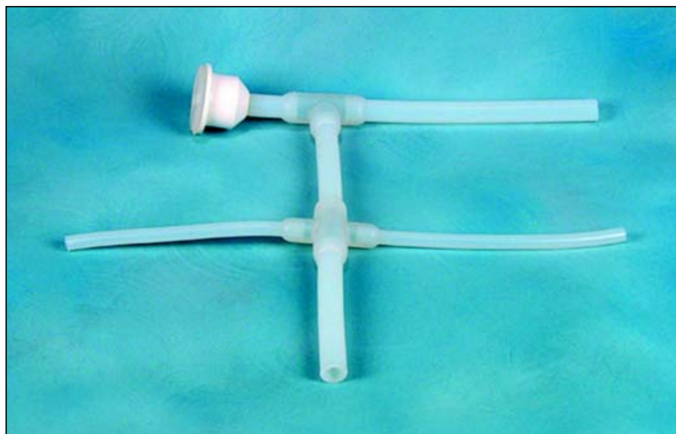

Figure 2. Basic silicone manifold assembly.

initiated the placement of filters down stream from the pumps to ensure any particles could be effectively removed. However, the introduction of a filter was sometimes self-defeating as it increased the pressure requirement to maintain flow volumes, which in turn increased the potential for spallation. The only solution was to monitor the tubing and move the pumping head at intervals to a new section of the tubing. This effectively ensures that the tubing is not exposed to the frictional forces for long enough to affect the physical integrity of the tubing.

The tubing also was required to be capable of maintaining its characteristics after sterilization in an autoclave prior to use, or possibly after gas or gamma sterilization. The use of peroxide tubing was common place until the early 1990s, when the continuing reduction in the active ingredients included within an injectable product was causing the level of inspection of leachables to move to parts per million (ppm) and sometimes parts per billion (ppb). During this period, the increased awareness of factors making up the possible transferences from the silicone by leaching, brought the 2,4,DCBA (di-chloral benzoylic acid), a bi-product of the peroxide tubing curing process, more into focus as a possible contaminant.

Peroxide cured tubing is cross-linked using a peroxide catalyst (hence the name) + heat and is commonly referred to as a Heat Cured Rubber (HCR). During this process, the volatiles, including most of the 2,4,DCBA, are driven off. However, the tubing must undergo additional post curing to drive off any remaining catalyst: this requires the tubing to be placed in ovens at 200°C for four hours. The time span needs to be monitored as the volume of material in the oven and additionally air changes can make differences in time required. Some care needs to be taken with this post-curing of peroxide tubing. Due to commercial time pressures, insufficient post curing sometimes can take place: the result being the “blooming” or development of a white deposit both on the interior and exterior surfaces of the tubing. This blooming represents the resident volatiles migrating from the silicone under normal room temperatures; and depending on storage temperatures, this can take weeks or possibly months to manifest itself. It should be noted that this phenomenon is well understood by most silicone manufacturers and as long as the recommended production processes are adhered to, blooming is unlikely to be seen.

We have seen the improvement of the physical properties of silicone rubber, from the original natural rubbers, through peroxide silicone, to platinum silicone, resulting in longer pump life. While the improvements in platinum silicone have allowed it to perform in almost all applications, there are some applications that require closed loop pumping for thousands of hours: beyond the range of normal tubing.

Specialized areas such as these have to accept the higher cost of the latest innovation that of a Silicone Teflon/Silicone hybrid pump tube. This hybrid tube holds the capability of increased pressure potential together with greatly increased pump life. Sadly, the current restrictions of maximum length (18") and cost limit the use of this tubing. However, the ability to develop much higher pressures which reduces the possibility of spallation for extreme periods of pumping makes this product a valuable addition to the industry.

The development of a cleaner platinum curing process, initially led by Dow Corning, took silicone tubing to a significantly lower extractable level. The new platinum curing system was enthusiastically adopted because the main bi-product of this cross-linking reaction is water. While there were slightly inferior mechanical properties compared to the earlier grades of peroxide tubing, the potential of a much “cleaner” silicone rubber material was quickly adopted by the pharmaceutical and biopharmaceutical industries.

Originally, the tubing was connected using stainless steel tubes made up into ‘T’, ‘Y,’ or ‘X’ configurations. This method of connecting silicone tubing predominantly remained throughout the 1950-90s; while the use of stainless steel progressed in part to plastic (PP, Acetyl, and nylon) barbed connectors. Even today we still see the use of these methods with the tubing attached with a double clip on each leg of these adaptors or connectors. Even after decades of experience with these connections, it is still common to hear of catastrophic failures costing not only re-filtering time, but sometimes invalidating a complete production campaign. Possible spills or leaks also have health and safety ramifications with regard to operator exposure and clean up. Furthermore, using these connection practices, particularly their assembly, creates another possible issue: that of Repetitive Stress Syndrome (RSS) to the operator. This has motivated novel introductions including pneumatic and hydraulic machines to secure the tubing to the barbs with steel or plastic clips.

In addition, there were difficulties involved trying to improve reliability which required more detailed quality procedures as well as testing routines that the novel introductions did not help. They did not address the issue of failures or difficulties with process validation, specifically the possible areas within the barb tubing void (Figure 1) that could harbor bacterial growth. Nor did they answer the concern of possible particulate generation because of the sharp edges of the barb scratching or scraping the inner diameter releasing small particles of silicone into the finished assembly.

The FDA focus on “Process Validation” in the later half of the 90s highlighted the need for a real solution to the connec-

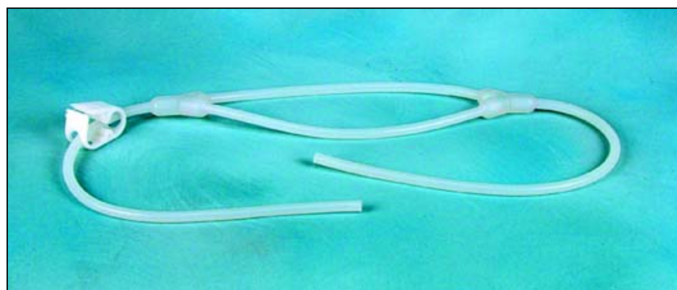

Figure 3. Molded double Y assembly.

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> *It is only in the past 10 years as focus on process validation has increased, that natural rubber stoppers, prone to cracking with age or exposure to heat, have started to be replaced with silicone.*

tion of silicone. The issues of possible bacteria entrapment, particulate generation, and sterility validation all became common points during FDA inspections. Because there was really no alternate to barb connections, there could be no effective process validation. As a result, several silicone fabricating companies started searching for solutions.

The completion and availability of the first aseptic all silicone end connection came in the early 90s. This allowed the tubing to be connected to a standard end fitting and incorporated the silicone gasket. The patented system relied on a premolded end being assembled on the tube using a Liquid Silicone Material (LSM) and vulcanized: a process that took 15 minutes and required some skill to achieve a bonded component.[1] There were initial attempts at manufacturing T, X, and Y connections, but the method required a time consuming destructible insert which could damage the internal diameter. This method also limited the length of the attached tube to 60".

In 1997, the first really practical manufacturing method of silicone assemblies was presented and patented.[2] This development allowed a complete aseptic connection system to be manufactured in one piece through a two part molding process. The new technology also allowed longer lengths to be connected. The connections were constructed of a platinum cured Liquid Injection Material (LIM) which allowed the manufacture of secure, aseptic connections from a silicone TC compatible end through the required transitions to a final silicone TC compatible end. This technology met the FDA concerns and resolved the issues raised by some inspectors. Safety questions were answered, leak concerns were resolved, and possible contamination reduced. There have been concerns that the two-step molding system could possibly allow dirt entrapment or liquid silicone to block the tubing. However, these concerns can be resolved through correct production procedures and diligent quality controls.

In 1999, a single step molding process was presented to the industry, which makes a connection in one simple operation. This patented method[3] resolves any lingering concerns the two-step process may have given with regard to both dirt entrapment and blockages. This system also has the advantage of being very portable with regard to manifold assembly, which is now able to take place on a small desktop molding unit. Molding silicone previously had been almost exclusively restricted to either heavy injection molding machines or presses. Complex assemblies can now be completed within the facility they are to be used in if desired – *Figure 2*. The open sharing of this technology was quite unique within the silicone industry, which is notorious for its secretive nature. The method also reduced the cost of tooling as it allows for a considerable range of tubing sizes to be accommodated. This progress in manifold handling in a range of tubing size also allows reductions in the minimum distance between connections. While distance is normally not a restriction, the possibility of closely connecting several filters in parallel within a manifold offers significant advantages.

When introducing platinum silicone, the initial materials increased cleanliness, but had a lower pump peristaltic life in comparison to peroxide silicone. The need to increase pumping volumes while reducing pump head speeds heralded the option of multi-head pumps. These allowed one inlet to be split into two pump heads timed 120 degrees apart which gave almost twice the volume in pumping at the same pump head speed. These also increased the tube life in a pump by effectively slowing the head speed for the similar volume transfer.

Several pump manufacturers have offered pump configurations to take advantage of this development; however, initially these “double-y” (Figure 3) assemblies had to use steel or plastic Y connectors and were notoriously vulnerable to connection failure. The development of an effective all silicone aseptic connection system occurred in 1997 and completely resolved this issue - *Figure 3*. The multi-head idea has been developed into a triple Y which maximizes peristaltic flow capacity on a specific tubing size, utilizing three pump heads each offset from the other by 120 degrees.

While manufacturing, tubing manifolds and aseptic silicone ends (Figure 4) together with lightweight clamps were maturing, there was a limitation on the possible working pressure capability. There was a need to incorporate the new technology with the increased pressure capability of polyester braid reinforced tubing. Initially, braid reinforced silicone required crimped stainless steel end fittings which is still the only method to utilize the maximum possible pressure capacity of the tubing. However, using steam at approximately 2 bar (35 psi) is the normal maximum requirement. The manifold technology has encompassed this need and now allows for braid reinforced tubing up to 1/2" (12.7 mm) to be used. There is also the choice of aseptic silicone ends to be molded to braid reinforced tubing up to 1" (25.4 mm) ID.

The use of braid reinforced tubing manifold systems allows them to be Cleaned-in-Place (CIP) or Sterilized-in-Place (SIP). The reassurance that there is a mechanical bond holding the tubing in place with a consistent pressure capability also has reduced the concern over potential leaks.

Over the past 10 years, the cost of the platinum cured silicone resin has reduced. This drop has been a direct result of increased usage and the introduction of several competing raw material suppliers entering the market place. In 1990,[1] Dow Corning was the almost exclusive raw material manufacturer to processors supplying the pharmaceutical, biopharmaceutical industry. However, in the early months of 1997, GE Silicones, through strategic partnerships, also became a significant raw material supplier. They were joined by Wacker, Applied Silicones, and Rhodia, as pharmaceutical companies validated multi-source procurement. These two facts occurring over the past four to five years also have allowed platinum silicone tubing to become a commodity. The standards and specifications also have improved even though the cost has either remained static or fallen.

However, the growing use of silicone versus the natural rubbers in tubing did not transfer to its use for closures or stoppers as quickly. It is only in the past 10 years as focus on process validation has increased, that natural rubber stoppers, prone to cracking with age or exposure to heat, have started to

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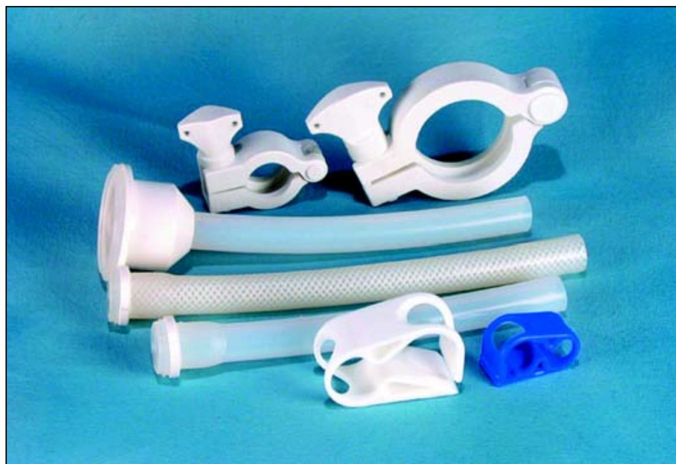

Figure 4. Molded sanitary ends with lightweight clamps.

be replaced with silicone. Initially, closures and stoppers were molded in peroxide cured silicone, but as standards improve, those made of platinum silicone are being adopted in line with the purity demanded of the tubing.

Continuing development has led to stoppers or closures with incorporated tubing and pinch clamps - *Figure 5*. The ability to have a pre-assembled stopper significantly reduces time and effort required in sampling or production. The capability of these simple closure assemblies to be supplied sterile (gamma irradiated) can give additional savings by eliminating the need to autoclave.

There are occasionally special requirements in the area of closures. In the late 90s, a need arose for a silicone stopper that had inert properties, but could accept long-term exposure to one large manufacturer's concentrates. The physical characteristics of the concentrate meant it was attacking the regular silicone closure in a similar manner to a solvent. Fluro-silicone was tested, passed USP 23 class VI together with internal requirements, and functioned well on a prolonged six-month contact assessment. Fluro-silicone has historically been used in applications where flexibility, heat resistance, and the ability to tolerate petro-carbons were required. As a material, its application is restricted because of its higher cost when compared to platinum cured silicone: the cost differential is almost 10 times the cost as a raw material. Overall, its excellent characteristics make it a viable material in some applications.

Cleaning and reuse of silicone is now a thing of the past as the cost of validation and cleaning, now far outweigh the cost of replacing the tubing or assembly. The advent of manifold technology also has brought possible costs into focus. The apparent additional cost of purchasing an assembly as opposed to the components should be carefully examined. When we take into account the labor to assemble the silicone manifolds, the possible exposure of employees to RSS as well as the potential for a leak, the cost variance is small. If we then look at the process validation of these connections and the confidence that these assemblies can be supplied exactly to a specification, the savings using pre-assembled manifolds can be considerable.

Silicone manifold production has evolved to include full manifold kits. Packaging all silicone assemblies required in a single production run or campaign together ensures handling is simplified for the production staff. Preparing all manifolds ready for autoclaving can aid in simpler SOPs as well as ensuring readiness at the set up of a system. Within the kits, pinch clamps may be incorporated in the assemblies. The introduction of pinch clamps can reduce the concerns of correct assembly, ensuring simpler compliance. The existence of a kit also allows for the option of a single lot number for materials within the assemblies, reducing the possible testing requirements. As the development of these products unfolds, simple, but sometimes important points come to light. Pharmaceutical processors are now acknowledging the true costs of leaks and resulting documentation, re-processing, and product loss. A secure system, possibly supplied sterile, which will allow the end connections to be steam sterilized in place is now the next prophesied step.

As a result of the potential national training program for the FDA field investigators via e-education currently being discussed, there will be a better understanding of new technologies. Because of the efforts being employed by the FDA, the developing partner EduNeering together with ISPE, we will see the introduction of comprehensive courses leading to accredited qualification on line. One possible result of this will be a common expectation for FDA inspections and audits. We now know that as an industry, pharmaceutical companies also can benefit from incorporating the very same educational tools within their training requirements that the FDA investigators employ. The site will clearly facilitate a common understanding of new technologies as they emerge. The time spent in detailed re-assessment of any improvements within individual pharmaceutical facilities will thus be reduced. As we continue to meet these challenges, it is comforting to know that not only are we reducing possible 483s (FDA observation or warning letters), but also reducing the need for re-evaluation inspections.

The development of an Internet based education and accreditation program was described at an ISPE meeting in Princeton, NJ on October 4, 2001 featuring the following speakers: Jerry Roth, ISPE VP of Education; Gary German, Director Division of Human Resources Development, Office of Regulatory Affairs; Doug Ellsworth, FDA NJ District Director, and Chairman Field Drug Committee. The FDA, together with its partner EduNeering, has initiated the first stage of the national e-education system which went live on-line September 27, 2001. Ellsworth stated that, supported by his department, he is committed to achieving a national education system for all FDA employees, which will include all investigators for the pharmaceutical industries.

From an initial view of the site and their comments, it seems clear that the FDA is encouraging any industry to make use of the same education system. The likelihood of non-compliance during an audit could be drastically reduced if both the company being audited and the auditors shared a common understanding of current regulations and requirements. They also mentioned, as a heads up, that because resources after the September 11 tragedy are understandably stretched, re-inspections would not be tolerated without penalty after the initial re-evaluation. German stressed that multiple re-assessment of cited non-compliances would not be acceptable in the future. His comment "Non-Compliance may be met with Disgorgement of Profits" emphasized the post 9/11 FDA policy.

It is clear that continuing efforts to understand the pertinent issues and learning about the possible solutions are likely to be high priorities within the industry. The quest for continuous improvement and its inherent understanding of issues together with potential solutions comes as a clear corollary to the constant pressure of time commitments of managers and

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supervisors within the pharmaceutical industry. Silicone connections are one particular area where we have the technology to resolve the known issues and as new challenges are discovered, there are specialist companies already poised to help provide solutions.

Looking at the past 50 years reveals that the major changes have taken place in the past 10 years. We also can see that faster information transfer has driven the new developments. With the development of the FDA "Team Biologics," combined with reductions in drug concentrations, the need for safer aseptic production systems continues to be an industry focus. These factors have spurred the development of the products needed to meet the current challenges. Silicone tubing, braid, and hose can now meet the more stringent aseptic requirements. Process engineers, system designers, and development specialists now have the option of improved, cleaner, safer silicone connection systems. Understanding the increased demand for improvements, the technology to transfer information in seconds will continue to drive the search for new innovations and solutions. Today, the biggest hurdle to incorporate these new techniques is the amount of documentation and time required to validate a change in process and its inherent cost. Perhaps soon we will see a simplified answer to this dilemma, allowing obvious improvements to be adopted into production in a timely manner. With the increasing demands placed on organizations, there may be a need for a standardized validation protocol, possibly developed by the cooperation of the processors and manufacturers of these systems. One thing is inevitable: change. The only variable is how we influence it.

Figure 5. Sampling bottle assembly.

Consideration to specific uses and applications will be given in a future article.

References

1. United States Patent, 5,320,389, Dupont Jr., Quick-Connect Sanitary Hose Fitting.

2. United States Patent, 6,290,265, Warburton-Pitt et al, Tubing and Connector Assembly and Method and Molding.

3. United States Patent (allowed patent application), Warburton-Pitt, Single-Step Method of Manufacturing a Silicone Tube Manifold.

About the Author

Stephen Warburton-Pitt attended Isleworth Polytechnic London where he received his HND in engineering, 1971. Founding his first company in 1972, he developed a reputation in Europe as a specialist in extruder screw design and manufacture. In 1979, he presented the first purpose built silicone extrusion system manufactured by another of his companies at Harrogate, England. In 1981, he received a commendation from Czechoslovakia for advancements to silicone processing. In 1982, British Aerospace adopted his silicone extrusion technology. During 1984, he introduced the first silicone extrusion system to Israel. During the period 1987-90, he was a supervisor/sponsor at Nottingham Polytechnic of doctoral research into Computer Integrated Manufacture (CIM) of extruder screws. Moving to Israel with his family in 1990, he was employed there as export marketing director for Degania Silicone. He was elected in 1991 to the International Baby Bottle and Nipple Manufacturers Association based in Paris, France, representing Israel. During his time in Israel, he developed several new medical devices including a new treatment for Keloid and Hypertrophic scarring. In 1996, he was employed by Nalge Nunc International to run the newly acquired Sani-Tech Silicone Division in Sparta, NJ where he introduced new processing technology as well as a fully compliant cGMP quality system. Throughout his 31 years within the plastic and rubber industries, Warburton-Pitt has held board level positions with 10 companies: six in Europe, one in Israel and three in the US. In nine of these companies, he was either president or managing director. A history of innovative developments has followed Warburton-Pitt throughout his diverse career. He is considered one of the worlds leading silicone-processing specialists. Warburton-Pitt, a member of the ISPE NJ Chapter, is currently the President of Truseal USA Inc. located in Dover, NJ.

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This article compares single-use technology and traditional stainless steel vessels.

Reprinted from
PHARMACEUTICAL ENGINEERING®
The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

Quantitative Economic Evaluation of Single Use Disposables in Bioprocessing

by Andrew Sinclair and Miriam Monge

Introduction

In a biopharmaceutical manufacturing environment where there are concerns about cost and available capacity, companies need to take time to analyze their processes and look at the technology available allowing process optimization.

In this article, a study comparing the following two technologies used in biomanufacturing is presented:

1. single-use disposable technology
2. traditional stainless steel vessels

An overview is presented of the concerns and issues facing the biomanufacturing industry in relation to facility design as well as an introduction to the concept of disposable bag technology. This is followed by a presentation of the Process Comparison and a Cost of Goods (COG) model comparing the two technologies.

Manufacturing Facilities for Biologicals

What are the major concerns for biologicals manufacturers in the case of a new facility design or indeed within their existing processes?

Safety

Today, the main Good Manufacturing Practice (GMP) deficiency reported from biopharmaceutical plant audits is linked to cross contamination, which represents 15% of total deficiencies.

Lack of Flexibility

Plants are designed for specific product processes.

Maintenance

Biologicals plants are extremely complex, requiring expensive maintenance.

Long Construction Time

Average construction time is two to three years or more followed by extensive validation - the commissioning phase may take several months. Companies may lose the battle to get their drug on the market ahead of the competition.

Lack of Production Capacity

As reported in Biopharm Europe,[1] in relation to the number of biologicals in the pipeline, the industry is facing a serious capacity shortage problem over the next five years.

Capital Intensive

Some of the sums spent on new biologicals facilities represent in excess of 1 billion Euros ($887,800,000).

Process Costs

One of the biggest costs in biomanufacturing is the cost of transfer of sterile fluids (such as product and reagents) through different process steps located in different parts of the facility. Traditionally, the logistics of fluid transfer have been handled through product piping, stainless steel vessels, routing manifolds, and valves. All this equipment has to be cleaned and sterilized. Equipment validation is required before re-use. As an example, a typical Clean In Place (CIP) cycle for vessels ranging between 100L to 1000L can take between 1.5 to 2.5 hours. If the process is classed as totally sterile, vessels need to go through an additional Steam in Place

Figure 1. Manifold of 10 x 50L bags stored in racking system for optimized use of space. The system can be moved around the facility easily.

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Step	Step (min)	Elapsed (min)
PW Rinse 1	5	5
Caustic Fill	1	6
Caustic Recirculate	10	16
PW Rinse 2	5	21
Acid Fill	1	22
Acid Recirculate	10	32
PW Rinse 3	15	47
WFI Rinse	5	52

Table A. Full CIP sequence.

(SIP) cycle, which with steaming, vessel cooling, and hydrophobic filter integrity testing may take an additional three hours, possibly more.

Traditional equipment preparation and validation for sterile fluid handling is extremely time consuming; meaning production capacity is not being optimized.

Before looking at the Process Comparison model, a short introduction to disposable bag technology will be presented.

The Concept of Single-Use Disposable Bag Technology

Single-use bag systems manufactured in a range from 50ml to 3000L are intended to replace glass bottles or stainless steel vessels used for sterile fluid handling in process. The bag systems are provided pre-assembled, sterile and pyrogen-free, ready for process-use. The systems can be customized according to customer specifications.

Suitable bag support systems are provided across the scale, designed for space saving and ease of maneuverability around the facility. Movable racking systems are provided for the bags 5L - 50L (Figure 1), and specially designed, stackable container systems (ideal for cold storage of buffers for example) have been developed for bags 100L to 3000L (Figure 2). A new modular range has been developed recently as a standard item allowing customers to choose the modules required to build the container according to their process requirements

The bag systems are manufactured according to GMP and are tested according to US and European Pharmacopoeias, biocompatibility testing is carried out according to ISO 10993.[2] Chemical compatibility testing of bags and solutions is carried out according to ASTM.[3]

Extractables

Extractables tests based on storage of WFI are carried out on the bags. All this information is supplied to customers who have validated the bags that are regularly used for sterile fluid handling in the monoclonal antibody manufacturing process. Customers who use the bags in their process include well-known and respected contract manufacturers.

Applications

The bags can be used for a wide variety of bioprocess applications in Upstream Processing (USP) and Down Stream Processing (DSP) the most common being:

- open bag systems for media and buffer formulation before sterile filtration
- closed bag systems for storage of media, buffer, intermediate, and bulk final product
- Manifold systems (Figure 3) which are a series of interconnected bags around a common central filling line allowing simultaneous distribution and sterilization of the fluid stream. In many cases, the manifolds are fitted with a disposable sterilizing filter capsule on the filling line, 0.2 or 0.1μ. Dispensing manifolds allow the dispensing of a volume of liquid made up for a given batch (cell culture media, buffers, intermediate, or final product) into several bags of smaller volume. The bags are then stocked and used according to the needs of the given process.

Sampling manifolds can be used to allow multiple samples to be taken from a perfusion bioreactor, for example.

So what impact can disposable technology have for biopharmaceutical manufacturers? These aspects can be seen more distinctly in the Process Comparison model, but can be outlined as follows:

Capital Investment

Equipment

There is a reduced requirement for media, buffer, and product hold vessels; as well as reduced number and size of CIP skids, and reduced utility systems capacity. Containers for the bags and tube welders will be required.

Reduced Floor Area in the Facility

The requirement for contained areas is reduced through use of tube fusing systems to make aseptic connections from bag to bag instead of making connections under LAF cabinets. The containers can be stacked up for maximum space saving within the facility.

Validation Requirements

IQ/OQ/PQ time required for disposable technology in the commissioning phase of a new facility is considerably less than the

Figure 2. Stackable container systems for space saving, allowing bag manipulation in place.

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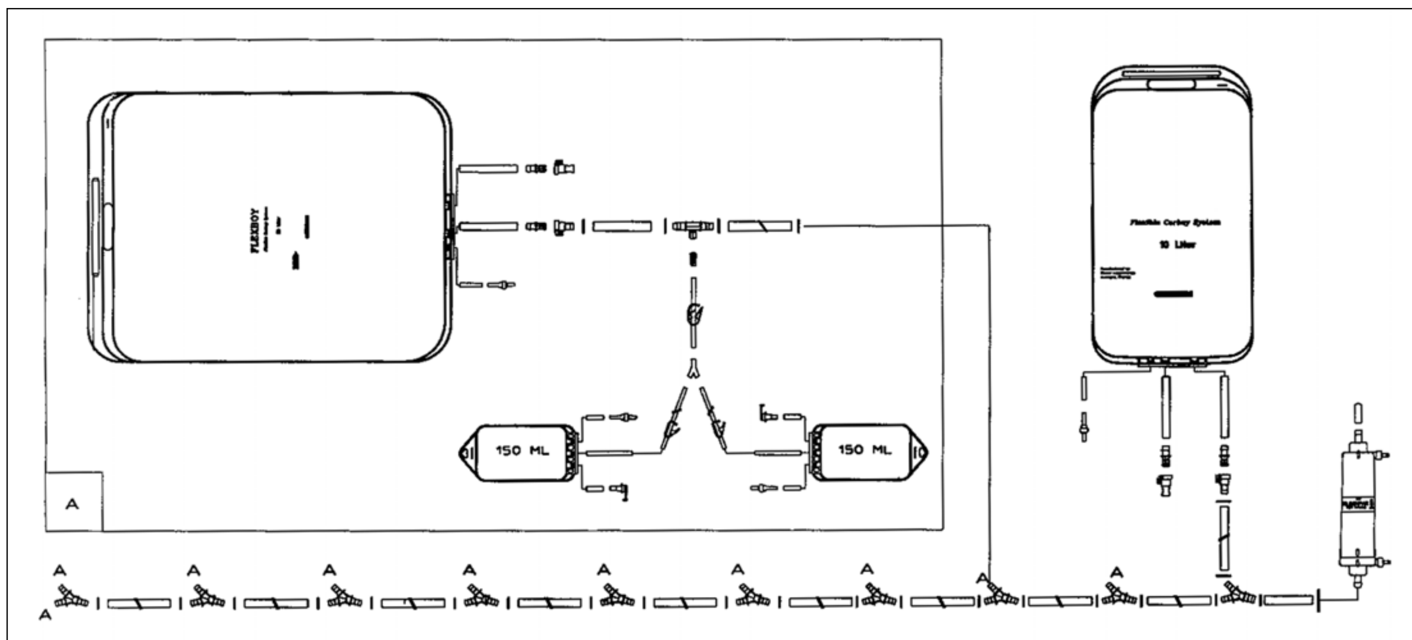

Figure 3. Manifold allowing simultaneous distribution and sterilization of the fluid stream.

time required for traditional equipment (this is not represented in the model, but will be part of a future study).

Operation

Productivity

Disposable bag technology allows immediate equipment turnaround with no cleaning, sterilization, or revalidation time required, and no downtime while vessels are cleaned.

Utility Consumption

With disposable technology, there is no cleaning required and a reduced requirement for utilities such as WFI/Pure Water, clean steam (only required for specific process equipment), and CIP chemicals

Maintenance

Stainless steel vessels require regular maintenance, which is not required with bag technology.

Validation

There are reduced annual validation requirements required for CIP and SIP.

Labor

There is reduced labor requirement as is shown in the model.

Flexibility and Efficiency in Process

The size of the bags can be adapted to a variety of batch sizes matching make up of buffers to requirements with no waste - buffers can be available just in time.

Safety

The bag technology functions in a closed circuit (no need for an air-vent filter) moving toward the total-containment concept that the FDA prefers. As a single-use system, bag technology eliminates the risk of cross contamination.

Limitations of Bag Technology

The specific limitations of the bag technology in bioprocessing are:

- maximum available single-use bag volume of 3000L
- Mixing in bags 100L and above is currently limited to recirculation loop systems; studies have shown mixing to be most effective in the Flexel 3D bag (100l and above) when recirculating from bottom to top. Mixing in bags up to 50L can be most effective using an oscillating tray system creating a wave effect in the bag.
- issues related to mixing such as mass transfer and heat transfer

Introduction to the Process Comparison and Cost of Goods Model

The features and benefits of single-use disposable technology for biomanufacturing described above are known to us and appreciated by those using the technology. However, as a technology representing a new concept, there is a need to present more scientific evidence to support the claims.

The proposition of a tool allowing direct comparison of the two technologies through process simulation was therefore most attractive.

The modeling technique used is discrete event modeling as opposed to continuous modeling. Discrete event deals with time related events, mapping process sequences as opposed to continuous modeling that simulates time through equations. With discrete event, processes are defined by time; sequences

Material Consumption/ Batch	Bags	Vessel	Vessel - Bags
WFI	19,500L	160,500L	141,000L
Purified Water	34,500L	240,000L	205,500L
Steam	33kg	181kg	148kg
Dilute caustic for CIP	450L	2,580L	2,130L
Dilute acid for CIP	450L	2,580L	2,130L

Table B. Reduction in consumption of key materials. Comparing a disposable bag with stainless steel vessel process (based on retrofit case).

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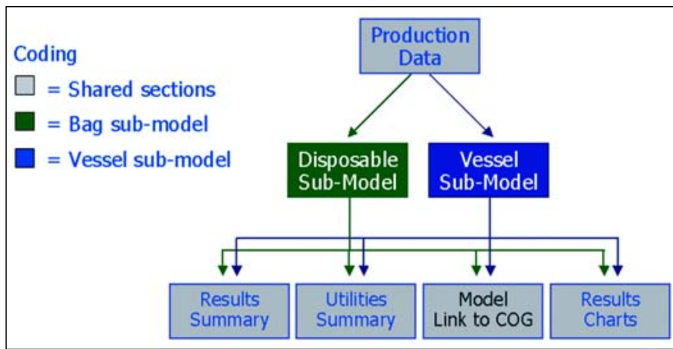

Figure 4. Process simulation structure.

of operations and scheduling constraints can be clearly seen in the simulation.

The objectives of the simulation comparing the two technologies for sterile fluid handling in process are:

- optimization of capital requirements by maximizing asset utilization
- Measuring production rates
- optimizing production

Basis

The basis for the comparison is a commercially relevant monoclonal antibody process. Using information in the public domain, a process operation was developed that mimics many industrial operations. The manufacturing route adopted consists of a fermentation system run at 2000L scale. This scale of operation was selected on the basis that this is a common size employed for the supply of clinical quantities of material and for in market supply for the smaller indications. It also is a scale of operation that can be accommodated by commercially available disposable bag systems ranging from 50L to 500L.

The modeled process consisted of the following operations covering the production of the monoclonal antibody from inoculation of the seed fermenter through to bulk purified sterile filtered product:

- Seed Fermenter
- Production Fermenter
- Harvest Centrifuge
- UF Concentration Diafiltration
- Protein A Chromatography
- UF Concentration Diafiltration
- Ion Exchange Chromatography
- UF Concentration Diafiltration
- Gel Permeation Chromatography

The output from this manufacturing operation would supply a fill finish operation. Disposable bag technology is used wherever it is technically feasible within the process operation. In particular, flexible bags are used to replace fixed sterile vessels where there is no significant mixing duty, namely:

- Product Hold
- Media Hold
- Buffer Hold

Vessel liners are used for all other operations where a quick changeover is required between batches and where the operation is not sterile, namely:

- Media Prep
- Buffer Prep

Analytical Approach

A resource constrained time based simulation was used as the basis for the analysis. This allowed a comparison of two parallel production lines of the same size to be made. The main difference between the two production lines is that one uses disposable bags and the other uses stainless steel vessels. The process steps and all the associated major equipment are included in the simulation model. Running the operations in simulated time allows us to compare the performance of the two production lines at start up and during normal operation under varying conditions. Quantitative data relating to the performance of each production line allows us to compare the alternative technological approaches. In this exercise, quantitative data was generated for:

- high quality utility systems, water and steam
  - assessing size of utility generator
  - assessing storage capacity requirements
  - calculating consumptions
- measuring production rates
- evaluating CIP requirements
- flexible bag model
  - number of the different bag types that are used per batch
  - the maximum number or bags (broken down by size) in use at any one time
  - the size of the utility systems required to support bag operation
- fixed vessel model
  - identify the size and number of fixed vessels required to run the plant
  - the size of the utility systems supporting fixed vessel operation

Data Structure

The database used by the simulation contains all the data relating to the simulation, specifically all process data, sequencing information and equipment details. This data is used as the basis of the process simulation. After the simulation is complete, performance data for the two production lines is automatically exported to a COG spreadsheet.

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Bag Size L	Number Used/Batch
50	51
100	18
200	12
500	12

Table C. Disposable bag requirements per batch.

Process Simulation

Each production line within the simulation shares the same production database - *Figure 4*. Each of them differs in the following ways.

Each sub model can have the following independently set parameters:

- Water Systems WFI and Purified Water
  - Storage Capacity
  - Distribution Limit
  - Generation Rate
- Clean Steam Generation Rate

In addition, the fixed vessel sub model has the number and size of vessels used for buffer, media, or product hold explicitly specified. Other parameters that can be changed by the user are titer in the cell culture vessel, failure rate for a cell culture vessel, and CIP failure rate.

CIP

Cleaning of equipment in biotech facilities is a major user of high quality utilities. The principle used as the basis of the cleaning regimes is that "All surfaces that come in contact with products shall be clean and free of surface solids."[4] This requires that the cleaning regime employed be based upon the nature of the materials handled. In this case, the following sequence was used for all vessels coming into contact with product or cell culture media.

The exact sequence differs from company to company, and this sequence is based upon an article published in1990.[5] The sequence in table A differs from the 1990 paper in that it does not allow for recycles of wash fluids and all rinses are once through. Air blow are included, but not shown in the sequence. All other cleans, particularly buffer preparation and hold vessels, employ a reduced cleaning regime comprising a single hot WFI rinse for seven minutes. CIP is required after every vessel use.

Cost of Goods Analysis

At the end of a simulated operation, the process simulation model automatically transfers key performance data to the COG spreadsheet. The same data set is used for both the stainless steel vessel sub model and the single use disposable sub model. This data set defines the process equipment used, scale of the operation, and schedule related information. In addition, specific data relating to each of the sub models is transferred relating to:

- Capital requirements for:
  - CIP
  - utility systems
  - number and size of hold vessels used by the vessel sub model
  - number of disposable bag containers required for the disposable sub model
- Material Consumptions per Batch:
  - All critical utility consumptions
  - CIP chemical usage
  - Number of disposable bags used by the disposable sub model
- Production rate

The data set is used to compare the two sub models. Figure 5 illustrates the relationship between the data generated by the simulation and the COG models modules.

Equipment

This module generates a priced equipment list for the major items for each of the sub models. The module function is to provide a cost estimate for the major equipment items for each of the sub models. The Disposable Sub Model equipment list includes specialist items such as tube welders. The costs for capital items are taken from an internal database of costs built up from recent projects (both US and European) and vendor information. Where equipment sizes do not match, cost estimating factors are used.[6]

Capital Estimation

The total capital requirement for each sub model is estimated by applying Lang factors[7] based on the major equipment cost estimate. The factors used were based upon recent projects of an equivalent technology and scale. This capital estimate is used to calculate an annuity charge based on eight-year plant life and a 15% cost of capital; this annuity charge is used for the COG comparison.

Materials Estimation

This estimates the requirements for consumables, materials, and indirects. Disposable bag costs are separated out into fermentation and purification consumables. (Cost for bags were supplied by Stedim, other materials are obtained from suppliers).

Labor Estimation

Labor head count is estimated within the COG model. Labor charge allocation to a manufacturing batch is based on the allocated time that direct operation staff spend in production on a particular batch. The allocated time is generated from the process simulation. The quality cost allocated to each sub model is a variable in the COG model and allows a rapid evaluation of likely potential savings and their impact when using disposable bags. Savings potentially accrue from re-

Capital Estimate	Euros (US Dollars)
Stainless Steel Vessel Sub Model	€24,970,532 ($22,168,838)
Disposable Bag Sub Model	€19,670,355 ($17,463,341)
Overall Capital Saving	21.2%

Table D. Capital requirements for the two sub models.

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duced cleaning and sterilization validation costs, reduced maintenance, etc. Estimates were made for direct production labor. Other labor overhead (quality, engineering, logistics, etc) was factored using figure from Biopharm Services industry benchmarking studies.

User Interface
This allows simple "what if" analysis to be carried out. For example, changing exchange rates, switching between existing and new investment scenarios etc.

COG Comparison
This is the main output, and for each sub model, compares capital requirements for new installations and cost of goods for both new and existing scenarios. The COG comparison includes:

- materials, fermentation materials, fermentation consumables, purification materials, purification consumables, engineering spares
- labor, direct fermentation, direct purification, plant overhead, QA/QC
- others, indirect materials, utilities
- capital charge

Outcomes

The evaluation identifies distinct differences in the performance of the disposable bag production line when compared to the fixed vessel production line. Much of the difference arises from the reduced requirement for CIP. This resulted in reduced consumption of utilities and chemicals per batch by the disposables sub model when compared to the vessel sub model - *Table B and Figure 6.*

This is offset by an increased consumption of plastic disposable bags by the disposables sub model expressed as number used per batch at each specific bag size, the exact number used is listed in Table C.

These tangible differences have economic and environmental implications both for new and existing installations. The exact extent will depend upon the nature of the actual process and the scale at which it is being operated. The following sections quantify the benefits with specific reference to the 2000L monoclonal antibody process.

New Build

In the situation of a new capital investment, the objective is to minimize capital investment required for a specific throughput. The size and capacity of the utility systems supplying the stainless steel sub model and the disposable sub model were optimized to suit each production lines' requirements. For both the sub models, the following systems were optimized:

- Water For Injection - optimize generation and storage capacity
- Purified Water - optimize generation and storage capacity
- Steam - optimize generation

For the stainless steel vessel sub model, the number of support vessels had to be determined for each of the following operations:

- Media Preparation
- Media Hold
- Buffer Preparation

Cost of Goods in Euros (US Dollars) per Batch		Bags	Vessel	Vessel - Bags
Materials	Fermentation materials	€26,048 ($23,125)	€26,048 ($23,125)	€0 ($0)
	Fermentation consumables	€46,069 ($40,900)	€48,214 ($42,804)	€-2,144 ($-1,904)
	Purification materials	€23,294 ($20,680)	€23,294 ($20,680)	€0 ($0)
	Purification consumables	€29,422 ($26,121)	€41,440 ($36,790)	€-12,018 ($-10,669)
	Engineering spares	€2,402 ($2,133)	€2,402 ($2,132)	€1 ($1)
	Sub total	€127,236 ($112,960)	€141,397 ($125,532)	€-14,162 ($-12,572)
Labor	Direct fermentation	€41,707 ($37,027)	€41,695 ($37,017)	€11 ($10)
	Direct purification	€8,604 ($7,639)	€8,604 ($7,639)	€0 ($0)
	Plant overhead	€38,620 ($34,287)	€38,610 ($34,278)	€10 ($9)
	QA/QC	€56,304 ($49,987)	€45,043 ($39,989)	€11,261 ($9,998)
	Sub total	€145,234 ($128,939)	€133,952 ($118,923)	€11,282 ($10,016)
Others	Indirect materials	€9,056 ($8,040)	€9,054 ($8,038)	€2 ($2)
	Utilities	€33,750 ($29,963)	€26,579 ($23,597)	€7,171 ($6,366)
	Sub total	€42,806 ($38,003)	€35,633 ($31,635)	€7,173 ($6,368)
Capital Charge		€210,806 ($187,154)	€166,016 ($147,389)	€44,790 ($39,765)
Total		**€526,082 ($467,056)**	**€476,998 ($423,479)**	**€49,084 ($43,577)**
	Overall Cost of Goods Saving Bags vs. Vessels			9%

Table E. COG savings comparing disposable bags sub model to stainless steel vessels sub model - new build.

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Cost of Goods in Euros (US Dollars) per Batch		Bags	Vessel	Vessel - Bags
Materials	Fermentation materials	€26,048 ($23,125)	€26,048 ($23,125)	€0 ($0)
	Fermentation consumables	€46,069 ($40,900)	€48,214 ($42,804)	€-2,144 ($-1,904)
	Purification materials	€21,636 ($19,208)	€21,636 ($19,208)	€0 ($0)
	Purification consumables	€29,422 ($26,121)	€42,650 ($37,865)	€-13,228 ($-11,744)
	Engineering spares	€2,651 ($2,354)	€2,401 ($2,132)	€250 ($222)
	Sub total	€125,827 ($111,709)	€140,949 ($125,134)	€-15,122 ($-13,425)
Labor	Direct fermentation	€41,697 ($37,019)	€41,681 ($37,004)	€16 ($15)
	Direct purification	€36,823 ($32,691)	€18,131 ($16,097)	€18,692 ($16,594)
	Plant overhead	€42,623 ($37,841)	€38,596 ($34,265)	€4,026 ($3,576)
	QA/QC	€62,139 ($55,167)	€49,711 ($44,133)	€12,428 ($11,034)
	Sub total	€183,282 ($162,718)	€148,119 ($131,500)	€35,163 ($31,218)
Others	Indirect materials	€14,134 ($12,548)	€10,766 ($9,558)	€3,368 ($2,990)
	Utilities	€35,136 ($31,194)	€27,701 ($24,593)	€7,435 ($6,601)
	Sub total	€49,269 ($43,741)	38,467 ($34,151)	€10,803 ($9,590)
Capital Charge		€0 ($0)	€0 ($0)	€0 ($0)
Total		**€358,379 ($318,169)**	**€327,535 ($290,786)**	**€30,844 ($27,383)**
	Overall Cost of Goods Saving	8%		

Table F. COG savings comparing disposable bags sub model to stainless steel vessels sub model - retrofit.

- Buffer Hold
- Product Preparation

For the single use disposable sub model, a number of capital sensitive parameters were evaluated specifically.

- CIP skid requirement (to match the reduced CIP requirement)
- Number of bag holders required containing in process disposable bags
- Specific bag processing equipment, i.e., tube welders
- Reduced floor area requirements (stacking bag systems minimizes floor area required to hold and process buffers)

There is a significant reduction in the capital requirement for the single use disposable bag production line compared to that required for the one based on stainless steel vessels - *Table D*. This is a result of a reduced requirement for CIP by the disposable bag sub model. For example, the WFI generation capacity required for the disposable bag sub model is around 400L/hour; however, the stainless steel vessel sub model requires 1500L/hour of WFI capacity to achieve the same production rate. This effect is seen throughout the utility systems. Together with the reduced vessel count and CIP requirement of the disposable bag sub model, this results in a capital saving of around 5 million Euros ($4,439,000).

The question remains as to whether this reduction in capital results in a reduced cost of goods for the monoclonal antibody. In this analysis, it does. In Table E, capital is factored in an amortized charge: this is based upon an eight-year plant life and 15% cost of capital and 5% residual value in the plant after the eight years. In addition, for the disposable bag sub model, there are higher consumable costs resulting from bag use. These are offset by utility savings and savings in quality costs. In this analysis, it estimated that the quality head count would be reduced by five for the disposable sub model case, resulting from a reduced ongoing cost requirement for CIP and SIP annual validation and reduced paper work. The overall impact is a saving of 9% on the cost of goods resulting from a new plant installation based on disposable bags when compared to one based on stainless steel vessels.

Retrofitting an Existing Facility

When considering the retrofit of disposable bags within an existing facility, attention has to be paid to the nature and the scale of the operation. By definition, if manufacturing is running to design capacity and constrained by the size of the equipment in the main production line, it is unlikely that the substitution of stainless steel vessels by disposable bags would have any positive impact on the cost of goods. The introduction of disposable bags by definition will not change the existing infrastructure. However, it is the case that in many facilities constraints are imposed by lack of adequate utilities and even floor space. The reason being that it is difficult, using current design methodologies, to rationally size utility systems. Our view is this can only be done by the adoption of tools that use resource constrained discrete event modeling as their basis.

In a situation where the support systems are constraining the throughput of the main production line, we would expect that capacity would improve once the constraint is released. In this case, this is what happens.

To evaluate how disposables may assist in the case where utilities constraints are impacting on production, we have constructed a case study where both the disposables sub model and the fixed vessel sub model share the same utility system sizes. In this case, the WFI is constraining the throughput of

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the fixed vessel model. WFI generation capacity is set at 950L/hr. We know that to operate without WFI constraint, the fixed vessel model requires a WFI generation capacity of 1500L/hr.

In this instance, we are reviewing the impact of substituting fixed vessels by bags and assessing the overall impact on the cost of goods of this substitution.

When the disposables are introduced, the production rate increases from 15.3 kg/yr to 16.4 kg/yr. The introduction of single use disposables reduces the CIP requirement, and as a consequence, the demand for water is reduced and can be met by the existing utility system. This reduction in requirements means that production can proceed at the design rate. The increase in this case amounts to about 7%. The actual improvement will be case dependent and situations do arise where that increase can be significantly higher.

This time, the capital charge difference is zero and the capital employed in both production lines is approximately the same. The resulting increase in throughput achieved through the introduction of disposable bags achieves a more efficient operation and results in a net reduction in cost of goods of about 8% - *Table F*. The increased consumables cost is more than offset by more efficient use of labor and reduced utilities and indirect costs.

Environmental Impact

An often quoted concern about the use of disposable bag technology is the environmental impact of the 'additional' consumption of the single use bags. These are a very tangible and visible manifestation of waste, and it is often assumed that the use of disposable bags will result in greater environmental damage when compared to the use of stainless steel vessels. Although it was not the aim of this exercise to carry out a full environmental impact comparison of the two process options, it was possible to gain an insight into likely environmental damage/benefits based on material consumptions and capital requirements.

The study compared the results between the two models on differences in material used per batch basis for:

Disposable sub model requires approximately 100 disposable plastic bags plus associated tubing per batch amounting to around 200kg of plastic waste. In a number of facilities, this material either goes off site for incineration or into landfill.

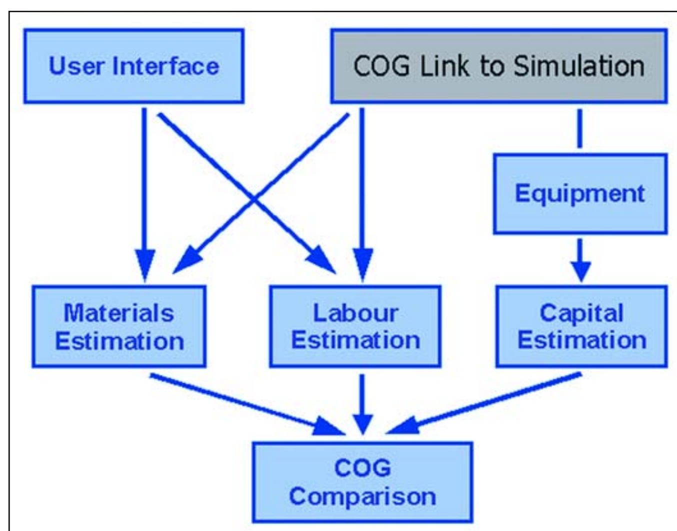

Figure 5. Structure of COG model.

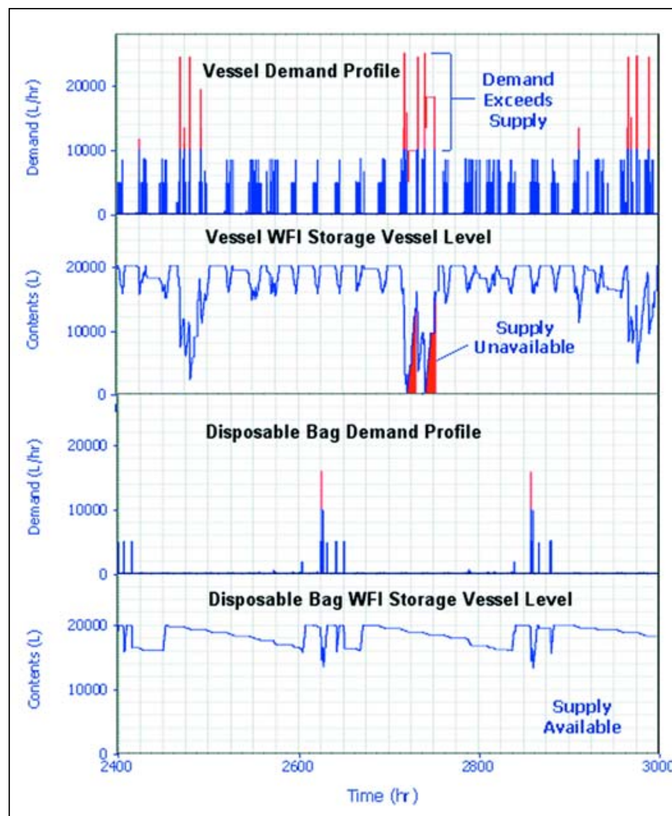

Figure 6. WFI demand and storage levels.

Fixed vessel sub model requires an additional 141 tons of WFI generated from 204 tons of Purified Water. In addition, about 4.2 tons of dilute CIP chemicals are needed (equivalent to about 100L of 40% caustic and 5L of 80% phosphoric acid). Overall, these additional materials will require treatment before discharge.

Where a new investment is being planned, the disposable-based production system also will require less plant and equipment compared to the stainless steel vessel based process.

Conclusion

By the application of discrete event modeling techniques, it is possible to simulate the whole operation of bioprocess manufacturing systems. This capability allows us to compare and quantify at a macro level the impact of new emerging technology (disposable single use bags) with a traditional technology (stainless steel vessels).

For the case considered (that of a 2000L monoclonal antibody process), it has been demonstrated that for a new installation, disposable bag technologies will result in reduced capital requirements of around 20%, which in turn result in an 8% reduction in the cost of goods. This has the benefit in reducing upfront capital spent early on in the product life cycle.

When considering substituting stainless steel vessels by bags in an existing operation, the situation is more complex. The net benefit very much depends on what is limiting production. In many bioprocessing facilities, there are problems with insufficient utility capacity, CIP capacity, and/or floor space. In these scenarios, the application of disposable bags can increase production throughput. The exact increase depends upon individual plant circumstances. In the case explored here, we saw an improvement of about 7% in throughput that equated to an 8% reduction in cost of goods.

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The commonly held view that the adoption of disposable bags results in an adverse environmental impact when compared to stainless steel vessel processing has been shown to be not necessarily true. Although not an objective of the study, the analysis showed that the increased consumption in plastic for the bags is offset by large reductions in water and CIP chemical requirements that also reduce capital requirement for new facilities. In fact, it may be the case that stainless steel based processes have a greater environmental impact, though a rigorous environmental audit is required to fully compare the two options.

References

1. Finnegan, Stephanie, Manufacturing: Biotechs Next Crisis, *Biopharm Europe,* November 2000.

2. ISO 10993-1, Biological Evaluation of Medical Devices.

3. ASTM D1239-98, Standard Test Method for Resistance of Plastics Films to Extraction by Chemicals.

4. Physical Establishment, Equipment, Animals, and Care, Code of Federal Regulations, Sec. 600.11, April 2001, 8.

5. Adams, G.A., and Agarwal, A., CIP System Design and Installation, *Pharmaceutical Engineering,* 10 No. 6, 1990.

6. Remer, D.S., and Idrove, H.J., Cost Estimating Factors for Biopharmaceutical Process Equipment, *Pharmaceutical Technology International,* March, 1991, 36-42.

7. Lang, H.J., Simplified Approach to Preliminary Cost Estimates, *Chemical Engineering,* 55, 1948, 112-113.

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Andrew Sinclair founded BioPharm Services in December 1998 to develop a technology based services business focused on all aspects of biopharmaceuticals manufacturing. The company has developed software tools and databases for streamlining and optimization of biomanufacturing systems. Sinclair has more than 20 years of experience within the fine chemicals and biotechnology sectors working for manufacturing operations (Lonza, Eli Lilly, GlaxoSmithKline) and contractors (Kvaerner, Jacobs Engineering).

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Miriam Monge has been working in the biopharmaceutical industry since 1989, based in the UK, Germany, and now in France. She joined Stedim, S.A. in 1994 as area sales manager for Northern Europe, then moved to a key account management role before taking up her new position as Product Marketing Manager, European Operations. Her aim is to make Stedim disposable bag technology a well recognized standard in the bioprocess industry.

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This article was developed from a presentation given to various ISPE Student Chapter members in conjunction with the Carolina/ Southeast Chapter Technology Show in March 2002.

Reprinted from
PHARMACEUTICAL ENGINEERING®
The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

What is Biotechnology?

by Jeffery N. Odum

Introduction

Biotechnology, as a science, is the culmination of more than 8,000 years of human experience using living organisms and the process of fermentation to make useful products for mankind. These products include such familiar items as beer, wine, cheese, bread, and more recently, biopharmaceutical drugs.

The term "Biotechnology" was first defined in 1917 by Karl Ereky as:

> *"All lines of work by which products are produced from raw materials with the aid of living thing."*

Biotechnology also may be described as:

> *"A combination of advances in our understanding of molecular and cell biology, human genetics, and how the human immune system fights disease."*

Biotechnology is the process by which 'biologics' are produced. Biologics is used as the short form of 'Biological Therapeutic Products' and generally encompass any protein, virus, vaccine, blood product, or gene transfer product.

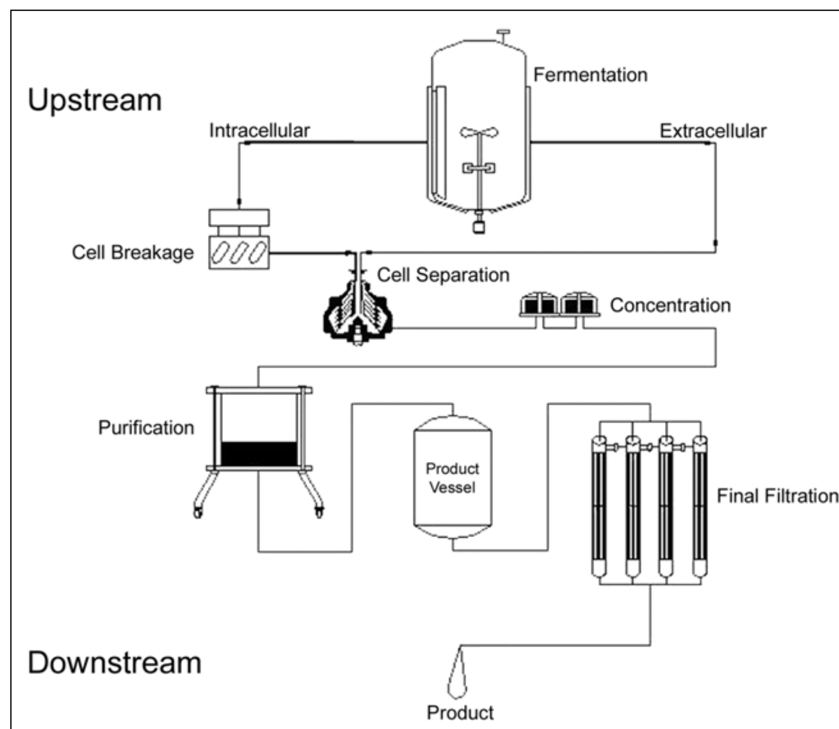

Figure 1. Upstream/downstream processing.

Biologics

Biologics are very fragile molecules that easily degrade in the digestive system, and as a result, they are injected into the bloodstream. Biologics are designed to interact with molecules outside the cell, but are typically more difficult and costly to manufacture than the traditional small molecule therapeutics.

In contrast to biologics, small molecule therapeutics comprise most of the traditional pharmaceutical drugs, which are developed via chemical synthesis. Such drugs are taken orally and absorbed through the intestine walls into the bloodstream. These are usually designed to block targets.

Therapeutic Proteins

Therapeutic proteins are produced in biological organisms through recombinant DNA technology. Such proteins include monoclonal antibodies (which recognize only a single antigen), cytokines (which act as chemical messengers), and growth factors.

Therapeutic proteins can more effectively interact with a large number of target receptors than small molecule therapeutics. This interaction is more effective in triggering the desired biological response (i.e., the biologics are more effective at "turning on the switch" to bring about the desired result).

How Does Biotechnology Work?

Recombinant DNA

Biotechnology is based on the fact that the same basic genetic material programs the cells of virtually all living organisms, regardless of the type of organism. Biotechnologists use recombinant DNA technology to transplant and/ or combine genetic material from one organism to another.

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That genetic material is Deoxyribonucleic Acid (DNA). DNA is a nucleic acid composed of two long chains of nucleotides. Each nucleotide is an organic compound, which consists of deoxyribose (a sugar) linked to one of the following four bases:

- Adenine
- Cytosine
- Guanine
- Thymine

The two chains of nucleotides are linked together by hydrogen bonds and wound round each other to form a spiral molecule, the familiar 'double helix.'

DNA may be divided into functional sections, consisting of a series of nucleotides, which are referred to as genes. Each gene contains the information required for a cell to produce a specific protein. Biotechnology requires the production of specific proteins and therefore requires the expression of the relevant gene. To isolate the gene required, the DNA containing that gene is split into fragments using restriction enzymes - a type of enzyme that can slice molecules of DNA at a precise known site in the DNA nucleotide sequence.

The fragments are inserted into cloning vectors, such as bacterial plasmids. Plasmids consist of DNA that can exist and replicate independently of chromosomes. Restriction enzymes are used to cut the plasmid DNA to allow the gene fragments to combine with the plasmid DNA. The combined plasmid and required gene is known as recombinant DNA, and the process of forming such DNA is known as genetic engineering.

Transformation

The engineered plasmid transports the required gene to host cells. The recombinant DNA is mixed with the host cells, which may be bacteria (E.Coli), yeast, or mammalian. Transformation occurs when a plasmid encounters a host cell and enters that cell through its membrane.

Cloning

Once the plasmid has entered a host cell, the cell replicates the recombinant DNA. When cell division occurs, the recombinant DNA is replicated in the daughter cells. This is called cloning.

As the host cells grow and reproduce, the presence of the recombinant DNA containing the gene for the required protein causes the host cells to produce that protein, via expression of the gene. The protein may be produced within the cell (intracellular), or it may be secreted from the cell and will be present in the growth medium.

Fermentation and Cell Culture

Fermentation is the process by which living cells obtain energy through the breakdown of glucose and other molecules in anaerobic (oxygen deficient) conditions, e.g., brewing beer. Microorganisms are cultured in fermentors and within biotechnology; fermentation refers to the large-scale cultivation of microorganisms. Fermentation is part of what is known as "upstream processing," literally, engineering and growing the cell line to be used.

Cell culture is the process of taking cells from living organisms and growing them under controlled conditions. Cell culture is a specific kind of fermentation that applies similar techniques for growing cells from plants and animals, which are cultured in bioreactors – *Figure 1*.

Once fermentation is complete, the desired product must be recovered, separated out, and purified.

Recovery

Recovery requires the separation of crude product from microbial mass and other solids and liquid medium to prepare it for purification.

Product recovery usually requires cell disruption. There are several methods by which this may be achieved, such as the use of high pressure (centrifugation – Figure 2), homogenization, mechanical grinding, or non-mechanical methods including the following:

- Freezing
- Detergents
- Enzymes

Once the cells have been successfully disrupted, separation may be performed by extraction and precipitation or filtration, which may involve microfiltration or ultrafiltration.

Purification of the required protein is achieved by using chromatographic techniques such as:

- Gel Filtration
- Ion Exchange
- Hydrophobic Interaction (HIC)
- Affinity

Filling

Filling is the process of putting the drug product into a sterile container. There are two general categories of filling:

- Bulk filling, which is defined as the placement of larger quantities (5L - 100L) of product into containers for shipment/storage.
- Final filling, which is defined as the placement of the sterile drug product into its final container/closure system, including vials, ampoules, syringes, or dental cartridges.

Bioanalysis

Nearly every process conducted in a biopharmaceutical company requires the support of analytical methods to prove safety, efficacy, and consistency. This is true in product development, process development, raw material testing, and validation. These analytical methods serve to back up regulatory submissions, support preclinical and clinical studies, monitor environmental conditions during manufacturing, and monitor the overall quality of the manufacturing process.

These analytical methods include:

- Microbial Testing for Bioburden
- Total Organic Carbon (TOC) Analysis
- Biocalorimetry
- Ultracentrifugation

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Figure 2. Centrifuge.

- Peptide Mapping
- Limulus Amebocyte Lysate (LAL) Assay
- Sterility Testing

All of these tests produce the data that forms the backbone of regulatory submissions to the FDA and other regulatory agencies.

Summary

Biotechnology is an exciting, technically complex field that holds untold promise in the potential cure of diseases. It is important for individuals to understand the basics of the science in order to appreciate the potential that it holds.

About the Author

Jeffery N. Odum, Biotech Team Leader/Senior Project Manager for CRB Consulting Engineers' Southeast Regional Office, Cary, NC, has more than 22 years of professional engineering and construction experience that has been primarily focused on process-driven technology projects. The last 12 years have been focused on the biotech and pharmaceutical industries with key assignments in both engineering and construction management. He is also a member of ISPE and PDA. Odum has authored numerous articles on GMP compliance and design and construction issues and is the author of an industry reference guide, *Sterile Product Facility Design and Project Management*, published by Interpharm Press.

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This article describes the development of a curriculum in a hands-on biomanufacturing laboratory environment. Data are presented on the production, purification, and analysis of a recombinant protein performed by undergraduate students at James Madison University.

Reprinted from
PHARMACEUTICAL ENGINEERING®
The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

Development of Biotechnology Curriculum for the Biomanufacturing Industry

by Robert L. McKown, PhD and George L. Coffman, PhD

Introduction

The biotechnology industry has come of age and is delivering on its promise to produce new drugs for the treatment of human disease. The biopharmaceutical product development pipeline is full, and the demand for new manufacturing capacity is expected to triple in the next five years. As the industry gears up for increased production, the demand for a skilled workforce to manufacture these products also will increase. The unique technological skills required for biomanufacturing are not readily found in traditional academic programs.

Historical Perspective

The term "biotechnology" was first introduced in 1917 by Karl Ereky and defined as "All lines of work by which products are produced from raw materials with the aid of living things."[1] Although the use of living systems to make a product has a long and established history, the modern definition of biotechnology is usually associated with genetic engineering and recombinant DNA technology. G. Steven Burrill, CEO, Burrill & Company (San Francisco, CA), defined biotechnology as "Recombinant genetic engineering…using biological processes to develop products," thereby preserving the original notion that biotechnology uses living systems to make products. Stanley Cohen and Herbert Boyer published the founding principles of recombinant DNA technology in 1973,[2] and in 1980, a US patent was issued describing a "Process for Producing Biologically Functional Molecular Chimeras." On April 7, 1976, the first independent biotechnology company, Genentech, Inc. (South San Francisco, CA), was founded to commercialize the newly discovered technology and the adventure began.[3]

Although many applications of recombinant DNA technology were considered, the first biotech product created with this technology was human insulin (Humulin) produced in the bacteria *Escherichia coli* to treat diabetes. Lacking manufacturing capabilities, Genentech licensed recombinant insulin to Eli Lilly and Company (Indianapolis) for production and marketing. Humulin was a major success (worldwide sales in 1999 reached $880 million) and set a paradigm that is still the driving force of the

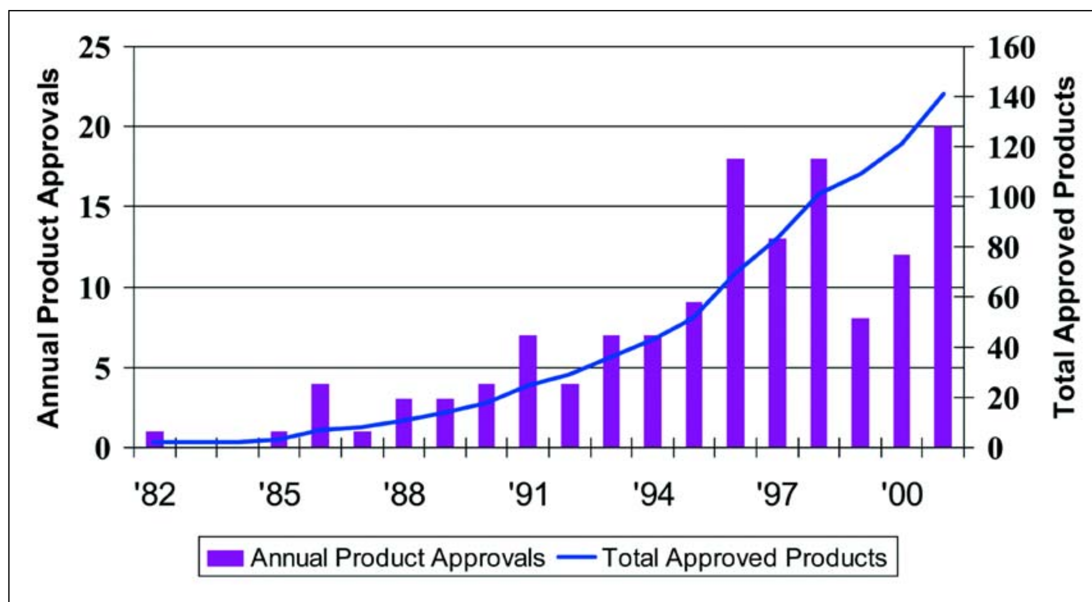

Figure 1. Annual biopharmaceutial product approvals from 1982-2001.

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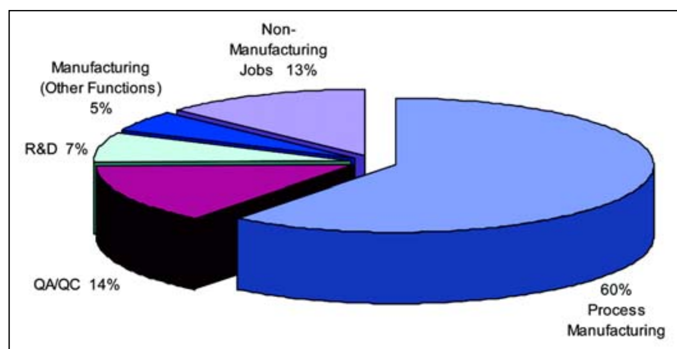

Figure 2. Distribution of the scientific and technical workforce in a typical biomanufacturing facility.

biotech industry today. Brandon Price, President, Goodwin Biotechnology, Inc. (Plantation, FL) captured the focus of the industry in the statement "The promise of biotechnology is the bioproduction of drugs so complex they can only be made in living systems."

As the first human therapeutic products generated through recombinant DNA technology began to emerge from research laboratories, it became apparent that a manufacturing infrastructure was needed to produce material for clinical trials and the commercial market. Unlike traditional pharmaceutical drugs that use chemical synthesis to make product, most biopharmaceutical drugs require viable biological host cells for the production of recombinant proteins. Using living systems as vehicles for manufacturing human therapeutics introduced issues of reproducibility, product identity, product purity, product potency, and possible contamination with human pathogens. These issues also resulted in new federal regulations governing the manufacture of biopharmaceuticals (biologics) that are still evolving. The first biopharmaceutical products to enter clinical trials utilized the established manufacturing technology of microbial fermentation; however, it soon was realized that certain products require mammalian cells grown in culture to produce a biologically active molecule. Although mammalian cell culture technology has an established history in laboratory research, it had never been scaled up to manufacture the quantities of product needed for commercial use. In addition, new purification technologies were needed to isolate a protein of interest from the complex mixture of molecules found within the cell. The unique requirements for production of pharmaceuticals in living systems presented a challenge to invent new manufacturing technologies. The early pioneers of the biotechnology industry not only discovered and cloned new genes; they also invented the technology needed to bring these products to market and in doing so provided a foundation for the biomanufacturing industry.

In the last five years, the biotech industry has come of age and is delivering on its promise to produce new drugs for the treatment of human disease. Since the approval of recombinant insulin in 1982, more than 100 new biopharmaceutical products have been approved for the market (Figure 1) and the pipeline is full. Approximately 400 new drugs requiring biological systems for production are in clinical development and the recent sequencing of the human genome combined with high throughput screening technologies will fuel the pipeline well into the future. As more new drugs enter the product development pipeline, additional biomanufacturing capacity will be needed to bring these products to market. Recent analysis suggests that demand for manufacturing capacity for protein-based drugs will triple in the next five years.[4] Certainly, the industry will respond and build the infrastructure needed to meet market demand, but bricks, mortar, and stainless steel tanks are only half the equation. A trained and technologically skilled workforce will be needed to execute the complex process of manufacturing a biopharmaceutical product.

Biotechnology Education

Biomanufacturing is a labor-intensive endeavor requiring unique skills that are not readily found in traditional academic programs. A common error made by academics, especially in the biological sciences, is that biotechnology means just molecular and cell biology.[5] While molecular and cell biology may provide a solid foundation for the research side of the biotech industry, additional highly technical skill sets are needed for the manufacturing side of the industry. Figure 2 shows the distribution of the scientific and technical workforce in a typical large biomanufacturing facility.[6] More than 90% of the jobs are in production-related areas while only 7% are in research and development. The biotech industry was founded on technology derived from basic academic research; however, it is the application of this technology that fuels the industry. The challenge in developing new curriculum in biotechnology is to find the correct balance between basic science education and the application of this knowledge to develop a market product.

Development of biotechnology curriculum is problematic in that it is an applied science rather than a basic science. Although techniques founded in biotechnology permeate research protocols in the life sciences, very few academic programs are devoted to biotechnology. In a recent survey of college programs, only 15 Bachelor of Science degree programs in biotechnology could be identified in the United States.[7] Although a few highly specialized training programs in bioprocessing have been established, biomanufacturing programs in the traditional academic sense are virtually nonexistent. This fact does not bode well for the biotech industry at a time when record numbers of new product candidates are entering the pipeline and manufacturing capacity is expanding.

A New Approach to Higher Education

In 1993, an innovative approach to higher education was launched with the enrollment of the first freshman class in the Integrated Science and Technology Program (ISAT) at James Madison University.[8] In response to a national call for fundamental change in science education, the ISAT Bachelor of Science degree program was created. A new curriculum was designed to provide students with a breadth of knowledge and skills across a variety of scientific and technological disciplines. Formal training in collaborative and leadership methods, problem-solving techniques from many disciplines, and use of the computer as a problem-solving tool were integrated into the curriculum. Strategic sectors were identified that reflect national critical technologies and include Biotechnology, Energy, Engineering and Manufacturing, Environment, Information and Knowledge Management, Health Systems, and Telecommunications. Highly qualified faculty members with industry experience in each of these strategic sectors were recruited to develop and teach this novel curriculum.

In May of 2001, the fifth ISAT class graduated and the program currently enrolls more than 800 students. By every index, the ISAT program has been a success. ISAT graduates are recruited for positions that are often filled by graduates of

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Figure 3. JMU students working on a production campaign in the biomanufacturing laboratory. Clockwise from top right; Dr. George Coffman and Curtis Jones, Maria Scherer (front) and Jo Maillet, Melissa Orr, Carl Randecker and Laura Pillor (front), and Megan Barber and Kevin Carlton (rear).

the traditional sciences, engineering, and business programs. Employers have been particularly impressed with the interdisciplinary skills and the project-orientated team approach to problem solving of ISAT graduates. It is in this environment of applied interdisciplinary education that the notion of creating a new curriculum in biomanufacturing has evolved.

The Biomanufacturing Laboratory at JMU

In considering the development of a new curriculum in biomanufacturing, it became apparent that experiential laboratory work would be critical for effective education, and the idea of a functional facility for the production and purification of recombinant proteins emerged. Planning for a Biomanufacturing Laboratory at JMU began in 1997 as an integral component of a proposed Center of Manufacturing Innovation to be located on the JMU campus. In 1998, Virginia's Manufacturing Innovation Center[9] was founded and awarded a Center Grant from Virginia's Center for Innovative Technology.[10] In 1999, the College of Integrated Science and Technology (CISAT) at JMU dedicated 2,300 square feet of wet laboratory space in three adjoining rooms for the creation of a functional biomanufacturing facility. CRB Consulting Engineers (Cary, NC) became a partner in the project and provided both design plans for the laboratory layout and a detailed list of equipment needs. JMU students were engaged to participate in the project and played an active role in the design and set-up of the laboratory. An ISPE JMU Student Chapter was founded and students attended training workshops and participated in international meetings.

In the fall of 2001, the Biomanufacturing Laboratory became operational. The laboratory has established capabilities for cloning and expressing genes in bacterial systems, pilot-scale fermentation, purification of recombinant proteins, and analytical testing - *Figure 3*. In addition, the laboratory supports basic and applied research in the areas of molecular biology, genetics, and biochemistry. Besides education and workforce development, the Biomanufacturing Laboratory offers pilot-scale production of recombinant proteins for research and product development on a contractual basis. Four ISAT faculty members currently use the facility for basic and applied research in the areas of biomanufacturing process and product development.

Curriculum Development: The Production Campaign

The production "campaign" is the manufacturing strategy of choice for most biopharmaceuticls. With this strategy, a production run is carefully planned from beginning to end. Genetically engineered cells are retrieved from a master cell bank, grown to produce a working cell bank, and tested for production

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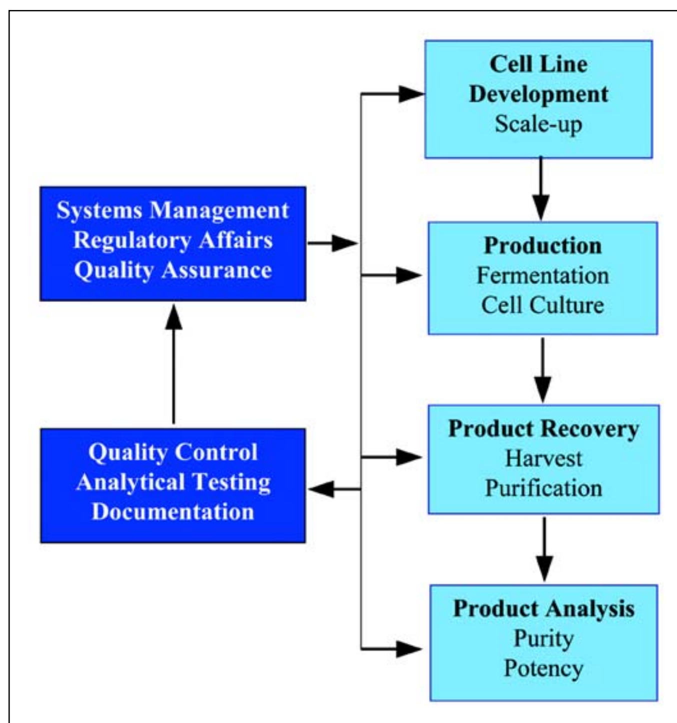

Figure 4. Components and organizational structure of the production platform and management systems in the biomanufacturing laboratory.

capabilities. The facilities and equipment are sanitized and validated. Media are prepared, the production platform is set up, and the entire process is tested and validated. The cell line is scaled up in increasing increments to inoculate the bioreactor. Cell growth within the bioreactor is carefully monitored, and at a precise moment, the cells are induced to express the protein product. The product is then harvested and isolated through multiple separation steps to achieve a high degree of purity. The production campaign itself is a highly orchestrated event with numerous checkpoints for quality control and quality assurance. The final product is subjected to an array of analytical tests and quarantined for safe storage. The entire process is carefully documented to assure quality control and regulatory compliance with current Good Manufacturing Practices (cGMP). Experiencing a production campaign is critical in understanding the purpose and function of each step involved and was a focal point in the development of a new curriculum.

For the purpose of curriculum development, the production campaign can be divided into a number of distinct interrelated components that are shown in Figure 4. The production train is a sequential series of steps that begin with the cell line, move through production, product recovery, and end with a purified product. Each of these steps is monitored for compliance with Standard Operating Procedures (SOPs), and product development is recorded with analytical testing for quality control. Detailed documentation is collected for regulatory compliance and standards are set for quality assurance. A systems management structure provides oversight for the entire manufacturing process.

A primary goal for any new curriculum development is to place abstract concepts in the context of practical applications. The biomanufacturing laboratory is the classroom, and the students are active players in the manufacturing process. The pedagogical approach involves the formation of teams to plan and execute specific aspects of the manufacturing process. Five teams with two to four members each are organized into the task related categories of cell line development, production, product recovery, product analysis/quality control, and systems management/regulatory affairs. Each team develops a detailed plan of action for the execution of their specific task and then the teams coordinate their plans to develop a campaign protocol. In the process of developing this protocol, teams learn how to create and follow SOPs, how to operate and monitor sophisticated equipment, and how to document the process for cGMP compliance. Troubleshooting and problem solving become an integral part of the learning experience. The production campaign is the high point of the learning experience and is executed according to the protocol. Following the production campaign, the student teams prepare written and oral reports for the class to review and analyze. In this manner, the students gain a global perspective of the manufacturing process and understand the role and importance of the various components.

Proof of Concept: The Green Fluorescence Protein (GFP)

A challenge in developing any biotechnology laboratory curriculum has been visualization of the molecular processes involved. It usually requires a leap of faith to believe that DNA has been cloned, genes have been expressed, and proteins have been purified when the evidence is bands on a gel or numbers on a graph. In considering a gene to clone, express, and purify for an educational experience in biomanufacturing, the Green Fluorescence Protein (GFP) from the jellyfish *Aequorea victoria* became an ideal candidate. The bright green fluorescence of the jellyfish is a distinctive phenotypic characteristic. Cloning and expression of a single gene transfers this phenotype to bacteria and the protein can be visualized throughout the purification process - *Figure 5*.

E. coli strain HB101 was transformed with pGLO, an ampicillin-resistance conferring plasmid in which the gene for GFP is under the control of the arabinose operon. Ampicillin resistance, restriction mapping, and visualization of bacterial colonies under long-wave ultraviolet light established proof of successful transformation. A master cell bank was created and stored for future use. A working cell bank was developed and scaled up for a production campaign in a 10-liter fermentation bioreactor. From the time of inoculation, the cells were induced to produce GFP by the addition of L-arabinose. The culture was grown to late log phase, and the cells were harvested by centrifugation and lysed by sonication. After the removal of cell debris by high-speed centrifugation, solid ammonium sulfate was added to the cleared lysate to 45% salt saturation. Precipitated proteins were removed by centrifugation, leaving a supernatant which glowed bright green under long-wave UV. This supernatant was loaded onto a hydrophobic interaction chromatography column, the column was washed with 1.5 M ammonium sulfate solution to remove the non-binding protein fraction, and the GFP was eluted with a linear salt gradient from 1.5 M ammonium sulfate to 10 mM Tris-HCl, 10 mM EDTA, pH 8. Fractions containing GFP were pooled and loaded onto a chromatography column, and proteins were eluted with a solution of 10 mM Tris-HCl, 10 mM EDTA (pH 8.0) - *Figure 6*. The crude cell lysate and the two chromatography purification steps were analyzed by SDS polyacrylamide gel electrophoresis - *Figure 7*. The entire production process was documented and reviewed by the campaign teams.

In recent work, the gene encoding GFP was cloned into an

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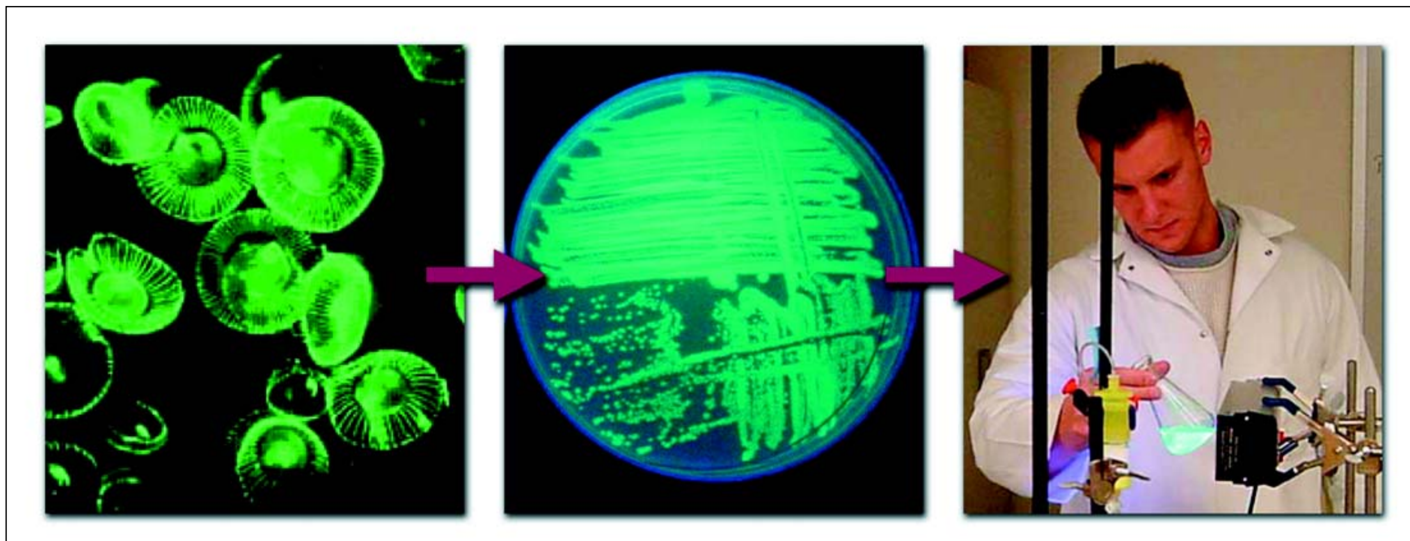

Figure 5. Cloning, expression, and purification of the Green Fluorescence Protein from the jellyfish Aequorea victoria.

inducible expression vector and was transformed into *E. coli*. Small-scale cultures were grown, and after cell lysis by sonication, crude (cleared) lysate was loaded onto a small column of chitin beads. After column washing, the chitin-bound intein-GFP hybrid protein was treated with a reducing agent and allowed to self-cleave. Finally, GFP was eluted from the column, and its purification was demonstrated by SDS-PAGE. A scaled up production and purification of GFP campaign is planned to allow further student experience in the art of protein production and downstream processing.

Over the past year, eight ISAT students have been working in three separate teams to develop and execute the GFP production protocol as part of their senior project requirement for graduation. The hydrophobic interaction chromatography step of the GFP purification protocol has been integrated into an ISAT laboratory course (ISAT 305) and performed by 114 ISAT students during the fall, 2001 semester. The entire campaign production curriculum will be introduced as a laboratory component to an existing ISAT lecture course (ISAT 451, Biotechnology in Industry and Agriculture) in the fall, 2002 semester that services approximately 48 students annually. A new ISAT course in biomanufacturing that includes cGMP regulatory issues will be introduced in the spring, 2003 semester. Biomanufacturing education has become an important component of the biotechnology concentration within the ISAT program. The Bachelor of Science ISAT degree program currently enrolls more than 800 students and approximately 170 students will be awarded a degree this year.

Summary

The promise of biotechnology to discover and produce a new generation of drugs to treat human disease is now a reality. The first 100 biopharmaceutical products to reach the market have demonstrated that the notion of manufacturing biological molecules in living systems to produce human therapeutic agents is a viable alternative to chemical synthesis.

The product development pipeline is full and the biotech industry is expanding manufacturing capacity to meet production demands. The rapidly evolving technology of biomanufacturing and the increasing demands for capacity present a challenge and an opportunity for the development of new curricula. The Integrated Science and Technology Program at James Madison University has taken an experiential approach to biomanufactureing education with the development of a functional bioprocessing facility. The biomanufacturing laboratory is the classroom and the students have taken an active role in the design process, set up, and development of a new curriculum.

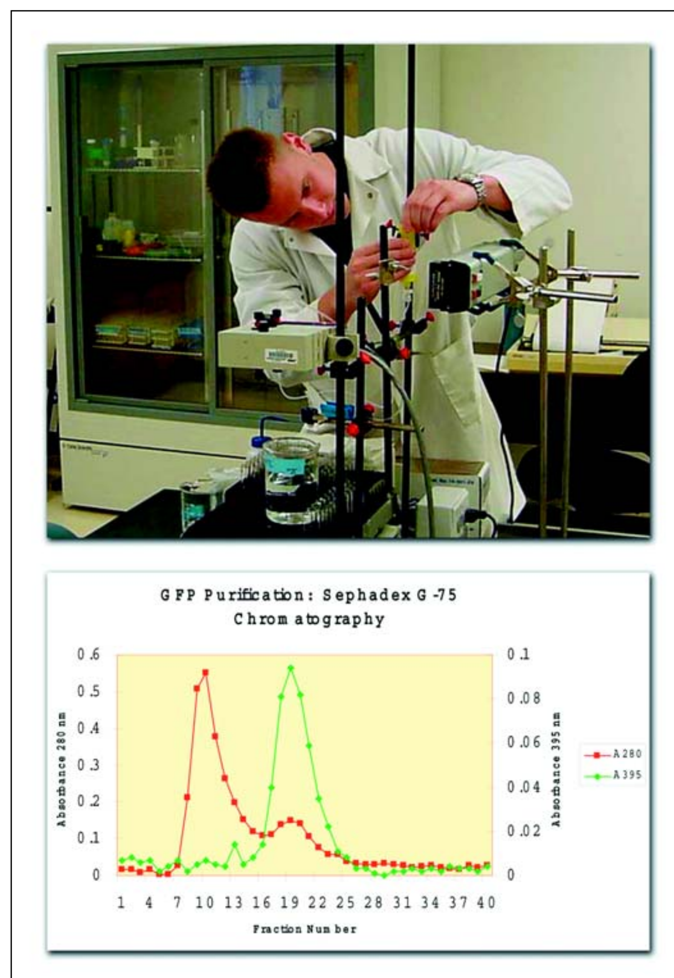

Figure 6. Purification of Green Fluorescence Protein with a chromatography column. JMU student Luke McGinty.

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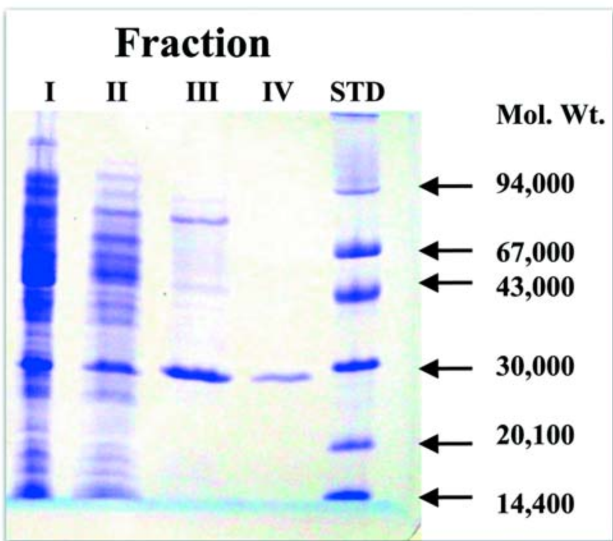

Figure 7. SDS Polyacrylamide Gel Electrophoresis of the purification fractions of GFP. JMU student Megan Barber.

References

1. Glick, B. R. and Pasternak, J. J. 1998. Molecular Biotechnology; Principles and Applications of Recombinant DNA, 2nd Ed., American Society for Microbiology, publishers, Washington, DC, 5-6.

2. Cohen, S., A. C. Y. Chang, H. W. Boyer, and R. B. Helling, 1973. Construction of Biologically Functional Plasmids in Vitro. *Proc. Natl. Acad.* Sci USA 70: 3240-3244.

3. http://www.genentech.com.

4. Odum, J. N. 2001. Biotech Manufacturing: Is the Crisis Real? *Pharmaceutical Engineering,* **21**: 22-33.

5. Dahms, A. S. 2001. The US Biotechnology Industry: The Importance of Workforce Quality in the Maintenance of Corporate Competitive Advantage. *Biochemistry and Molecular Biology Education,* **29** 206-208.

6. Windows on the Workplace. 1997. A report published by North Carolina Biotechnology Center.

7. The College Board College Handbook 2001, 38th Edition. College Board Publications, New York, NY.

8. http://www.isat.jmu.edu

9. http://www.vmic.org

10. http://www.cit.org

Acknowledgements

The College of Integrated Science and Technology at James Madison University, Virginia's Manufacturing Innovation Center, and Virginia's Center for Innovative Technology have sponsored development and outfitting of the Biomanufacturing Laboratory. ISPE has provided valuable instructional literature on pharmaceutical manufacturing and the ISPE Carolina South Atlantic Chapter has sponsored the establishment of the first Student Chapter at JMU. Jeffery N. Odum and CRB Consulting Engineers have provided valuable design information and equipment needs for the Biomanufacturing Laboratory. The authors would like to especially thank all the students who have taken part in the design, set up, and operations of the Biomanufacturing Laboratory over the past three years.

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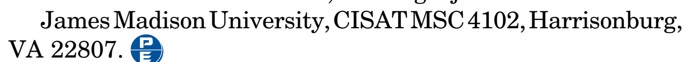

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This article outlines the scope of process validation for biotech Active Pharmaceutical Ingredient (API) manufacturing. Definitions, validation approaches, and requirements for validation documents are described.

This article is dedicated to Dr. Walter Dürckheimer on the occasion of his 70th birthday.

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The Official Journal of ISPE
May/June 2002, Vol. 22 No. 3

Requirements for Process Validation of Biotech Active Pharmaceutical Ingredients (APIs)

by Dr. Reiner Kirrstetter

Introduction

Process validation of Active Pharmaceutical Ingredients (APIs) is still one of the most challenging topics for both pharmaceutical industry and regulatory authorities. It is the clear expectation of regulatory authorities, especially the FDA and EMEA, that production processes, cleaning procedures, analytical methods, computer and utility systems that have an impact on product quality and purity are validated. Facilities and equipment used in conjunction with production and testing of APIs must be qualified. The principles of validation are progressively applied from development through to full-scale production. For new products normally, process validation must be completed at the time of launching the product into the market. For biologics filed with the Center for Biologics Evaluation and Research (CBER), the FDA expects that process validation is finalized prior to the license submission.

Definitions of Process Validation

The "classical definition" for process validation is presented in an FDA Guideline (Guideline on General Principles of Process Validation, 1987):

> "Process validation means establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality characteristics."

In the ICH Guideline Q7A "GMP for APIs," issued as a final version in November 2000, this definition of process validation is slightly modified to read as follows (as item 12.40):

> "Process validation is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API meeting its pre-determined specification and quality attributes."

Approaches to Process Validation of Biotech APIs

A very rational approach for the control of a biotech API production process is one that requires application of appropriate GMP controls for all steps beginning with the establishment and maintenance of master and working cell banks, and with validation of those steps, during fermentation, isolation, and purification, identified to be critical to the quality and purity of the final API. These critical steps should not be limited to the final stages of the process, but should include also those steps that could introduce or remove impurities or contaminants, or change a physical parameter of the final API. This approach also may require data to demonstrate that a particular step is not critical to the manufacturing process. General principles of API process validation are shown in Figure 1.

Before starting process validation activities, appropriate qualification of equipment and facilities, and validation of utilities systems, like water and air systems, must be completed. This includes Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ), as defined in the ICH Guideline Q7A. Successful process validation programs do normally cover DQ/IQ/OQ/PQ. It should be mentioned that DQ is the first element of the qualification of new facilities and equipment, and requested for prospective qualification according to the ICH Guide Q7A. DQ is the documented verification that the proposed design of the facili-

General Principles of API Process Validation
Apply controls to all manufacturing steps, beginning with the use of starting materials, or master cell bank
Increase controls as process proceeds to the final isolation and purification steps.
Validate all process steps identified to be critical to quality and purity of the final API.

Figure 1. General principles of API process validation.

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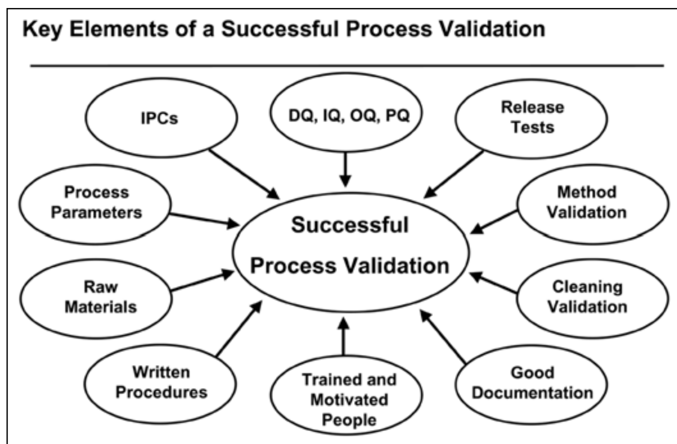

Figure 2. Key elements of a successful process validation.

ties or equipment is suitable for the intended purpose. DQ includes the review of the specific requirements for equipment and facilities design, specifications, construction, and performance up to the point of purchase to ensure that user requirements and functional specifications are met. Other key elements for a successful process validation are presented in Figure 2.

Concerning the performance of process validation, there are three specific approaches:

- Prospective Validation
- Concurrent Validation
- Retrospective Validation

Prospective validation is the preferred approach and should be conducted for all new API processes or after major changes of older API processes. For prospective validation, "it is generally considered acceptable that three consecutive batches within the finally agreed parameters, would constitute a validation of the process."[2]

Concurrent validation can be performed in situations where a single or limited number of API batches are produced for commercial production. The decision to carry out concurrent validation should be justified, documented, and approved by the quality unit. This procedure involves obtaining data from thorough monitoring and extensive in-process and end product testing to demonstrate that each batch meets the established specifications and quality attributes. Process validation should be completed when additional commercial batches are manufactured under replicated conditions.

Retrospective validation is only acceptable for an existing API process that has not been previously validated and no significant changes have been made in the raw materials, equipment, systems, facilities, or the production process itself. According to the ICH Guideline Q7A, this validation approach may be used where:

- critical quality attributes and critical process parameters have been identified
- appropriate in-process acceptance criteria and controls have been established
- there have not been significant process/product failures attributable to causes other than operator error or equipment failures unrelated to equipment suitability; and
- impurity profiles have been established for the existing API

Batches selected for this validation approach should be representative of all batches made during the review period, including any batch that failed to meet specifications. A sufficient number of batches (normally 10 to 30) should be considered to demonstrate process consistency.

Change Control and Revalidation

In theory, a validation exercise needs to be carried out only once for a given process. However, in practice, the process rarely remains static. Changes occur in components (raw materials, intermediates, packaging materials), equipment is modified, or the process environment may change. Therefore, an effective change control system needs to be in place to evaluate the impact on the API quality and purity after the changes. The changes must be documented and approved, and the need for revalidation assessed. There is no regulatory requirement to revalidate at a specific time interval. The requirement is that facilities, systems, equipment, and processes are periodically evaluated to verify that they are still operating in a valid manner.

Validation Documentation

The company's overall policy, intentions, and approaches to validation should be described in a site-specific Validation Master Plan (VMP). This overall plan should include the following topics as a minimum:

- overall validation policy of the company
- organizational structure of and responsibilities for validation activities
- summary of facilities, equipment, systems, and processes to be qualified or validated
- plans and schedules for validation activities
- validation approaches for different products including the documentation formats for protocols and reports
- revalidation requirements as dictated by time or by changes
- definition of terms used in the master plan

Main Contents of a Validation Protocol
- scope and objective
- responsibilities and accountabilities
- validation strategy and rationale
- brief description of the process
- critical process steps identified
- acceptance criteria
- variables to be monitored, samples to be taken
- time schedules
- details of methods for recording and evaluating results

Figure 3. Main contents of a validation protocol.

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> *"In process validation, there's a lot of common sense. You need to fully understand the process you use to make your product." (FDA)*

In cases of larger projects, separate validation master plans can be created.

Process validation must not take place until a written validation protocol is established which specifies how validation will be performed for a particular process. This protocol needs to be reviewed and approved by the quality unit before it can be executed. The specific content of a validation protocol depends on the complexity of the process. Main contents are presented in Figure 3.

The batches under validation have to be documented comprehensively in a validation report with cross references to the validation protocol. A detailed summary of the results obtained from in process and final testing, commenting on any deviations observed, should lead to the conclusion that the process is considered to be validated. Any variations from the approved validation protocol need to be documented and justified with a rationale. In the event of failure of a validation study, an investigation should be conducted to determine the cause of the failure. Conclusions should be drawn including a statement in the report on resolution. Validation cannot be considered finalized until an approved validation report is available. An overview of validation timelines for APIs and Drug Products (DPs) in relation to regulatory submission and launch is presented in Figure 4.

As mentioned before, process validation of biologics should be completed prior to the submission to CBER.

Specific Requirements for Process Validation

Although the same validation principles, approaches, and procedures apply as for all APIs, some differences exist for the process validation of biotech APIs because the technology is different. The term "biotech process" refers to the use of organisms or cells which were generated or modified by recombinant DNA, hybridoma (cell clone), or other similar technology to produce APIs. These APIs normally consist of high molecular weight substances, e.g., proteins or polypeptides. Their production involves biological processes, such as cultivation of cells or isolation and purification of materials from living organisms. The raw materials used (media or buffer components) may lead to microbiological contamination. Therefore, the control of bioburden, endotoxins, and viral contamination during the manufacturing process is essential. In addition, appropriate controls for equipment, utility systems (water, air, nitrogen, steam), and the microbiological environment are necessary to minimize the risk of contamination. Equipment sterilization is an area which must be studied in depth

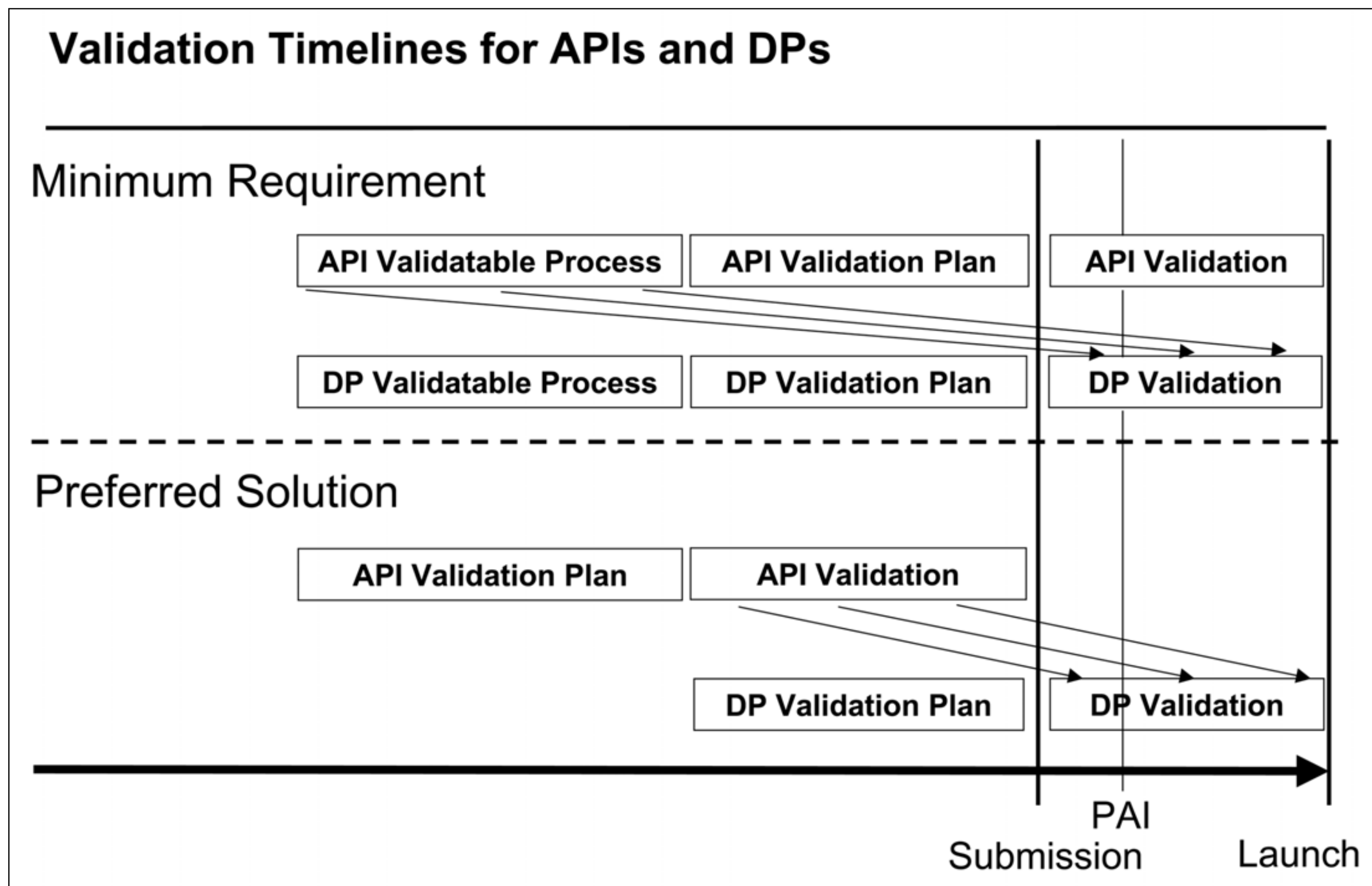

Figure 4. Validation timelines for Active Pharmaceutical Ingredients (APIs) and Drug Products (DPs).

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and fully understood in the validation process for biotech APIs. The acceptance criteria for environmental control and the frequency of monitoring should depend on the production conditions (open, closed, contained systems), and on the stage in production. Compared to other APIs, the amount of process validation activities for biotech APIs should be higher during process development and the scale-up period and therefore performed earlier in the timeline. The general process controls to be considered for biotech APIs are presented in Figure 5.

Before starting process validation of a biotech API, qualification of the facilities, equipment, and utility systems including microbiological aspects have to be performed. Elements associated with process validation, such as controlled areas, filter systems, sterilization procedures, cleaning validation including holding times before/after cleaning and sterilization, stability of intermediates, and computer validation must be addressed thoroughly. The main focus of the process validation program is to demonstrate and document the:

- removal of host cell proteins and other process-related impurities
- removal and control of product-related impurities
- consistent product quality and purity
- consistent process yields, and
- avoidance of contamination

The following issues, process-related and product-related impurities, contaminants, and viral removal, are of great importance to be considered during process validation of a biotech API.

Process-related impurities are derived from the manufacturing process, i.e., cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing (e.g., oxidizing or reducing agents, cyanogen bromide, guanidine, inorganic salts, column leachables).

Product-related impurities (e.g., precursors, certain degradation products) are molecular variants arising during production and/or storage. They normally do not have comparable properties to those of the desired product with respect to activity, efficacy, and safety. The acceptance criteria for these impurities should be based on data obtained from batches used in preclinical and clinical studies and as consistency batches.

Process Control Principles for Biotech APIs
- proper establishment and maintenance of Master and Working Cell Bank
- controlled inoculation and expansion of the culture
- defined critical variables and steps during fermentation
- monitoring of the cell growth process
- harvesting steps and purification procedure to remove cells or cellular components while minimizing degradation and contamination
- monitoring of bioburden and endotoxin levels, where necessary
- viral removal and viral inactivating steps, where needed

Figure 5. Process control principles for biotech APIs.

Contaminants include all adventitiously introduced materials not intended to be part of the manufacturing process, such as chemical and biochemical materials (e.g., microbial proteases), and/or microbial species.

Contaminants must be strictly avoided and/or suitably controlled with appropriate in-process acceptance criteria or additional limits for API specifications.

Viral removal and viral inactivation steps are critical processing steps for some biotech processes and should be included in the validation process as appropriate.

It also is important to identify and define critical process parameters and critical variables as early as possible for a biotech process. In principle, each step in the fermentation and purification process could be viewed as critical. But, as a typical biotech process contains hundreds of operational variables, all of which are important, the identification of the critical variables is essential, and they must be addressed during process validation.

An adequate change control program and the concept of revalidation also apply for biotech products to maintain the process in a controlled and validated state.

The Importance of Process Validation - A Conclusion

Process validation is a basic cGMP requirement and expected to be in place for a launched product. The pharmaceutical manufacturer must evaluate which validation activities are needed to demonstrate and to prove control of the critical aspects of the manufacturing operations. When processes are validated, products are repeatedly produced under a state of control which ensures operational consistency over a long time period.

One statement from Robert C. Coleman, National Drug Expert of the FDA, indicates that performance of process validation is also a good business practice - *Figure 6*:

> "In process validation, there's a lot of common sense. You need to fully understand the process you use to make your product."

References

1. **ICH Harmonized Tripartite Guideline Q7A:** GMP Guide for Active Pharmaceutical Ingredients, Final Version, November, 2000 (Chapter 12 on Validation).
2. **EU Guide to GMP, Annex 15:** Qualification and Validation, Final Version, July 2001.
3. **CPMP Note for Guidance on Process Validation,** March 2001 (valid from September 2001).
4. **FDA Guideline on General Principles of Process Validation,** May 1987 (reprinted February 1993).
5. Berry, I.R., and Harpez, D., (editors), **Validation of Active Pharmaceutical Ingredients,** IHS Health Group, Denver, Colorado 2001, ISBN 1-57491-119-8.

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Validation as Good Business Practice

It is Good Business Practice, because it ...

- is also Good Scientific Practice
- is a helpful tool to predict and control the manufacturing results
- helps to keep the process under a "state of control"
- is a requirement by regulatory authorities

Figure 6. Validation as good business practice.

6. Lanese, J., "Three Times is Not Even the Beginning," **Validation Technology,** Vol. 7, Feb. 2001, pp. 124-129.

7. Amer, G., "Process Validation Issues - A Discussion with Robert C. Coleman," **Validation Technology,** Vol. 6, May 2000, pp. 665-675.

8. Amer, G., "Validation and Change Control," **Validation Technology,** Vol. 5, August 1999, pp. 302-309.

9. Gibson, W., and Powell-Evans, K., **Validation Fundamentals - How to, What to, When to Validate,** Interpharm Press,Inc., Buffalo Grove, Illinois, 1998, ISBN 1-57491-070-1.

10. Rivera Martinez, E., "An FDA Perspective on Bulk Pharmaceutical Chemical GMPs, Control and Validation," **Pharmaceutical Engineering,** Vol. 14, 1994, pp.8-14.

11. Sawyer, Ch. J., and Stotz, R.W., "Validation Requirements for Bulk Pharmaceutical Chemical Facilities," **Pharmaceutical Engineering,** Vol. 12, 1992, pp. 44-52.

12. For specific biotech guidance see Reference 1, Chapter 18.

13. **ICH Harmonized Tripartite Guideline Q6B:** Specifications: Test Procedures and Acceptance Criteria for Biotechnical/Biological Products, Final Version, March 1999.

14. **FDA Biotechnology Inspection Guide,** November 1991.

15. Amicetti, V., and Lyda, J., "Process Validation for Biologic and Biotechnology Products," **PDA Letter,** November 2001, pp. 38-43.

16. Murphy, R., Seely, R.J., **Validation of Biotechnology Active Pharmaceutical Ingredients,** in Reference 5, Chapter 17, 2001, pp. 451-474.

17. "The Gold Sheet": Biologic Process Validation Challenges, **Gold Sheet,** Vol. 35, January 2001, pp. 1-30.

18. Martin-Moe, S.W., Kelsey, W.H., Ellis, J., and Kamarck, M.E., "Process Validation in Biopharmaceutical Manufacturing," in **Biopharmaceutical Process Validation,** edited by Sofer, G. and Zabriskie, D.W., Dekker, New York, 2000, pp. 287-298.

19. Seely, R.J. Hutchins, H.V., Luscher, M.P., Sniff, K.S., and Hassler, R., "Defining Critical Variables in Well-Characterized Biotechnology Processes," **Bio. Pharm.,** April 1999, pp. 33-36.

20. Vincent, D.V., and Revie Nabi, D.R., "The Validation Life Cycle of a Biotechnology Derived Drug," Part I-III, **Validation Technology,** Vol. 5, February 1999, pp. 167-188; May 1999, pp. 272-293; August 1999, pp. 365-402.

About the Author

Dr. Reiner Kirrstetter studied chemistry and pharmacology and took his doctorate from the Department of Organic Chemistry of the University of Heidelberg in October 1976. After that, he spent about three years at the University of Kiel as Post Doc and Assistant Professor before he joined Hoechst AG in April 1980. After several years in pharmaceutical research, process development, and API production in Hoechst AG, he transferred to the GMP/Inspections Department of Hoechst AG in September 1988. Since that time, he has gained experience in the GMP and regulatory compliance area. In July 1992, he became Head of Quality Assurance/APIs, and then in July 1996 Head of QA/QC for Active Pharmaceutical Ingredients of Hoechst Marion Roussel Germany. In June 1999, he joined the Global Quality Operations/International Quality Assurance organization. In December 1999, he became the global head of Process Development Quality Management in Aventis Pharma AG, and in July 2001, he joined a global Aventis project to represent Quality Management. Dr. Reiner Kirrstetter has published about 45 scientific articles, submitted 15 patent applications, and was/is a member of different working groups dealing with GMPs of APIs. He was involved in eight FDA inspections, heading three as a team leader.

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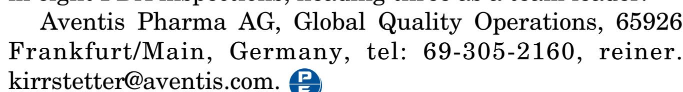

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This article describes the history and content of the Guidelines for Assessing the Particulate Containment Performance of Pharmaceutical Equipment currently approaching the final draft stage.

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May/June 2002, Vol. 22 No. 3

Guidelines for Assessing the Particulate Containment Performance of Pharmaceutical Equipment

by Paul Gurney-Read and Martin Koch

Many of the major pharmaceutical manufacturing companies have presented information, based on both their product history and R&D pipelines, showing a clear trend toward increasing drug potency.

The speed of introduction of competitive products has reduced the life expectation of new drugs. This, coupled with the need to have variable manufacturing capacity available for new products and shorter campaign lengths to reduce inventory, has seen a marked increase in new bulk active facilities being of multi-product design.

The equipment purchased to handle dry products in such facilities is usually specified for the most potent of the likely Active Pharmaceutical Ingredients (APIs) processed. For the most recent facilities built, these substances have exposure limits of between 1-10 μg/m^3, as a time weighted average over eight hrs.

The response by the pharmaceutical equipment suppliers has resulted in marked improvements in the containment levels achievable, using both established solids-handling equipment, and new innovative techniques. Unfortunately, the pressure to sell in this highly competitive arena also has led to performance claims that appear exaggerated or the use of confusing terminology that cannot be substantiated, such as 'dust free' or 'nanogram levels possible.'

The issue for the prospective purchasers of such equipment is to match their requirement to the actual expected performance of the equipment available. This has been difficult because of the lack of good plant-based performance data and the fast pace of equipment developments, meaning that few relevant reference installations exist. Also, the attempts by the equipment manufacturers to test their equipment either in-house or with a third party has produced results which are not comparable, due to the variation in equipment test methods and test facility design.

Therefore, it seemed vital to create a guideline for a standardized method of testing the containment efficiency of solids handling equipment that would allow direct comparison of test results both for similar and different equipment types.

The challenge to produce such a guideline was initiated by Buck Valve GmbH in the summer of 2000, who formed a working group from representatives of the end users, contractors, consultants, suppliers, and test laboratories. The group contains a number of occupational hygienists and engineers and is represented by people based in Europe, the US, and Japan.

In order to ensure the group was steered from an independent viewpoint, the Chair was passed to Kvaerner. The working group has met four times and the guideline has been a discussion topic at two ISPE conferences, namely Amsterdam, December 2000, and Tampa, February 2001. Publication is scheduled for late 2002.

Continued on page 56.

Figure 1. Typical test enclosure with HEPA filtered air extraction unit on lower right-hand side.

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Guideline Contents

The following sections have been reproduced from the guideline with further explanation where this is considered helpful.

Introduction

The main factors that affect the test results include the effects from the room environment, air quality, and ventilation. These parameters have either been fixed or an allowable range of operation specified. The test material and details of sampling, sampling equipment, and sampling location are all specified based on best practice within the industry. The testing includes measuring airborne and surface contamination to provide relevant data about the material released during operation. This will then allow direct comparison of test results and enable users to better assess equipment performance and suitability.

The test information can be used together with the guidance given in 'interpretation of results' to assess whether the equipment tested is suitable for the containment levels required for a particular material. The test results are not directly comparable to the Occupational Exposure Limit (OEL) of the material. Such a comparison requires a work place risk assessment to be carried out; e.g., how many operations are performed with the equipment within one shift. The full set of test data should provide designers with better information to engineer a suitable containment system. It also should help to identify where additional facilities or procedures are required, e.g., the need for manual cleaning to remove surface contamination prior to removing solids containers from the transfer room.

Scope

The test method, at this time, is valid for:

- powders only (this may be extrapolated for larger elements such as tablets, but is not appropriate for liquids or vapor containment)
- airborne and surface contamination
- test conditions and materials only. The test methodology is developed to provide a guide only to performance, although under simulated 'typical' operating conditions, and should not in any case be considered as a guarantee of performance. It is essential that users verify and confirm the performance of equipment in their own installations with their own materials and conditions, since this may differ significantly from the test materials and conditions.

The results of trials based on this guidance are expressed as an airborne concentration over the period of the test, along with real time peak concentrations and residual surface contamination. These factors have been chosen to reflect the exposure potential that can occur from use of the equipment. It is essential that users understand the effect of specific operating procedures, (e.g., frequency of operation, rotation of operators etc.), before using the data reported.

Methodology

Equipment and Equipment Preparation

The equipment test assembly should be configured to simulate intended production situations. A full description of the equipment must be included in the test report.

Consideration should be given to the need for earthing/grounding so that static charge cannot cause excessive particle build-up on the working surfaces of the equipment which might result in increased emissions.

Figure 2. IOM sample head.

It is important that the test facility provides sufficiently low background dust levels compared to those expected to be generated by the process during the measured tests. Prior to the start of measured tests, the equipment should be cleaned internally to a standard suitable for correct operation of the equipment. Externally, both the equipment and the surrounding test environment (including handling and any other ancillary equipment) should be thoroughly cleaned. During testing, it is possible for air movement, as well as equipment movement or vibration, to cause surface particles (either room dust or test powder deposited during pre-test trials) to become airborne with adverse effects on the test results. A dusty environment also will cause delays in bringing background aerosol readings down to the level needed for the tests to begin. Thorough cleaning will minimize these problems.

The surface areas, which are intended to be used for surface contamination measurement, must be swabbed clean prior to taking a baseline swab sample.

Testing Enclosure Design

Layout

The layout of the testing enclosure (Figure 1) should accommodate the following:

- the equipment item under test
- sufficient access for normal operation of the equipment
- access to allow placement/operation and removal of the sampling heads
- storage space for placement of any clean articles required during the test to prevent them having to be introduced

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during the test and hence disturb the integrity of the enclosure

- seating or standing area for observers. This should be positioned to allow observation of the test, but not interfere with the normal movements of the operator performing the test or interruption of the airflow patterns, which will have been used to position the test equipment. Excessive personnel movements will create or disturb particles, which may be recorded by the continuous monitoring devices.

- a layout table for resting and recording procedures, static instrumentation, swab samples, etc.

- unimpeded inlet and outlet extract ventilation points

- the necessary safety features, e.g., access ladders and working platforms to safely operate the equipment under test. This may include lifting devices to reduce the risk of manual handling injuries, low oxygen, or high solvent concentration alarms where appropriate.

Environmental Conditions

The environmental conditions within the testing enclosure are created to:

- minimize the influence of the environment external to the enclosure

- provide a consistent environment across test facilities

- provide a level of control that is comparable to the end users' facility

- provide consistent conditions throughout the test

- not adversely influence the results

The recommended environmental conditions are:

- temperature range to provide operator comfort: 20°C +/- 5°C

- Relative Humidity (RH): 40% maximum

- air change rate: 5/HR +/- 1

- positive room pressure: + 25 Pa. (minimum) over the adjacent space

These conditions should be recorded for the duration of the test.

The inlet and exhaust air should be filtered by an EU10 filter or better to prevent contaminated air from entering the test enclosure.

To prevent the background levels adversely influencing the test, the background concentration of test material in the enclosure should be no higher than the level expected to be liberated during the tests.

Test Material

The equipment should be challenged using a surrogate.

Micronized lactose is the recommended surrogate material based on the following criteria:

- flow characteristics

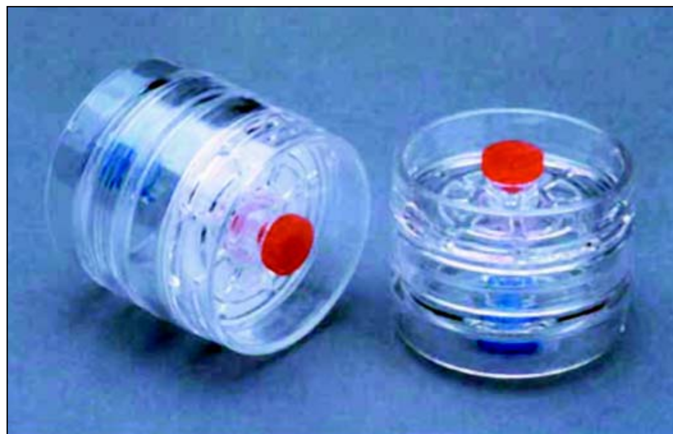

Figure 3. Three-piece cassette.

- detection sensitivity

- pharmaceutical activity (effect on testing personnel and cross-contamination risk)

- cost

- availability

- cost of analysis

- particle size range available

- consistency of particle shape, from different suppliers

- disposability (environmental)

- Solubility in water (post test cleaning)

- stability (test material and sampled material storage)

Milled lactose < 50 microns average particle size.

Note:

A common grade will be chosen to give a consistent particle/shape.

Moisture content of standard grades will be determined - This is available on the Certificate of analysis for the material.

Measurement of Airborne Dust and Surface Contamination

Conventions for Health Related Sampling

For size selective aerosol, sampling definitions of three health-related aerosol fractions: inhalable, thoracic, and resipirable have been adopted by EN 481, ISO 7708 and ACGIH (1994-1995). For pharmaceuticals, inhalable dust is the relevant fraction.

Dust Sampling Equipment

Personal Sampling for Inhalable Dust Using IOM Sampler

The performance of aerosol samplers against the above sampling conventions varies and a number of these may give satisfactory performance; however, the Institute of Occupational Medicine (IOM) sampler has been shown to give agreement with the EN 481 target specification for inhalable fraction, under the widest range of workplace conditions. The IOM

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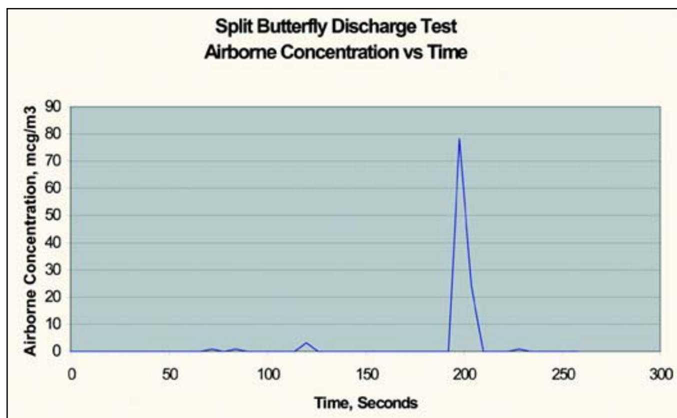

Figure 4. Typical results from a real-time monitor.

sample head is pictured in Figure 2.

The airflow rate for the pump is set at 2 liters/minute using a calibrated portable flow meter (e.g., rotameter) capable of measuring the desired volumetric flow rates to within 0.1 liter/minute and calibrated against a primary standard (i.e., a flow meter whose accuracy is traceable to national standards).

Other sampling methods such as a glass fiber or PTFE filter in a three-piece cassette give satisfactory performance although the results may not be comparable to the IOM results.

A three-piece cassette is shown in Figure 3.

Fixed (Static) Sampling for Inhalable Dust

The sampling head also can be used as a static sampler to determine the background levels of dust in the atmosphere and particulate concentrations at specific locations relative to a source of emission. The sampling procedures are the same as for personal sampling. Static samplers can be used to identify the areas where most of the dust emissions are being generated. It is important to stress that it is not appropriate to compare fixed point-monitoring results with the exposure limit because the distribution of dust in the workplace is not uniform. In addition to this discrepancy, because of aerodynamic effects, fixed-samplers will not exhibit the same characteristics as the same device mounted on the body and may underestimate inhalable dust concentration.

Real-Time Aerosol Monitors

Real-time aerosol monitors (Figure 4) are normally direct-reading, light scattering photometers capable of measuring total particulate in the atmosphere and are not substance specific. The real-time monitors provide useful information on the pattern of exposure and also may be used to help identify the sources of dust emissions.

A real-time aerosol monitor can be used side by side with a sampler for comparison purposes and can help identify when peak exposures occur. They also can provide useful information on dust levels during a short cycle of operations when other sampling methods may not be adequately sensitive.

A real-time aerosol monitor also can be used to determine when a stable baseline level of airborne dust has been achieved in the test enclosure prior to the start of testing.

Sampling Equipment Location

Sampling location will be determined based on the likely source of emission as described in the annexes.

Swab Sampling (Surface Monitoring)

Swab sampling is a technique that can be used to assess the amounts of a chemical contamination on a surface. It is not a standardized technique for establishing health risks; however, it is an important measure in establishing the containment performance of the equipment. Planning is required and great care has to be taken to avoid cross contamination during sampling.

It must be noted that swab samples do not give a measure of individual exposure nor can they be extrapolated to give this information.

Their guideline also contains sections on construction materials, analytical methods, report format, and interpretation of results, which for reasons of brevity have been omitted.

Equipment annexes will portray typical equipment test set-ups within a test enclosure, indicating the sample positions. Equipment annexes have been produced for a number of containment systems:

1. Single Point - Make and Break (Valve Type)
2. Keg Emptying
3. Down Flow Booth
4. Isolator/Bag-Type Enclosure FIBC Fill/Empty
5. Keg Filling

Additional annexes will be added as required or as new systems are developed. The diagram in Figure 5, from the single point annex, illustrates the positions of fixed samplers.

Acknowledgements

The excerpts taken from the guideline have been written and edited by members of the guideline working group. They are: Marc Abromovitz, GSK; Dave Ainsworth, Foster Wheeler; Francis Boland, Aventis; Silke Buechl, Novartis; Neil Cocker, Extract Technology; Dave Drew, Matcon; Dave Eherts, Aventis; John Farris, Safe Bridge; Hari Floura, Ace; Andreas Flückiger, Roche; Yukio Fukushima, Hitachi; Paul Gurney-Read, Kvaerner; David Harrison, Sanofi-Synthelabo; Donna Heidel, J & J; Martin Koch, Buck Valve; Ralf Kretzschmar, Glatt; Vittoriano Lunardi, Romaco Zanchetta; Peter Marshall, AstraZeneca; Peter McHugh, Sanofi-Synthelabo; Matthew Meiners, NATLSCO Laboratory;

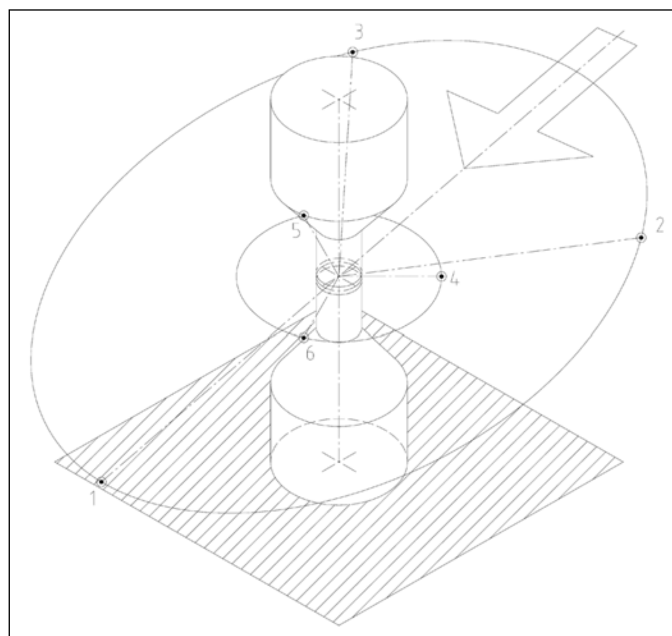

Figure 5. Positions of fixed samplers from the single point annex.

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Suzuki Osamu, JGC; Edward Sargent, Merck; Julian Wilkins, Pharma Consult; Jan Wren, GSK; and Andreas Znidar, Glatt.

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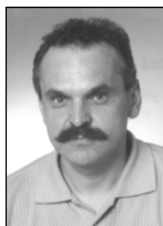

Martin Koch was born in 1959 in Germany, is a certified engineer, and has been working as a mechanical engineer for the last 16 years, eight years of which have been associated with the pharmaceutical industry. He is accredited with the invention of the Buck Valve in 1994. Since then, he has been responsible for all technical issues concerning the valve. He is currently working as the Managing Director for GEA Buck Valve GmbH, a small company within the Niro Pharma Systems group. Koch is the initiator of the guideline discussed above, brought the idea into the ISPE organization, and composed the committee to produce this guideline. He has been a member of ISPE since 2000.

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This article provides a high-level overview of the current content and structure of the Biopharmaceuticals Baseline® Guide, which is the sixth volume in the Baseline® Guides series.

Editor's Note: A first draft of the Guide is complete (May 2002) and will be reviewed by members of ISPE's Technical Documents Steering Committee. The final publication of the Guide is anticipated by mid-2003.

Reprinted from PHARMACEUTICAL ENGINEERING® The Official Journal of ISPE May/June 2002, Vol. 22 No. 3

Baseline® Pharmaceutical Engineering Guide Series: Biopharmaceuticals Outline

The Biopharmaceuticals Baseline® Guide is composed of nine chapters followed by six appendices, and begins by introducing the concepts, reasoning, scope, goals, and regulatory aspects encompassed by the Guide. The following chapters consider process and facility issues, including equipment, process integration, and automation. The main volume is supplemented by appendices which consider aspects such as European regulatory differences, and possible future developments within the biopharmaceutical industry.

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Appendix 1 - considerations for products not covered by this guide
Appendix 2 - References
Appendix 3 - European and other GMP differences
Appendix 4 - Summary of NIH guidance
Appendix 5 - Glossary
Appendix 6 - future developments in the bio industry

Many of these terms have more general definitions as well. Those given here are specific to their application in biotechnology.

Biotech Glossary of Terms

by Jeffery N. Odum

Absorption - Removing a particular antibody or antigen from a sample (e.g., from serum) by adding the corresponding antigen or antibody.

Adsorption - Nonspecific adherence of substances in solution or suspension to cells or other particulate matter.

Adventitious Agents - Acquired, sporadic, accidental contaminants.

Aerobe - An organism that can live and grow only in the presence of oxygen.
1. Facultative aerobe: one which normally thrives in the absence of oxygen, but which may acquire the faculty of living in the presence of oxygen.
2. Obligate aerobe: one that cannot live without air.

Aggregate - A clustered mass of individual cells - solid, fluffy, or palletized - that can clog the pores of filters or other fermentation apparatus.

Amino Acids - Any of a group of twenty hydrocarbon molecules (containing the radical group NH2) linked together in various combinations to form proteins in living things. Synthesized by living cells or obtained as essential components of the diet of human and animals, these twenty amino acids are divided into four (4) groups on the basis of their side-chain properties:
1. Neutral, hydrophobic side chains
2. Neutral, hydrophilic side chains
3. Acid, hydrophilic side chains
4. Basic, hydrophilic side chains

In addition to the twenty common amino acids there are less common derivatives (e.g. hydroxyproline, found in collagen) formed by the modification of a common amino acid.

Anaerobe - A microorganism that thrives best, or only, when deprived of oxygen.
1. Facultative anaerobe: one able to grow in the presence or absence of free oxygen.
2. Obligate or obligatory anaerobe: one that will grow only in the absence of free oxygen.

Animal Testing - Before researchers test pharmaceuticals in human clinical trials, they test them in animals to determine toxicity, dosing, and efficacy. What they learn in animal models helps them determine if it is safe and worthwhile to proceed to human trials, and how best to design those trials.

Antibody - An infection-fighting protein molecule that tags, neutralizes, and helps destroy foreign microorganisms or toxins. Also known as immunoglobulins, antibodies are produced by the immune system in response to antigens, which are often bacterial or viral particles or components.

Antifoam Agent - A chemical added to the fermentation broth to reduce surface tension and counteract the foaming (bubbles) that can be caused by mixing, sparging, or stirring.

Antigen (antigenicity) - Any agent, often a large molecule, that stimulates production of an antibody that will react specifically with it. Each antigen may contain more than one site capable of binding to a particular antibody. An immunogen can cause the production of a number of antibodies with different specificities. Antigenicity is the capacity of a substance to function as an antigen – to trigger an immune response.

Artificial Chromosome - Synthesized DNA in chromosomal form for use as an expression vector.

Aseptic - Sterile, free from bacteria, viruses, and contaminants.

Attenuated - Weakened (attenuated) viruses often used as vaccines; they can no longer produce disease but still stimulate a strong immune response similar to the natural virus. Examples include oral polio, measles, mumps, and rubella vaccines.

Bacteriophage - A virus that infects bacteria, sometimes used as a vector.

Base Pair - Two bases on different strands of nucleic acid that link together. In DNA,

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cytostine (C) always pairs with guanine (G) and adenine (A) always links to thymine (T). In RNA molecules, adenine joins to uracil (U).

Batch Culture - Large-scale cell culture in which cell inoculum is cultured to a maximum density in a fermenter, harvested, and processed as a batch.

Bioactivity - A protein's ability to function correctly after it has been delivered to the active site of the body (*in vivo*).

Bioavailability - Measure of the true rate and the total amount of drug that reaches the target tissue after administration.

Biologic - A therapeutic agent derived from living things.

Biological and Chemical Assay - Once a pharmaceutical protein is isolated from the cells in which it was grown, researchers perform tests to measure the protein's biological activity. It must maintain a certain minimal level of biological activity to be issued for animal or clinical testing, or later, for market. Researchers also test to confirm that the isolated protein is identical to the desired protein.

Biopharmaceutical - A therapeutic product created through the genetic manipulation of living things, including (but not limited to) proteins and monoclonal antibodies, peptides, and other molecules that are not chemically synthesized, along with gene therapies, cell therapies and engineered tissues.

Bioprocessing - Using organisms or biologically derived macromolecules to carry out enzymatic reactions or to manufacture products.

Bioreactor - A vessel capable of supporting a cell culture in which a biological transformation takes place (also called a fermenter or reactor).

Broth - The liquid culture medium in which fermentation or cell culture takes place.
The contents of a microbial bioreactor: cells, nutrients, waste, etc.

Cascade Effects - A series of events that result from one initial cause.

Catabolites - Products of catabolism, by which organisms convert substances into excreted compounds.

Cell Culture - Cells taken from a living organism and grown under controlled conditions ("*in vitro*"). Method used to maintain cell lines or strains.

Cell Lines - When cells from the first culture (taken from the organism) are used to make subsequent cultures, a cell line is established. Genetic or other manipulations, allow immortal cell lines to replicate indefinitely.

Chemostat - A growth chamber that keeps a bacterial culture at a specific volume and rate of growth by limiting nutrient medium and removing spent culture.

Chromosome - A long and complex DNA chain containing the genetic information (genes) of a cell. Prokaryotes contain only a single chromosome; eukaryotes have more than one, made up of a complex of DNA, RNA, and protein. The exact number of chromosomes is species-specific. Humans have 23 pairs.

CIP (clean in place) - A way to clean large vessels (tanks, piping, and associated equipment) without moving them or taking them apart, using a high pressure rinsing treatment, sometimes followed by steam-in-place (SIP) sanitization.

Clean Room - A room in which the concentration of airborne particulate matter is controlled at specific limits to facilitate the manufacture of sterile and high-purity products. Clean rooms are classified according to the number of particles per volume of air.

Clearance - Demonstrated removal according to specified parameters.

Clone - To duplicate exactly, whether a gene or a whole organism; or, an organism that is a genetically identical copy of another organism.

Cloning Vectors - Methods of transferring desired genes to organisms that will be used to express them. Cloning vectors are used to make recombinant organisms.

Creutzfeld-Jacob Disease - A disease affecting the human nervous system, believed to be caused by a prion that also causes bovine spongiform encephalopathy (BSE) or "Mad Cow Disease" in cattle.

Cryopreservation - Maintenance of frozen cells, usually in liquid nitrogen.

Cytokine - A protein that acts as a chemical messenger to stimulate cell migration, usually toward where the protein was released. Interleukins, lymphokines, and and interferons are the most common.

Cytopathic - Damaging to cells, causing them to exhibit signs of disease.

Cytoplasm - The protoplasm of a cell outside the nucleus (inside the nucleus it is called nucleoplasm). Protoplasm is a semifluid, viscous, translucent mixture of water, proteins, lipids, carbohydrates, and inorganic salts found in all plant and animal cells.

Cytostat - Something that retards cellular activity. This can refer to cytostatic agents or to machinery, such as those that would freeze cells.

Dalton - The unit of molecular weight, equal to the weight of a hydrogen atom.

Downstream Processing - Bioprocessing steps following fermentation and/or cell culture, a sequence of separation and purification activities needed to obtain the required drug product at the necessary level of purity.

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DNA (deoxyribonucleic acid) - The nucleic acid based on deoxyribose (a sugar) and the nucleotides guanine, adenine, thymine, and cytosine. Occurring in a corkscrew-ladder shape, it is the primary component of chromosomes, which thus carry inheritable characteristics of life.

DNA Fingerprinting - Sequences of nucleic acids in specified areas (loci) on a DNA molecule are polymorphic, meaning that the genes in those locations may differ from person to person. DNA fragments can be cut from those sequences using restriction enzymes. Fragments from various samples can be analyzed to determine whether they are from the same person. The technique of analyzing restriction fragment length polymorphism (RFLP) is called DNA typing or DNA fingerprinting.

DNA Vaccine - A nucleic acid vaccine. Genes coding for specific antigenic proteins are injected to produce those antigens and trigger an immune response.

Efficacy - The ability of a substance (such as protein therapeutic) to produce a desired clinical effect; its strength and effectiveness.

ELISA: Enzyme linked immunosorbent assay - a test to measure the concentration of antigens or antibodies.

Endogenous - Growing or developing from a cell or organism; or arising from causes within the organism.

Endonuclease - A restriction enzyme that breaks up nucleic acid molecules at specific sites along their length. Such enzymes are naturally produced by microorganisms as a defense against foreign nucleic acids.

Endoplasmic Reticulum - A highly specialized and complex network of branching, interconnecting tubules (surrounded by membranes) found in the cytoplasm of most animal and plant cells. The rough endoplasmic reticulum is where ribosomes make proteins. It appears "rough" because it is covered with ribosomes. The smooth endoplasmic reticulum is the site for syntheses and metabolism of lipids, and is involved in detoxifying chemicals such as drugs and pesticides.

Endotoxin - A poison in the form of a fat/sugar complex (lipopolysaccharide) that forms a part of the cell wall of some types of bacteria. It is released only when the cell is ruptured and can cause septic shock and tissue damage. Pharmaceuticals are tested routinely for endotoxins.

Enzymes - Proteins that catalyze biochemical reactions by causing or speeding up reactions without being changed in the process themselves.

Epithelium (epithelial) - The layer(s) of cells between an organism or its tissues or organs and their environment (skin cells, inner linings of lungs or digestive organs, outer linings of kidneys).

Establishment License Application (ELA) - Submitted concurrently with the PLA. The ELA provides data demonstrating the acceptability of the facilities and personnel for manufacturing the protein pharmaceutical.

Eukaryotes - Complex organisms, often multicellular, whose cells contain nuclei.

Exogenous - Developing from outside, originating externally. Exogenous factors can be external factors such as food and light that affect an organism.

Express - To translate a cell's genetic information, stored in its DNA (gene), into s specific protein.

Expression System - Organisms chosen to manufacture (by expression) a protein of interest through recombinant DNA technology.

Expression Vector - A way of delivering foreign genes to a host, creating a recombinant organism that will express the desired protein.

Fermenter - A vessel used to grow bacteria or yeasts in liquid culture.

Floc - A fluffy aggregate that resembles a woolly cloud.

Food and Drug Administration (FDA) - The U.S. Regulatory agency, which, among other responsibilities, evaluates pharmaceutical candidates for safety and efficacy, and determines whether to authorize them to be marketed in the United States. Other countries have similar regulatory agencies that must grant marketing authorization before a drug can be sold there.

Fusion Partner - When making a small protein or peptide in E. coli, it is often necessary to produce the protein fused to a larger protein to get high levels of stable expression. The resulting fusion protein must be cleaved (chemically or enzymatically broken) to yield the desired protein or peptide. The nonproduct fusion partner is left over and usually discarded.

Gene - The unit of inheritance consisting of a sequence of DNA, occupying a specific position within the genome. Three types of genes have been identified: structural genes encoding particular proteins; regulatory genes controlling the expression of the other genes; and genes for transfer RNA or ribosomal RNA.

Genetic Engineering - Altering the genetic structure of an organism (adding foreign genes, removing native genes, or both) through technological means rather than traditional breeding.

Genotype - The genetic composition of an organism (including expressed and non-expressed genes), which may not be readily apparent.

Germ Cell - The "sex cells" in higher animals and plants that carry half of the organism's genetic material and can combine to develop into new living things.

Glycosylation - Adding one or more carbohydrate molecules onto a protein (a glycoprotein) after it has been built by the ribosome; a posttranslational modification.

GMPs - Good manufacturing practices required by FDA regulations.

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Golgi Body - A cell organelle consisting of stacked membranes where posttranslational modifications of proteins are performed; also called Golgi apparatus.

Growth Hormone - A protein produced in the pituitary gland to control cell growth.

Hemocytometer - A device for counting blood cells.

Hormone - A protein released by an endocrine gland to travel in the blood and act on tissues at another location in the body.

Host Cells - The cells in which recombinant DNA is inserted so that it may be cloned as the host cells divide, so that the recombinant protein it codes for can be reproduced in large quantities.

HPLC - High-performance liquid chromatography or high-pressure liquid chromatography, a commonly used method for separating liquid mixtures.

Human Clinical Trials - Controlled clinical studies in human volunteers to test the safety and efficacy of pharmaceutical candidates.

Hybridoma - An immortalized cell line (usually derived by fusing B-lymphocyte cells with myeloma tumor cells) that secretes desirable antibodies.

Immortalize - To alter cells (either chemically or genetically) so that they can reproduce indefinitely.

Inoculate - To introduce cells into a culture medium.

Inoculum - Material (usually cells) introduced into a culture medium.

Interferon - A cytokine that inhibits virus reproduction. Interferons also affect growth and development (differentiation) in certain normal and tumor cells.

In Vitro - Performed in the laboratory rather than in a living organism (*in vivo*).

Investigational New Drug (IND) - An IND application containing laboratory study results of the drug candidate is submitted to the FDA to request permission to conduct studies in humans.

Ligase - An enzyme that causes fragments of DNA or RNA to link together; used with restriction enzymes to create recombinant DNA.

Lymphocytes - White blood cells that produce antibodies.

Lysosomes - Cell organelles containing enzymes, responsible for degrading proteins and other materials ingested by the cell.

Mab: Monoclonal antibody - A highly specific, purified antibody that recognizes only a single antigen.

Macrokinetics - Movement of whole cells and their media within a bioreactor.

Media - A (usually sterile) preparation made for the growth, storage, maintenance, or transport of microorganisms or other cells.

Metabolites - Chemical products of metabolism, the chemical process of life.

Microbiology - The study of microscopic life such as bacteria and viruses.

Microcarrier - A microscopic particle (often, a 200µm polymer bead) that supports cell attachment and growth in suspension culture.

Microencapsulated - Surrounded by a thin, protective layer of biodegradable substance referred to as a microsphere.

Microheterogeneity - Slight differences in the amino acid sequence of a protein. For example, to produce a recombinant protein in E. coli, a methionine (met) must be added to one end of the protein sequence to act as a signal that initiates protein synthesis. In most cases, that met is removed once the protein is made. Sometimes the met is removed for only some of the molecules. The purified product is then a mixture of a protein with the native sequence and a protein with the native sequence plus the extra amino acid.

Microinjection - Manually using tiny needles to inject microscopic material (such as DNA) directly into cells or cell nuclei; computer screens provide a magnified view.

Microkinetics - Movement of chemicals into, out of, or within the cell.

Microorganism - A microbe; a living thing too small to see with the naked eye.

Microtubules - Cellular organelles common in microorganisms; thin tubes that make structures involved in cellular movement.

Mitochondria - Animal-cell organelles that reproduce using their own DNA. They metabolize nutrients to provide the cell with energy and are believed to have once been symbiotic bacteria. Chloroplasts are their plant-cell equivalents.

Multicellular - Referring to organisms composed of more than one cell - often billions of them, arranged in various organs, tissues, and systems.

Mutagen - An agent (chemical, radiation) that causes mutations in DNA.

Mutation - A permanent change in DNA sequence or chromosomal structure.

Mycoplasma - Parasitic microorganisms that infect mammals, possessing some characteristics of both bacteria and viruses.

Myeloma - Lymphocytic cancer; a malignance normally found in bone marrow.

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New Drug Application (NDA) - An application to the FDA for approval to market a drug in the United States. The FDA determines if a protein pharmaceutical is considered a drug or a biologic and whether an NDA or PLA, respectively, should be filed to obtain marketing approval.

Nucleic Acids: DNA or RNA - long, chainlike molecules composed of nucleotides.

Nucleotides - Molecules composed of a nitrogen-rich base, phosphoric acid, and a sugar. The bases can be adenine (A), cytosine (C), guanine (G), thymine (T), or uracil (U).

Nucleus - The largest organelle, a sphere that contains all the cell's genetic material and a nucleolus that builds ribosomes.

Oncogen - A gene that, when expressed as a protein, can lead cells to become cancerous, usually by removing the normal constraints on its growth.

Organelle - A structurally discrete component that performs a specific function inside a cell.

Organism - A single, autonomous living thing. Bacteria and yeasts are organisms; mammalian and insect cells used in culture are not.

PCR - Ploymerase chain reaction, a method of duplicating genes exponentially.

Peptides - Proteins consisting of fewer than 40 amino acids.

Phenotype - The part of an organism's genotype that is expressed, and thus is generally apparent by observation.

Pilot Plant - A medium scale bioprocessing facility used as an intermediate in scaling up processes from the laboratory to commercial production.

Plasmid - Hereditary material that is not part of a chromosome. Plasmids are circular and self-replicating and found in the cytoplasm of cells (naturally in bacteria and some yeasts). They can be used as vectors for introducing up to 10,000 base pairs of foreign DNA into recipient cells.

Polymerase - An enzyme that catalyzes production of nucleic acid molecules.

Posttranslational Modifications - Protein processing done by the Golgi bodies after proteins have been constructed by ribosomoes.

Product License Application (PLA) - An application to the FDA for approval to market a biologic in the United States. The FDA determines if a protein pharmaceutical is considered a biologic or a drug and whether a PLA or an NDA, respectively, should be filed to obtain marketing approval.

Product Recovery - The process of separating a desired recombinant protein from the growth medium and the other elements in the host cells in which it was grown.

Prokaryotes - Simple organisms with no cell nuclei and very few organelles.

Protein - Macromolecules whose structures are coded in an organism's DNA. Each is a chain of more than 40 amino acids folded back upon itself in a particular way.

Proteolytic - Capable of lysing (denatureing, or breaking down) proteins.

Quality Control - In biotechnology, quality control is essential to ensure purified protein pharmaceuticals are indeed pure and that they are intact and maintain their biological activity.

Recombinant - Containing genetic material from another organism. Genetically altered microorganisms are usually referred to as recombinant; plants and animals so modified are called transgenic (see transgenics).

Recombinant DNA - DNA from one organism that has been combined with DNA from another organism. In biotechnology, individual human genes are often isolated and combined with a "DNA transporter," such as a plasmid, and this recombinant plasmid DNA is inserted into host cells so that it can be cloned.

Restriction Enzyme - A bacterial enzyme that cuts DNA molecules at the location of particular sequences of base pairs.

Ribosome - Cell organelles that translate information from the RNA to build proteins.

RNA - Ribonucleic acid; similar to DNA but based on ribose, and with the base uracil (U) in place of thymine (T). Various forms of RNA are found: mRNA (Messenger RNS); tRNA (transfer RNA); and rRNA (ribosomal RNA). Most RNA molecules are single stranded, although they can form double-stranded units.

Roller Bottle - A container with large growth surfaces in which cells can be grown in a confluent monolayer. The bottles are rotated or agitated to keep cells in suspension, but they require extensive handling, labor, and media. In large-scale vaccine production, roller bottles have been replaced by microcarrier culture systems that offer the advantage of scale-up.

Scale-Up - To take a biopharmaceutical manufacturing process from the laboratory scale to a scale at which it is commercially feasible.

Seed Stock - The initial inoculum, or the cells placed in growth medium from which other cells will grow.

Sequence - The precise order of bases in a nucleic acid or amino acids in a protein.

Serum - The water portion of an animal or plant fluid (such as blood) remaining after coagulation. When cheese is made, whey is the milk serum that is left.

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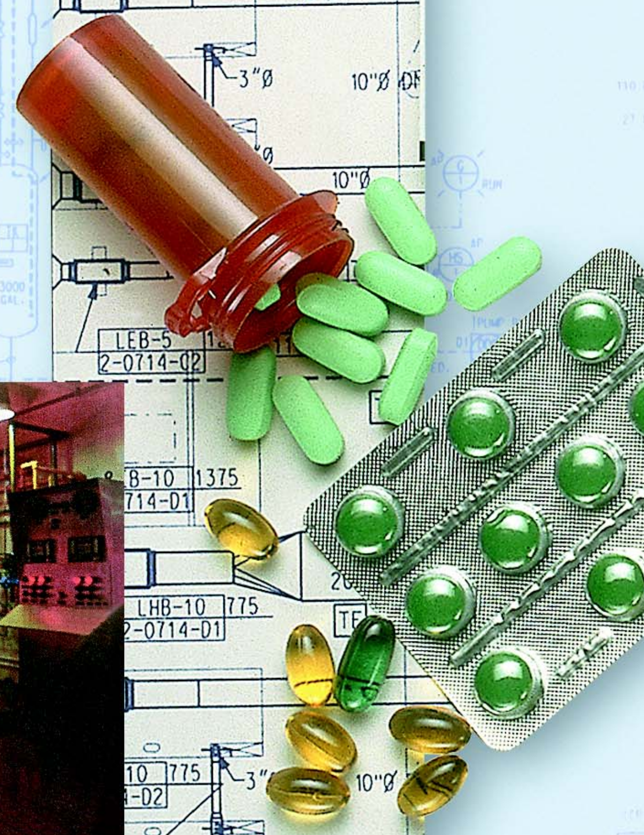

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SIP - Steam in place or sterilize in place (see CIP).

Somatic Cell - In higher organisms, a cell that, unlike germ cells, carries the full genetic make-up of an organism.

Sparge - To spray. A sparger is the component of a fermenter that sprays air into the broth.

Strain - A population of cells all descended from a single cell.

Substrate - Reactive material, the substance on which an enzyme acts.

Substratum - The solid surface on which a cell moves or on which cells grow.

Supernatant - Material floating on the surface of a liquid mixture (often the liquid component that has the lowest density).

Surfactant - Any substance that changes the nature of a surface, such as lowering the surface tension of water.

Suspension - Particles floating in (not necessarily on) a liquid medium, or the mix of particles and liquid itself.

Symbiotic - Living together for mutual benefit.

Synthesis - Creating products through chemical and enzymatic reactions.

Tissue Culture - Growing plant or animal tissues outside of the body, as in a nutrient medium in a laboratory; similar to cell culture, but cells are maintained in their structured, tissue form.

Titer - A measured sample (to draw a measured, representative sample from a larger amount is to titrate).

Transformation - Getting host cells to take up DNA that has been added to its medium, such as recombinant plasmid DNA.

Transgenics - The alteration of plant or animal DNA so that it contains a gene from another organism. There are two types of cells in animals and plants, germ line cells (the sperm and egg in animals, pollen and ovule in plants) and somatic cells (all of the other cells). It is the germ line DNA that is altered in transgenic animals and plants, so those alterations are passed on to offspring. Transgenic animals are used to produce therapeutics, to study disease, or to improve livestock strains. Transgenic plants have been created for increased resistance to disease and insects, as well as to make biopharmaceuticals.

Translation - The process by which information transferred from DNA by RNA specifies the sequence of amino acids in a polypeptide (protein) chain.

Trypsin, Tryptic Digestion - Trypsin allows the growth of cells as independent microorganisms distinct from tissue culture by causing cell disaggregation. Excised tissue is softened and treated with a proteolytic enzyme, normally typsin, then washed and suspended in a growth medium to produce a primary culture. Subculturing from the primary culture usually involves treatment with an antitrypsin (such as serum) to produce a secondary culture. Cell lines are established by repeated culture through cycles of growth, trypsinization, and subculture. Trypsin is also used to remove anchorage-dependent cells from their attached substratum.

Tryptic Fragment Analysis - Quantitating the resultant fragments caused by tryptic digestion.

Turbidostat - A variation on a chemostat. Whereas a chemostat is designed for constant input of medium, a turbidostat is designed to keep the organisms at a constant concentration. A turbidity sensor measures the concentration of organisms in the culture and adds additional medium when a preset value is exceeded.

Turbulent Flow Field - The state that results from mixing the contents of a fermenter or bioreactor to provide oxygen to the cells. That must be balanced against the shear that causes cell damage and death.

Unicellular - Composed of only a single cell.

Vaccines - Preparations of antigens from killed or modified organisms that elicit immune response (production of antibodies) to protect a person or animal from the disease-causing agent.

Vacuolation - In cell and tissue culture, excess fluid, debris (aggregates), or gas (from sparging) can form inside a cell vacuole. A vacuole is a cavity within the cell that can be relatively clear and fluid filled, gas filled (as in a number of blue-green algae), or food filled (as in protozoa).

Vector - The plasmid, virus, or other vehicle used to carry a DNA sequence into the cell of another species.

Vessel Jacket - A temperature control method consisting of a double wall outside the main vessel wall. Liquid or steam flows through the jacket to heat (or cool) the fluid in the vessel. Because biopharmaceutical products are so sensitive and vessel jackets can cause uneven heating (hot or cold spots), shell-and-tube heat exchangers are more common in biopharmaceutical production systems.

Viability - Life and health, ability to grow and reproduce; a measure of the proportion of live cells in a population.

Virus - The simplest form of life - RNA or DNA wrapped in a shell of protein, sometimes with a means of injecting that genetic material into a host organism (infection). Viruses cannot reproduce on their own, but require the aid of a host.

Viscosity - Thickness of a liquid; determines its internal resistance to shear forces.

Water-For-Injection - Very pure water, suitable for medical uses.

Yeast - A single-celled fungus.