Chapter 26

The Biomanufacturing of Biotechnology Products

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What is biologics manufacturing? How is it different from small molecule pharmaceutical manufacturing? Biologics manufacturing, or biomanufacturing for short, is a complex process that produces a product largely derived from discoveries using recombinant DNA technology to develop processes and analytics to manufacture biotherapeutic products. These recombinant products were developed from several platforms such as whole *multicellular* systems encompassing transgenic plants, animals and *unicellular* microbials (bacteria and yeast), and insect and mammalian cell cultures. The discovery and "proof-of-concept" from the research bench is transferred to a process and analytical group that will use science and engineering as well as regulatory experience to scale-up the product efficiently with sufficient product yield to support the clinical program and a quality product expressing the quality attributes of the product as well as profile any product-associated impurities. The process for different biologic platforms are complex and unlike traditional chemical synthesis the biologic product resulting from a living system is not as an exact science as chemistry.

The long-existing paradigm for biologics was "the process is the product" and any variation in the process could impart a change in the product's safety and efficacy. Although today's raw materials, process and analytics are better defined and allow a lot more flexibility in the design and development of the process. Changes in the process or materials could severely alter the product's safety and characteristics, thus one may end up with a product with a different profile. Changes in the manufacturing process can alter the "impurity profile" of a biologic, thus imparting changes in the product's purity which can have an adverse effect on safety. It has been demonstrated that endogenous adventitious viruses may result from processing changes or the extension of the production process. Therefore, end of production processes and genotypic studies on the cell line have been required to understand the implications of changes in the production process that can affect the product's quality attributes, impurity profiles that could impact the safety of the therapeutic. Today's biologic manufacturing facilities incorporate analytical and process development capabilities to develop and test the scale-up of the process to deliver sufficient productivity of a quality product. The development will support a Phase 1 clinical study focusing on safety and efficacy of the product. If the product can demonstrate safety and efficacy, the product with regulatory agency and business-positive feedback will continue the manufacturing of the biologics until reaching final approval and licensing.

THE HISTORY OF BIOTECHNOLOGY AND BIOMANUFACTURING

Biotechnology is technology based on biology. It utilizes the biology of living systems and their genetic manipulations of these systems and processes to develop technologies and products that help improve the lives and health of individuals worldwide. These biological processes of microorganisms have been used for thousands of years to make food products from fermentation, such as bread, beer, wine, pickles and cheese—these processes are still used today. Modern biotechnology provides discoveries of recombinant DNA technology to discover and develop

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therapeutic biologics. The following is a chronological history of significance to the field of biotechnology and biomanufacturing (http://www.bio.org/articles/history-biotechnology?page):

500 B.C.—In China, the first antibiotic, moldy soybean curds, is put to use to treat boils.
A.D. 100—The first insecticide is produced in China from powdered chrysanthemums.
1761—English surgeon Edward Jenner pioneers vaccination, inoculating a child with a viral smallpox vaccine.
1870—Breeders crossbreed cotton, developing hundreds of varieties with superior qualities.
1870—The first experimental corn hybrid is produced in a laboratory.
1911—American pathologist Peyton Rous discovers the first cancer-causing virus.
1928—Scottish scientist Alexander Fleming discovers penicillin.
1933—Hybrid corn is commercialized.
1942—Penicillin is mass produced in microbes for the first time.
1950s—The first synthetic antibiotic is created.
1951—Artificial insemination of livestock is accomplished using frozen semen.
1958—DNA is made in a test tube for the first time.
1978—Recombinant human insulin is produced for the first time.
1979—Human growth hormone is synthesized for the first time.
1980—Smallpox is globally eradicated following a 20-year mass vaccination effort.
1980—The U.S. Supreme Court approves the principle of patenting organisms, which allows the Exxon Oil Company to patent an oil-eating microorganism.
1981—Scientists at Ohio University produce the first transgenic animals by transferring genes from other animals into mice.
1982—The first recombinant DNA vaccine for livestock is developed.
1982—The first biotech drug, human insulin produced in genetically modified bacteria, is approved by the FDA. Genentech and Eli Lilly developed the product.
1985—Genetic markers are found for kidney disease and cystic fibrosis.
1986—The first recombinant vaccine for humans, a vaccine for hepatitis B, is approved.
1986—Interferon becomes the first anticancer drug produced through biotech.
1988—The first pest-resistant corn, Bt corn, is produced.
1990—The first successful gene therapy is performed on a 4-year-old girl suffering from an immune disorder.
1992—The FDA approves bovine somatotropin (BST) for increased milk production in dairy cows.
1993—The FDA approves Betaseron®, the first of several biotech products that have had a major impact on multiple sclerosis treatment.
1994—The first breast cancer gene is discovered.
1994—The Americas are certified polio-free by the International Commission for the Certification of Polio Eradication.
1995—Gene therapy, immune-system modulation, and recombinantly produced antibodies enter the clinic in the war against cancer.
1996—A gene associated with Parkinson's disease is discovered.
1996—The first genetically engineered crop is commercialized.
1997—A sheep named Dolly in Scotland becomes the first animal cloned from an adult cell.
1998—The FDA approves Herceptin®, a pharmacogenomic breast cancer drug for patients whose cancer overexpresses the HER2 receptor.
1999—A diagnostic test allows for the quick identification of Bovine Spongicorm Encephalopathy (BSE, also known as "mad cow" disease) and Creutzfeldt-Jakob Disease (CJD).
2000—Kenya field-tests its first biotech crop, a virus-resistant sweet potato.
2001—The FDA approves Gleevec® (imatinib), a gene-targeted drug for patients with chronic myeloid leukemia. Gleevec is the first gene-targeted drug to receive FDA approval.
2002—The EPA approves the first transgenic rootworm-resistant corn.
2002—The banteng, an endangered species, is cloned for the first time.
2003—China grants the world's first regulatory approval of the gene therapy product, Gendicine (Shenzhen SiBiono GenTech), which delivers the p53 gene as a therapy for squamous cell head and neck cancer.
2003—The Human Genome Project completes sequencing of the human genome.
2004—The United Nations Food and Agriculture Organization endorses biotech crops, stating biotechnology is a complementary tool to traditional farming methods that can help poor farmers and consumers in developing nations.
2004—The FDA approves the first antiangiogenic drug for cancer, Avastin®.
2005—The Energy Policy Act is passed and signed into law, authorizing numerous incentives for bioethanol development.
2006—The FDA approves the recombinant vaccine Gardasil®, the first vaccine developed against human papillomavirus (HPV), an infection implicated in cervical and throat cancers, and the first preventative cancer vaccine.

2006—The U.S. Department of Agriculture grants Dow AgroSciences the first regulatory approval for a plant-made vaccine.
2007—The FDA approves the H5N1 vaccine, the first vaccine approved for avian flu.
2009—Global biotech crop acreage reaches 330 million acres.
2009—The FDA approves the first genetically engineered animal for production of a recombinant form of human antithrombin.

A TYPICAL BIOMANUFACTURING PROCESS

Figures 26.1 to 26.2 show typical biomanufacturing processes for upstream processes, and regulatory milestones from preclinical, clinical, BLA, and NDA submissions.

A Typical Biologic Product Development Diagram and Regulatory Milestones from Preclinical, Clinical, BLA, and NDA Submission

There are various regulatory opinions in the United States, European, Japanese pharmaceutical industries, and other countries that regulate biologics. It is quite confusing and overlaps in opinions and redundancies. In order to bring together these regulatory authorities, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was formed in 1990. This organization brings together these regulatory authorities to discuss scientific and technical aspects of drug registration. Since 1990 the increase in drug development has grown globally and at an incredible pace. ICH has harmonized regulatory guidance documents to help biopharmaceutical entities to register and develop safe, quality, and effective drugs. There are guidances covering quality, safety and efficacy guidelines. Below is a list of ICH guidances that are relevant to the manufacture of a common biologic, a "therapeutic protein" known as a monoclonal antibody (mAb). The following slides lists the pertinent ICH guidance document for a mAb biomanufacturing process and the diagram illustrates the typical biomanufacturing process flow for a mAb. (See Figures 26.3A and B.)

Biotechnology products are derived from manipulation of various cells and their subsequent products these engineered cell systems produce. These biologic products are produced from various biomanufacturing platforms. One platform produces therapeutic proteins such as monoclonal antibodies, cytokines, fusion proteins, and a number of therapeutic and process enzymes. There are also vaccines, whole cell, and gene therapy products being developed and biomanufactured.

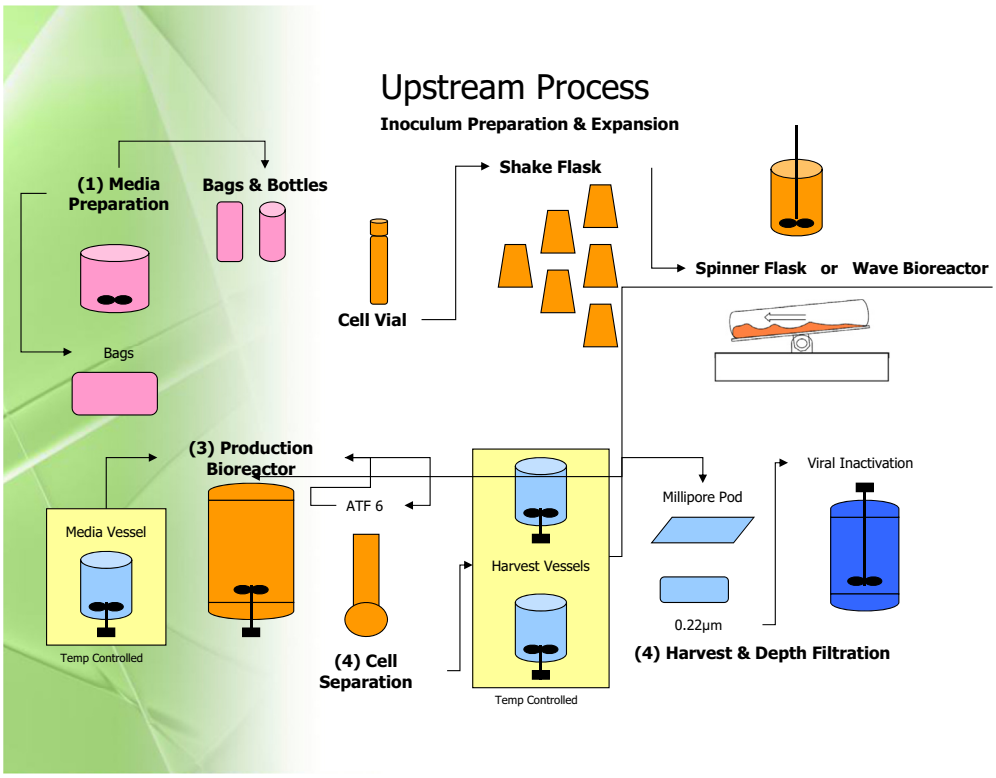

FIGURE 26.1 A Typical biomanufacturing diagram of an upstream process. *(Source: Cytovance Biologics Inc., John Conner, 2013.)*

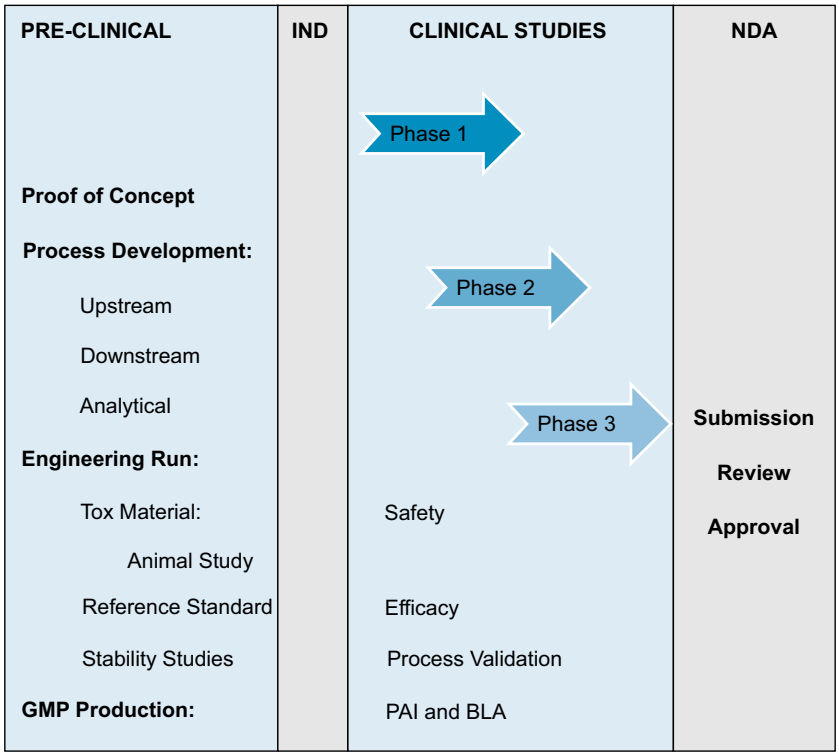

FIGURE 26.2 A typical biologic product development diagram and regulatory milestones from a preclinical, clinical, BLA, and NDA submission. *(Source: Cytovance Biologics Inc., John Conner, 2013.)*

A biologic therapeutic product, also known as a biologic, is a therapeutic product developed to treat a variety of diseases. This biologic product can be a monoclonal antibody, a vaccine, a tissue, or various proteins such as cytokines, enzymes, fusion proteins, whole cells, and viral and nonviral gene therapies. Biologic products are derived from living systems that may or may not be altered. Recombinant DNA technology has produced many biological products and has allowed research an avenue to discover many more. The paradigm for biologics "the process is the product" still is viable today. Biologics are also known as large molecules (nucleic acid and protein platforms). It is generally known that a therapeutic biologic is a product derived from or part of a cell or tissue. While the term biologic is used more often when the medical product consists of a cellular or tissue

There are several classes of biologic products. Some are naturally occurring biologics such as whole blood and blood components, organ and tissue transplantations, vaccines and recently stem cell therapy. Those therapeutic biologics that are derived via recombinant DNA technology have developed a large number of therapeutics and have replaced older naturally derived drugs with recombinant DNA technology such as "insulin" that was originally derived up until the 1980s from insulin extracted from cattle and pig pancreas (When comparing non-recombinant insulin, there are only three amino acid differences between cattle and human insulin. There are also only one amino acid difference between human and pig insulin). Today, most insulin is biomanufactured via recombinant DNA technology or "genetic engineering."

Recombinant DNA technology products are wide ranging and include proteins derived that are monoclonal antibodies, signaling-type proteins, and receptor-type proteins.

In general here are a number of types of biologics derived from recombinant DNA technology:

- Article I. Monoclonal antibodies
- Article II. Cytokines
- Article III. Process or intermediates used in manufacturing
- Article IV. Recombinant proteins
- Article V. Therapeutic vaccines
- Article VI. Growth hormones
- Article VII. Blood production-stimulating proteins
- Article VIII. Insulin and analogs
- Article IX. Therapeutic enzymes
- Article X. Fusion proteins
- Article XI. Therapeutic peptides
- Article XII. Therapeutic oligos
- Article XIII. Vaccines
- Article XIV. Gene therapy
- Article XV. Whole cells
- Article XVI. Tissues
- Article XVII. Biosimilars

A biologic can be defined as a large complex molecule produced from or extracted from a biological or living system.

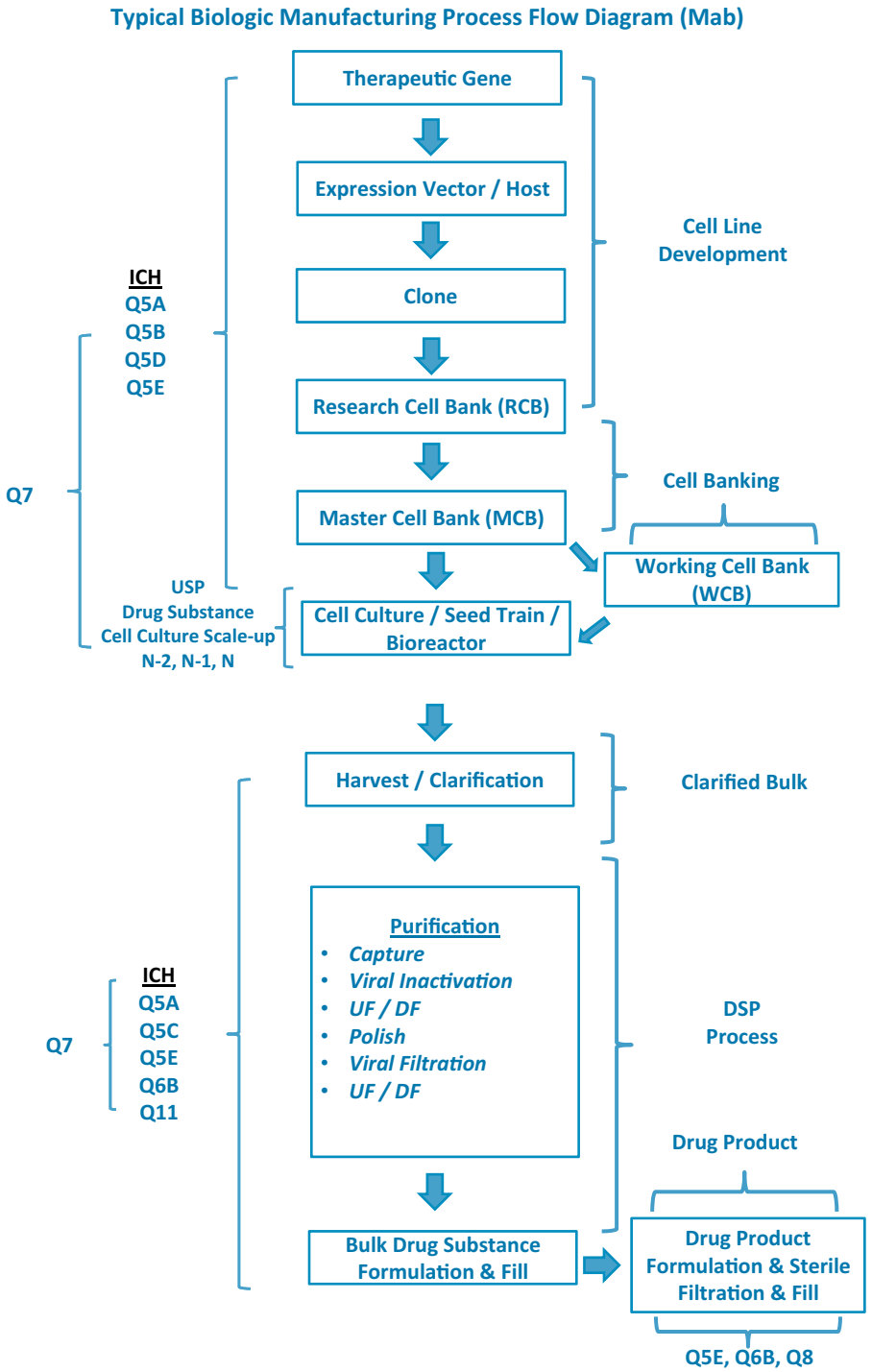

FIGURE 26.3 A typical biologic manufacturing process flow diagram with appropriate ICH guidance per steps. *(Source: Cytovance Biologics Inc., John Conner, 2013.)*

The biomanufacturing process, process controls as well as the complete physiochemical and biological testing all together characterize the biologic product. For example, in regards to the biomanufacturing process, a biologic can be derived from biotechnology or it may be prepared using more conventional methods as is the case for blood- or plasma-derived products and a number of vaccines. In regards to the nature of a biologic's active substance, it may consist entirely of microorganisms or mammalian cells, nucleic acids (DNA or RNA), be a protein (antibody, cytokine, enzyme, protein-like, etc.) all originating from either a microbial, animal, human, or plant sources.

TABLE 26.1 A Variety of Biologics

Designation	Name	Indication	Technology	Mechanism of Action
Abatacept	Orencia	Rheumatoid arthritis	Immunoglobin CTLA-4 fusion protein	T-cell deactivation
Adalimumab	Humira	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease	Monoclonal antibody	TNF antagonist
Alefacept	Amevive	Chronic plaque psoriasis	Immunoglobin G1 fusion protein	Incompletely characterized
Erythropoietin	Epogen	Anemia arising from cancer chemotherapy, chronic renal failure, etc.	Recombinant protein	Stimulation of red blood cell production
Etanercept	Enbrel	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis	Recombinant human TNF-receptor fusion protein	TNF antagonist
Infliximab	Remicade	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease	Monoclonal antibody	TNF antagonist
Trastuzumab	Herceptin	Breast cancer	Humanized monoclonal antibody	HER2/neu (erbB2) antagonist
Ustekinumab	Stelara	Psoriasis	Humanized monoclonal antibody	IL-12 and IL-23 antagonist
Denileukin	Ontak	Cutaneous T-cell	Diphtheria toxin	Interleukin-2
Diftitox		Lymphoma (CTCL)	Engineered protein combining Interleukin-2 and Diphtheria toxin	Receptor binder

(**Source:** *http://en.wikipedia.org/wiki/Monoclonal_antibody_therapy#FDA_approved_therapeutic_antibodies*.)

A biologics mode of action may describe a biologic platform such as an immunotherapeutic, gene therapy or a cellular therapeutic. (See Table 26.1.)

BIOSIMILARS

What is a biosimilar? How is it different than a generic? Generic is the designation for (off-patent) small-molecule drugs that are exactly identical to the properties and the process to manufacture is the same and reproducible. In essence, chemically synthesized drugs are pretty much: add component A + B = C (product) almost all of the time. Biosimilars are much more complex and larger molecules can be influenced by the manufacturing process. Because of the biologics complexity, biosimilar manufacturers cannot guarantee that their biosimilar is exactly identical to the original manufacturer's version, but rather it is similar to the original biologic. The biosimilar manufacturer's manufacturing process may be slightly different. This may produce significantly different effects which may impact product quality, have no effect, or in some cases elicit additional quality attributes—now you have a biobetter, thus requiring clinical development. A disadvantage of the biosimilar developer is that in developing the innovator's process to produce the biosimilar, the innovator's starting material, i.e., the recombinant cell line or clone, is not available to the developer unlike generics where raw materials and process chemicals are known or can be derived. Finally, the impurity profiles may impart a variety of similar impurities or degradation products that may elicit harmful side-effects.

Thus, biosimilar manufacturers produce products that are slightly different than the innovator's and cannot guarantee that their biosimilar is as safe and effective as the innovator's product. So, unlike generics, biosimilars were not authorized in the United States or the European Union through the procedures that allowed generic approvals. So as a result, to date all biosimilar drugs have targeted well-known approved and coming off patent biologic drugs. The regulatory agencies have required biosimilar therapeutics to undergo a very detailed comparability review and testing. In 2012, the United States Federal Drug Agency (U.S. FDA) published a guidance as part of the "Patient Protection and Affordable Care Act of 2010" part of the Public Health Service Act (PHS Act) which was created to provide an

approval pathway known as the Biologics Price Competition and Innovation Act (BPCI Act). This approach or law allows for a potential approval of biological products that can demonstrate "biosimilar" properties that are "very similar" to the original biologic or one that "closely resembles" the FDA-licensed biological product.

The European regulatory authority (EMA) has provided an approach and coined their biosimilar as "similar biological medicinal products." Their document guides biosimilar companies in the manufacture and approval of these complex biologics to demonstrate comprehensive comparability of the biosimilar to the innovator's product. The European Medicines Agency (EMA) accomplished this well before the FDA. Thus, the EU has been further ahead of the United States in biosimilars with the EMA approving the first biosimilar "Omnitrope" in 2006. In June 2010, a biosimilar copy of Amgen's Neupogen was approved and since then a total of 12 biosimilars have been approved between 2006 and 2012. In July 2013, two monoclonal antibody (mAb) biosimilars (Remsima-Celltrion and Inflectra-Hospira) were approved by the EMA. These mAbs were very similar to the innovator molecule known as Remicade (infliximab) originally approved in 1999 for rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriatic arthritis, and psoriasis. All of these diseases are classified as autoimmune diseases.

Therefore, a biosimilar product is similar to a biologic reference or innovator product, has the same mechanism of action for the intended use, preapproved label use, has the same route of administration, dose formulation, and has similar potency or strength as the reference or innovator product. The biosimilar also should show no clinical differences between the biosimilar and the innovator product in terms of the safety, purity, and potency.

Therefore, the regulatory theme for the manufacture and approval is midway between testing and comparability for a new therapeutic biologic and more than the testing of a generic drug. Comprehensive comparability is the key! Below is a list of classes of biologics that are in the current biologic development or have been approved:

- Epoetins
- Filgrastims
- Insulins
- Growth hormones
- Monoclonal antibodies
- Low-molecular weight heparins
- Beta interferons

Key Phases of Biologics Development

The biologic product to be developed and manufactured starts with the discovery and proof-of-concept studies characterizing the product as well as defining the actual molecule and its mode of action. The study and knowledge of the molecule's characteristics will be important in designing cell culture and bioreactor parameters in order to scale-up and produce a sufficient yield of product to satisfy early experiments and analytical development. This crude product will also be used for early clarification, downstream process development, and formulation studies. During the discovery phase *in vitro* and *in vivo* (small animal) studies will be performed. Once the product proof-of-concept is achieved, a decision to move forward with process development and the eventual investigational new drug (IND) finally is agreed upon. In today's drug development world, the next step is to secure funding to continue developing the therapeutic. This usually involves forming a company and securing funding from private sources, venture capital or government grants. Most of these companies don't have sufficient funding to build development, manufacturing and quality laboratories nor the time it takes to build this infrastructure. These companies partner with Contract Manufacturing Organizations (CMOs) to help develop and manufacture the drug as well as offer Quality and Regulatory support.

DISCOVERY

Target identification of a therapeutic biologic molecule involves choosing a disease with an unmet need or an improvement of a current therapeutic, and usually the group or person may have a personal or scientific interest in studying and developing a drug candidate. These biologic drugs are usually discovered in academic and biotech research labs. Once a molecule is chosen, the biochemical mechanism and other biological characteristics are tested for their interaction with the drug or disease target. There are thousands of biologic molecules studied and they go through a very arduous process to profile and characterize the molecule and its potential drug or disease target. Proof-of-concept *in vitro* and *in vivo* (small animals such as rodents) must be validated before any drug can move from discovery and into a clinical development program. Once a promising therapeutic candidate or several leading candidates are validated, the company or group will set up and budget a preclinical development program.

PROCESS DEVELOPMENT

All biologic products transition from bench scale research and proof-of-concept to the next stage of product development called process and analytical development. The therapeutic protein candidate's characteristics, purification strategies, analytical methods for in-process and final product characterization, and any stability data are supplied to the process and analytical development groups. This is typically called the technology transfer or "tech transfer." The tech transfer is the most important phase of product development as it is the hand-off to the scale-up team (process

and analytical development—PD and AD respectfully), manufacturing science and technology (MST), manufacturing and quality assurance (QA), and quality control (QC) teams. A thorough understanding of the product's characteristics and the process at bench scale is necessary in order to facilitate the transfer of all of the critical parameters.

Once the tech transfer is initiated from the client, the upstream process development (USP), or cell culture group, embarks on accessing the media and cell culture conditions relative to the correct identification (ID) of the cell line, the growth and viability of the cell line is robust, and the productivity of the cell line's product (therapeutic protein) titer (yield) is adequate to scale-up. Once, the cell line and growth conditions are confirmed, the PD group will make a development cell bank from the research cell bank (RCB) and will use this to develop a scale-up process and optimize the media and supplements used in the cell culture. The USP development will also make material to be used in the downstream process development (DSP) which is the purification and formulation development group. This group will also work on the postharvest clarification (removal of the cells and other large molecules as well as some large protein and DNA aggregates) prior to the first chromatography or purification steps. The first steps post clarification is usually a capture- or affinity-binding step. In essence the therapeutic protein is bound to a chromatographic resin's (example Protein A mAbSelect Sure™ from General Electric (GE) packed in a column allowing for impurities to flow through the column. The bound therapeutic protein (TP) is then pulled off or eluted from the resin and collected for the purification steps which takes the TP through various chromatographic column steps to remove impurities and aggregates such as host cell proteins (HCP) and host DNA. All of these chromatographic purification and polishing steps are designed based on the protein properties and characteristics described during the discovery bench scale or preclinical phase of the product development. The DSP also will include viral clearance studies to reduce the risk of viral contaminants. Several resins or filters significantly reduce viral load or give "x amount of Log reduction of virus." The DSP team also develops the formulation steps for bulk drug substance (BDS) and the final formulation of the drug product (DP) and excipients (other materials in the formulation that impart a property such as providing stability to the DP). During the DSP process, the development and optimization of the DSP team will provide the analytical development team process/product material to work on designing, confirming, qualifying, or validating analytical methods needed for in-process and release testing.

As the process development phase of the product development proceeds the manufacturing science and technology group will work with the PD and AD teams to capture and work on the tech transfer from the development groups into the manufacturing and quality groups.

CLINICAL MANUFACTURING

Preclinical Trials

In the preclinical phase the product is further characterized. The biologic molecule's phenotypic, genotypic, and biochemical profiles must be determined. Attributes and parameters useful in determining its strengths and weaknesses such as shape, amino acid sequence, isoelectric point (PI), drug candidates mechanism of action, its potential bioactivity or availability, and any possible toxicity issues are some of the characterizations that will need to be studied or elucidated. These will be the building blocks of information that will be transferred to other groups responsible for process and analytical development.

How are we going to manufacture the novel therapeutic biologic? During this phase a process is developed to scale-up the process to manufacture a quality product that can be used in a Phase 1 clinical trial. Typically a master cell bank (MCB) will be produced from a research cell bank (RCB) or a pre-MCB. A working cell bank (WCB) may be produced from a MCB that has been fully tested for bio-safety and has also been fully characterized. If a WCB is produced it to will be tested as the MCB was tested for bio-safety and characterized as defined per the Q7 ICH Guidance. These cells will be used in PD DEMO (demonstration) runs and the data from the PD work will be transferred (tech transfer) to manufacturing. Concurrently analytical development will be working on developing and qualifying in-process and release analytics which will then be transferred into quality control (QC).

Manufacturing scientists and engineers will then take the process and design a scale-up plan and tech transfer into manufacturing. The first run will be a non-GMP engineering run that will lead to a GMP run. Typically the engineering run is a process dress rehearsal for the Good Manufacturing Practice (GMP) run. This run will manufacture products for:

- Toxicology-primate study
- Viral clearance/validation
- Reference standard
- Stability studies
- Storage
- Shipping
- Container closure DBS and DP
- Analytical development (ex bioassays)
- Other analytical methods work
  - Compendial
  - Method qualification/validation work

The GMP run will be prepared by reviewing the engineering run outcome based on the process review, in-process data, and final testing of the product. The engineering run will be deemed successful and ready for GMP production if

there are no major process or testing issues and the product has met all of its quality parameters allowing for the release of the drug substance and if the drug product is formulated to pass all of the acceptance criteria which would allow for the product to be released. The documents would be revised and updated from the engineering run redlined batch records. The final production records would be reviewed and approved for GMP production. The GMP run would provide material to support the following needed for IND filing and the start of the Phase 1 clinical trial:

- Clinical Trial Material
- Stability
- Reference standard
- End-of-production (EOP) cell bank
- Genetic stability

The GMP clinical trial material is held in quarantine at the biomanufacturing site and when it passes all of the release testing and document review, the GMP lot will be released to the in-house clinical distribution group for clinical studies once the IND is approved. The clinical trials are usually managed by the in-house clinical group or outsourced to companies like Almac, Quintiles, or Covance.

Another important part of the preclinical phase of the product development is drug formulation and drug delivery. How are we going to present this drug to the patient's biologic system? Formulation, delivery, and container/closure development must be studied at this early stage. If you cannot develop a stable formulation matrix and do not know the delivery path, the drug candidate development will be delayed or stopped. Formulation and the product's delivery parameter development is a critical element that must be developed and understood. The key is stability (biosafety and product maintains its quality attributes) of the drug candidate's formulation, safety as determined initially by biosafety testing of the bulk, and final product. It must also be noted that this formulation and drug delivery system may be revised during the development of the product.

Once formulated, several other important studies are important to develop and understand the formulated biologic drug. Pharmacokinetic (PK) studies look at absorption, drug distribution, metabolism of the drug, and the excretion or elimination of the biologic drug. Why is this important? PK data from animal toxicity (TOX) studies (product produced from non-GMP scale-up or engineering run) will be used to compare to the eventual early-phase studies. These preclinical TOX studies are typically dosing studies (acute and chronic dosing that help determine the specific dose and range in the animal TOX and first in the human Phase I clinical study). Other toxicity studies look at carcinogenic, mutagenic, and reproductive toxicity. This should give an idea of the potential degree of safety and efficacy of the formulated drug candidate as well as a foundation to support the process development, analytical development, and the investigational new drug (IND) application filing. Once filed and approved, the preclinical and discovery information will help support the clinical development of the biologic drug. *Note*: all of the proposed preclinical toxicity studies are actual guidances from various regulatory agencies.

In order to understand and support the product characteristics and quality attributes and support preclinical and clinical in-process and release testing bioanalytical testing must be developed in the preclinical phase of the biologics development. Thus the analytical development group will support methods for cell culture, fermentation, assay to determine titers or process yields as well as bioassays, and other assays to elicit the identity, potency, purity, and safety of the product (e.g., in monoclonal antibodies [mAbs]) to use size exclusion chromatography (SEC) to determine the percent of purity by evaluating the amount of aggregation and other product impurities. It is important to understand the impurity profile of the product and the final formulated product.

CLINICAL TRIALS

Clinical studies are grouped according to their objective into three types or phases (Phase 1, 2, and 3). How does biomanufacturing activities correlate to the different phases of a clinical trial leading up to the commercialization of a biologic product? Manufacturing of the therapeutic biologic will continue through the prelinical and clinical phase of the trial to supply the clinic with the trial product to the patients enrolled in the clinical study, stability programs, additional reference standards, additional process optimization studies, and additional studies as needed to support investigations.

Phase 1 Clinical Development

Thirty days after a "biotech" company has filed its IND, it may begin a small-scale Phase 1 clinical trial to demonstrate human pharmacology and safety. Phase 1 parameters such as pharmacokinetic (PK) and tolerance in healthy recruited volunteers will be studied. These studies include acute and chronic dosing studies including initial single-dose, a dose escalation, and repeated-dose studies.

Phase 2 Clinical Development

Phase 2 clinical studies are small-scale trials to continue to evaluate the safety and PK of the biologic and also to evaluate the efficacy and possible side-effects in a small set of patients (commonly 100 to 200). Typical Phase 2 objectives are:

- Safety
- Efficacy
- Risk assessment
- Process review
- Raw materials

- Analaytical methods
- BDS container closure
- DP container closure
- Storage
- Shipping
- Phase 2b (Phase 2a and b review and requirements to move to Phase 3)

Phase 3 Clinical Development

Phase 3 studies are large-scale clinical trials for continued safety and efficacy in a larger patient population. While Phase 3 studies are in progress, there are several interim analyses available to show continued safety and efficacy. During this phase the final process is determined and "locked down." A gap analysis of the process to support process validation is conducted and any gaps or risks are addressed. Process validation commences, and if all goes well the company will commence manufacturing of the three registration or conformance lots prior to the filing of a Biologics License Application (BLA) or a New Drug Application (NDA). Typical Phase 3 objectives are:

- Safety
- Efficacy
- Risk assessment
- Lock down process
- Process and analytical gap analysis (FMEA)
- Process validation
- Minimum 3 conformance or registration lots
- BLA submission
- PAI facility and quality assessment inspection
- BLA approval
- Secondary labeling and packaging-approval
- Inventory build

Phase 4 Marketing

Prepare for Commercial Launch and Commercial Manufacturing

Once a BLA and NDA have been approved, the biotech company will be seeking to launch the released drug product in the approved market. The inventory to launch may be the three conformance lots or other released lots manufactured in anticipation of an approved license to manufacture, distribute, and market the new drug.

GOOD MANUFACTURING PRACTICES

Requirements

Biologics manufacturing requires the use of good manufacturing practices (GMP) or current good manufacturing practices (cGMP) to ensure that adequate history is maintained for each product run. As the product manufacturing process is developed and defined and as it matures from the lab bench development through Phases 1, 2, and 3, the regulatory expectation is that appropriate GMP be applied to help ensure subject safety. This is especially critical at Phase 1 where the clinical trial focus is safety and efficacy.

In order to support clinical trial drugs, manufacturers are expected to implement manufacturing controls that reflect product and manufacturing considerations, evolving process and product knowledge, and manufacturing experience. As the process becomes better-defined, critical control points are identified, and experience in the process increases, increased GMP documentation must be implemented and maintained. This means that information that is gained from the lab development bench scale all the way through Phase 3 trials must be translated into compliant GMP documents that house product production history.

Code of Federal Regulations and European Regulations

The United States Food and Drug Administration (FDA) and the European Union provide regulations for the manufacture of drug products. These are the laws that biologics manufacturers are required to follow.

The FDA regulations are defined in 21 CFR 210, "Current Good Manufacturing Practice in Manufacturing, Processing, Packaging, or Holding of Drugs; General" and 21CFR211, "Current Good Manufacturing Practices for Finished Pharmaceuticals." EU regulations are defined in the EudraLex, Volume 4, "EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use."

Additionally, the United States publishes guidance documents that provide the agencies current thinking on topics. These guidance documents provide valuable details into how the agencies expect manufacturers to show compliance to the regulations. The most used guidance documents can be found at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065005.htm. Here is a list of the ICH Guidances specifically related to Quality and Manufacturing of Biologics:

FDA Guidance for Industry—Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, August 2001

FDA Guidance for Industry—Q8 (R2) Pharmaceutical Development

FDA Guidance for Industry—Q9 Quality Risk Management

FDA Guidance for Industry—Q10 Pharmaceutical Quality System

FDA Guidance for Industry—Q11 Development and Manufacture of Drug Substances

FDA Guidance for Industry—Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practices
FDA Guidance for Industry—CGMP for Phase 1 Investigational Drugs
FDA Guidance for Industry—Investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production
FDA Guidance for Industry—Process Validation: General Principles and Practices
EU Guidelines to Good Manufacturing Practice—Annex 1, Manufacture of Sterile Medicinal Products
EU Guidelines to Good Manufacturing Practice—Annex 12, Investigational Medicinal Products.

Oversight and Compliance

Regulatory compliance in biologics manufacturing requires that the implemented quality unit and system be robust enough to support product production throughout its clinical phase maturation.

The quality unit is expected to be independent of the operations/manufacturing unit and that it fulfills both the quality assurance and quality control responsibilities. Their roles and responsibilities should be defined and documented. Critical roles of the quality unit are the review and approval of all quality-related documents; disposition of raw materials, intermediates, packaging, labeling materials, and the final product; conduct internal and supplier audits; review completed batch production and laboratory control records before determining disposition; approve changes that could potentially affect the intermediate and final product; and ensure the complete investigation and resolution into deviations and complaints.

In biologics manufacturing, especially with Phase 1 material, not all critical parameters, control points, and at times raw material may all be defined or identified. Knowing this and knowing that the process will continue to grow through its clinical phases, oversight from the quality unit must ensure that the manufacturing process adheres to the foundational components of GMPs. These foundational components are those that ensure full support of the product production and are maintained within the quality system. All components of the quality system are controlled through written and approved policies and procedures.

Documentation

Change control—A system established to evaluate all changes that could affect the production and control of the product.
Personnel—A system for maintaining and evaluating personnel qualifications and training for job-related functions
Building and facilities—Systems that provide evidence of the adequacy of the facility. These systems include at minimum, design, qualification, calibration, cleaning, maintenance, and monitoring.
Laboratory control records—Systems that ensure records include complete data derived from all tests conducted to ensure compliance with established specification and standards.
Batch records and specifications—A system that ensures that documents related to the manufacture of intermediates and product be prepared, reviewed, approved, and distributed according to written procedures.
Materials management—A system for the receipt, identification, quarantine, storage, handling, sampling, testing, and disposition of material.
Production and process controls—A system for the control of critical steps such as weight and measurement; time limits; in-process sample testing; and contamination control.

FACILITY REQUIREMENTS

The facility and the utility requirements are the fundamental backbone of the process flow and production effectiveness. In facility design, it is important to consider the regulatory nature of the industry and seeking some in-depth knowledge of the requirements will pay big dividends in the long run. Let's briefly look at a few of these systems and their importance to the overall health of the business platform. (See Figures 26.4A and B.)

Air-Handling Equipment

Large volumes of air are required to properly satisfy the international standards for clean room air. This is an area where many people may underestimate their current and future needs. This can be a costly upfront mistake and can further restrict a company's ability to grow additional revenue streams. Industry air flow standards are dictated by international standards. Air flows dictate cleanroom classifications. Current classifications and room air changes per hour are found in Table 26.2.

These clean air room classifications must be considered as they dictate the allowable operations in each area. Due to the large volumes of air flows, building chilling capacity must be a strategic part of the facility design as well.

Process Water (Purified Water [PW] and Water for Injection [WFI])

Another crucial element in the success of your production processes is the demand and need for ultra-pure water, which cannot be overstated. Purified water is to be used for

(A)

(B)

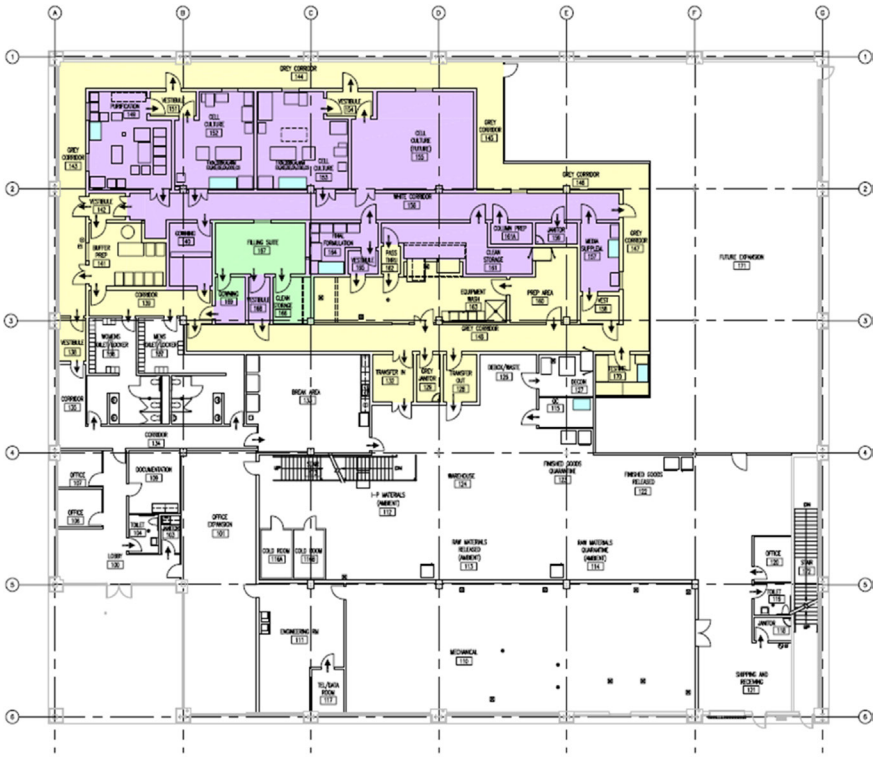

FIGURE 26.4 A and B. A typical biomanufacturing facility. (A) Outside the facility. (B) A schematic of the first floor. *(Source: Cytovance Biologics Inc., 2013.)*

TABLE 26.2 International Air Flow Standards for Cleanrooms: Current Classifications and Room Air Changes

FS Cleanroom Class	ISO Equivalent Class	Air Change Rate (per hour)
1	ISO 3	360–540
10	ISO 4	300–540
100	ISO 5	240–480
1,000	ISO 6	150–240
10,000	ISO 7	60–90
100,000	ISO 8	5–48

Source: IEST. *http://www.iest.org/Standards-RPs/ISO-Standards/ISO-14644-Standards*.

FIGURE 26.6 Typical water for injection system. *(Source: Water Sciences. http://www.watersciences.biz/Purified_Water_Generation_Plant.html.)*

FIGURE 26.5 A typical purified water system. *(Source: Cytovance Biologics Inc., 2013.)*

the production of USP products. Purified water and sterile purified water may be obtained by any suitable process (see Figure 26.5). Water for injection is water purified by distillation or reverse osmosis (see Figure 26.6). As cost is an important factor in the implementation of these systems, at a minimum, a healthy Reverse Osmosis Deionized water (RO/DI) or distillation system should be utilized. Attention should be given to the design and installation of this critical system. Details such as storage, sanitization, number of water loops (ambient and hot), and the number of water drops need to be carefully planned in advance. In addition, the maintenance of the purified water systems is the most important element of continuously producing USP-acceptable water to support the facility's biological manufacturing processes.

Building Automation and Alarm Systems (BAS)

Proper building automation and alarming is paramount to effective and efficient operations. Clients and auditors will want to see if you have control of critical facility and process-related parameters such as differential pressures, temperatures, humidity, pressure, flows, and more. There are many off-the-shelf systems that provide control and capture of important and useful information. Skillful use of automation and alarms can quickly pay dividends in facility utility costs. In addition, these automation software packages provide tools for trending, tracking, and troubleshooting various production-related applications. Building automation and alarm systems is an essential part of a current good manufacturing practices enterprise.

Additional Facility Requirements

In addition to the above referenced critical systems, there are many areas of consideration when designing or operating a compliant good manufacturing facility and organization. Topics noteworthy of additional study are included, but not limited to: cleanliness, validation and commissioning, process-material personnel and air flows, cold storage, dry storage, emergency procedures, safety, security, pest control, environmental monitoring, preventive maintenance and calibrations, and all possible redundancies that may be required to support utility and processes.

THE BIOMANUFACTURING TEAM—THEIR TYPICAL ROLES AND RESPONSIBILITIES IN A BIOLOGICS MANUFACTURING FACILITY

Manufacturing-related Functions

Manufacturing-related positions typically make up the majority of positions in a biologics manufacturing facility. These can be classified as positions that directly interact with the manufacturing process. Typical functions include

FIGURE 26.7 Upstream manufacturing: bioreactor operations. *(Source: Cytovance Biologics Inc., 2013.)*

FIGURE 26.8 Downstream manufacturing: chromatography operations. *(Source: Cytovance Biologics Inc., 2013.)*

upstream manufacturing, downstream manufacturing (purification), fill/finish operations, a manufacturing support team, and a manufacturing technical support function.

Upstream Manufacturing

Upstream manufacturing responsibilities routinely include operations related to cell expansion steps starting with a single vial of frozen cells and growing these exponentially into larger and larger systems eventually reaching your large-scale terminal reactor where the targeted protein is expressed. These operations require highly skilled specialists trained in microbiological processes, Good Manufacturing Practices (GMPs), fermenter and bioreactor systems, automation systems, and in-process analysis instruments. While not always required, typical employees will have a bachelor's degree in biology, microbiology, or a similar science. (See Figure 26.7.)

Downstream Manufacturing

Downstream manufacturing, or commonly referred to as purification, is focused on the capture and isolation of a targeted molecule and the removal of impurities. This is accomplished through several different processes to include filtration, column chromatography, and tangential flow filtration (TFF). These operations also require highly skilled specialists trained in chemical properties, chromatography, TFF, filtration systems, Good Manufacturing Practices (GMPs), automation systems, and in-process analysis instruments. Typical employees will have a bachelor's degree in chemistry, chemical engineering, biology, or a similar science. (See Figure 26.8.)

Production Support

The production support function is commonly utilized in biologics manufacturing facilities. While these functions can be performed by the upstream and downstream

FIGURE 26.9 Manufacturing support: glass washer, autoclave (steam sterilizer), and prep area. *(Source: Cytovance Biologics Inc., 2013.)*

functions, the amount of coordination and activity in these areas would usually warrant a separate team. These functions perform a variety of supporting tasks to include: media and buffer preparation, equipment and component preparation, chemical dispensing, equipment and environmental cleaning, and some in-process testing. Associates on this team are trained in the use of glass washers, autoclaves, solution prep equipment, and Good Manufacturing Practices (GMPs). Typical employees will have a bachelor's degree in science or engineering. (See Figure 26.9).

Fill/Finish Operations

The fill/finish team is a specialized function within the manufacturing team dedicated to the manufacture of the drug product. A drug product refers to the final formulated product in its delivery container. In most cases for biologic products, these will be traditional glass vials. Other systems include prefilled syringes or intravenous (IV) bags. As these steps in the process are the last manufacturing

FIGURE 26.10 (A) Fill/finish operations: Chase Logeman automated vial fill machine. (*Source*: *Cytovance Biologics Inc. and Chase Logeman, 2013.)* (B) Fill/Finish Operations. *(Source: Cytovance Biologics Inc., 2013.)*

steps, they must be performed in highly contained environments. Typically these areas will be the cleanest areas in the facility with the highest levels of control. (See Figure 26.10A and B.)

Manufacturing Technical Support (MTS)

The MTS function provides scientific support to the manufacturing team. Their roles include tech transfer activities from the process development group, new equipment identification and qualification, on the floor support for complex process steps, troubleshooting complex process related issues, and support technical investigations. Skilled specialists and engineers on this team are familiar with the scientific principles related to one or more areas of a traditional process. They are also skilled and knowledgeable on the process equipment utilized throughout the process train. Specialists on this team will typically have many years of relevant experience as well as bachelor's level degrees in a scientific discipline (many have advanced degrees). (See Figure 26.11).

FIGURE 26.11 Manufacturing technical support. *(Source: Cytovance Biologics Inc., 2013.)*

Quality Assurance

One of the most important functions in a biologics manufacturing facility is the quality unit. As this is a heavily regulated industry, a robust internal quality system is required to ensure adherence to all regulations as well as patient safety. Quality assurance provides a fully independent look at all documentation, production areas, and supply chain functions to ensure compliance. Quality is responsible for ensuring all raw materials, procedures, and areas are released for production. They will also perform reviews of all documentation, quality control samples, and final release specifications before approving a batch for release. Typically the quality unit will be the second largest function in the facility behind the manufacturing staff. Specialists on this team will typically have bachelor's degrees in a scientific discipline.

Quality Control

The quality control unit is responsible for performing testing on raw materials, in-process and final product testing, and environmental monitoring activities in a biologics facility. Analysts on this team will typically have bachelor's or master's degrees in chemistry, biology, microbiology, or engineering. (See Figure 26.12).

Facilities and Engineering

The facilities and engineering teams oversee all of the key systems required to keep the manufacturing plant operational. This includes base building systems, process equipment, utilities, building automation, and heating, ventilation, and air conditioning (HVAC). They also perform routine and nonroutine maintenance activities on the aforementioned systems. These teams are staffed with skilled

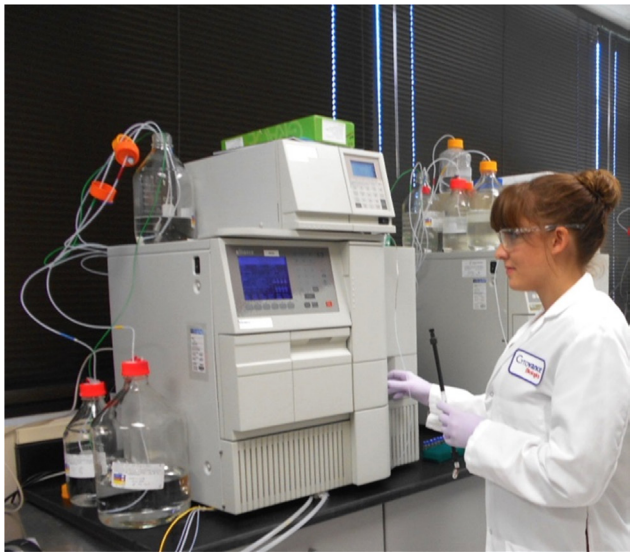

FIGURE 26.12 A quality control laboratory. *(Source: Cytovance Biologics Inc., 2013.)*

trade's people, plant and process engineers, and calibration (metrology) professionals. (See Figure 26.13.)

Supply Chain

The supply chain function is responsible for the procurement, warehousing, delivery, and management of all materials used in the process. This includes all shipping and receiving activities for raw materials as well as finished products.

MATERIAL MANAGEMENT

Materials utilized in the production of biological manufacturing is required to be controlled through documented systems that ensure adequate controls beginning from vendor selection through receipt, inspection, and release for use.

All raw materials should be acquired from a reputable source that has been audited and approved by quality assurance. The raw materials should be animal-component free. An audit of all product contact materials should be performed and animal-component free statements acquired from the manufacturer. All materials should have manufacturer specifications and certificates-of-acceptance (COAs) when received. Material specifications are required to be established for all materials utilized in the manufacture of the product. These specifications should include COAs, certificates-of-sterility, and requirements for any testing and release of the material. The specifications will be used by manufacturing and the supply chain or purchasing department to acquire the appropriate material for GMP manufacturing. Once the material is ordered, the receiving department will use the purchase order and the material specifications to review the receipt of the material and ensure that it meets all material specifications.

FIGURE 26.13 Facilities and engineering. *(Source: Cytovance Biologics Inc. 2013.)*

It is important that the quality system that governs material management include controls to determine sampling requirements and quantities required per the vendor lot. These requirements can be found in the United States and the EU regulations and guidance documents. Raw material sampling and testing is required for all phases of clinical trials and the expectation is that a robust sampling program be implemented prior to commercialization of the product.

Raw material specifications will spell out the incoming sampling for ID testing and the storage conditions, and will require a COA from the vendor. All raw materials should be sampled and tested per the material specifications. The impact of a raw material that is not ID tested or any quality attribute could have a significant impact in the production of the product or its release. Not vetting the raw material ID or quality will impact Phase 2 and 3 clinical trials. Any issues during these late phase trials could have a significant setback to the development of the drug product. (See Figures 26.14 and 26.15.)

BIOLOGICS DRUG SUBSTANCE MANUFACTURING

An example of a typical biologics manufacturing process is shown in Figure 26.16.

Upstream Biologics Manufacturing

Cell Banking

Biotechnology requires the creation of a cell that has the capabilities to produce the monoclonal antibodies (mAbs) or proteins desired for use. These cells are created by transfecting the required genes with a marker into a host cell. Once this transfection has been completed, cells that exhibit the traits of the marker that was introduced are isolated and

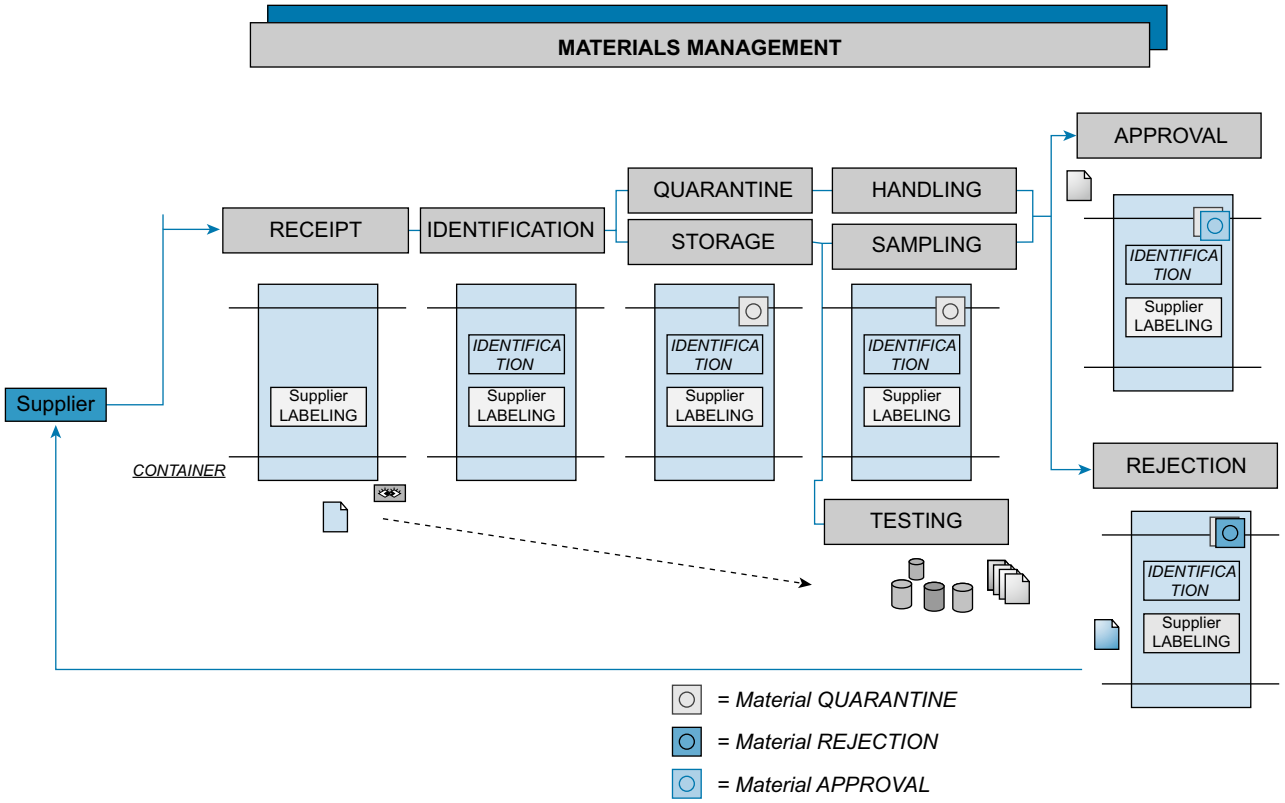

FIGURE 26.14 Materials management flow chart. *(Source:* http://2.bp.blogspot.com/-HLm1RlaWsN8/UArpSsteZII/AAAAAAAABJk/xLZIA9oulow/s1600/MATERIAL+MANAGEMENT.JPG.*)*

FIGURE 26.15 Materials quarantine. *(Source: Essential Medicines and Health Products Information Portal: A World Health Organization resource.)*

cloned. These clones are then frozen in small quantities and therefore producing a research cell bank (RCB). RCBs are evaluated for production capabilities and normally one RCB will be selected to move forward with testing and/or production.

Once a specific RCB has been selected, a two-tiered cell banking system is utilized (see Figure 20.17A and B). The first tier is referred to as a master cell bank (MCB). This cell bank is manufactured from the selected research cell bank. One vial of the RCB is thawed and expanded until a required number of cells are available for the creation of the cell bank. These cells are then aliquoted into small-volume cryopreservation vessels and frozen using DMSO (5 to 10 percent) at less than −130°C. These conditions limit the cellular activity which allows for long-term storage. Once produced, the MCB is tested for cell growth, cell viability, characterization, sterility, and various other tests as deemed necessary.

Once the MCB has been tested, the second tier or working cell bank (WCB) is initiated. One vial of the MCB is thawed and expanded until the required number of cells are available. These cells are again aliquoted into cryopreservation vessels for storage in DMSO (5 to 10 percent) at less than −130°C. Each WCB that is produced undergoes testing (cell growth, cell viability, characterization, sterility, etc.) before using further manufacturing processes.

Production of the targeted substance (mAb or protein) begins by preparing the inoculum to be used in the production bioreactor. This inoculum begins by thawing a vial of the tested working cell bank. The WCB culture is suspended

in a specialized growth medium within a shake flask. The culture is then maintained in a monitored incubator that controls temperature, CO_2 concentration, and shaking rate. As the initial culture grows, it proceeds through scale-up increasing the volume of the shake flasks. This allows the inoculum volume to increase with each step. The scale-up process might use any number of vessels that allow for increasing the inoculum volume.

Once the inoculum culture has reached a sufficient cell density and volume, it is used to inoculate the production bioreactor. Bioreactors allow for the control of various conditions, including pH, dissolved oxygen (DO), gas flow, and temperature. The controlled conditions allow for the creation of an ideal environment for the cell culture to produce the targeted substance.

Bioreactors

There are two main types of bioreactors: multiple-use (stainless steel) or single-use bioreactors (disposable).

Multiple-use bioreactors are made of stainless steel and currently are the predominant version of bioreactors used in production settings. Multi-use bioreactors generally require a large capital investment for purchase and installation. They also require validated processes for cleaning, and sterilization increases cost and time of maintenance and a skilled staff for operation. For this reason, in smaller-volume operations, disposable bioreactors are being used increasingly. (See Figures 26.18 and 26.19)

Disposable bioreactors utilize a disposable sterilized cell chamber in which the cell culture is maintained. This cell chamber minimizes the risks of cross-contamination as it is only used for one growth operation. The use of disposable bioreactors decreases the amount of validation, cleaning, sterilization, and maintenance needed per bioreactor run. For this reason, disposable bioreactors runs are able to be scheduled closer together allowing for an increase in plant production.

After the bioreactor has been inoculated there are three main types of bioreactor processes that are used: batch, continuous, and fed-batch. Batch bioreactor processes consist of filling the bioreactor with medium and inoculum and operating the bioreactor without additions of nutrients or medium until the growth profile is finished. Continuous bioreactor processes continually feed nutrients and medium into the bioreactor while also continually harvesting material from the bioreactor. Being as material is continuously being harvested, these processes can result in larger amounts of harvested material and longer bioreactor campaigns. Unfortunately, the longer bioreactor campaigns greatly increase the chance for contamination.

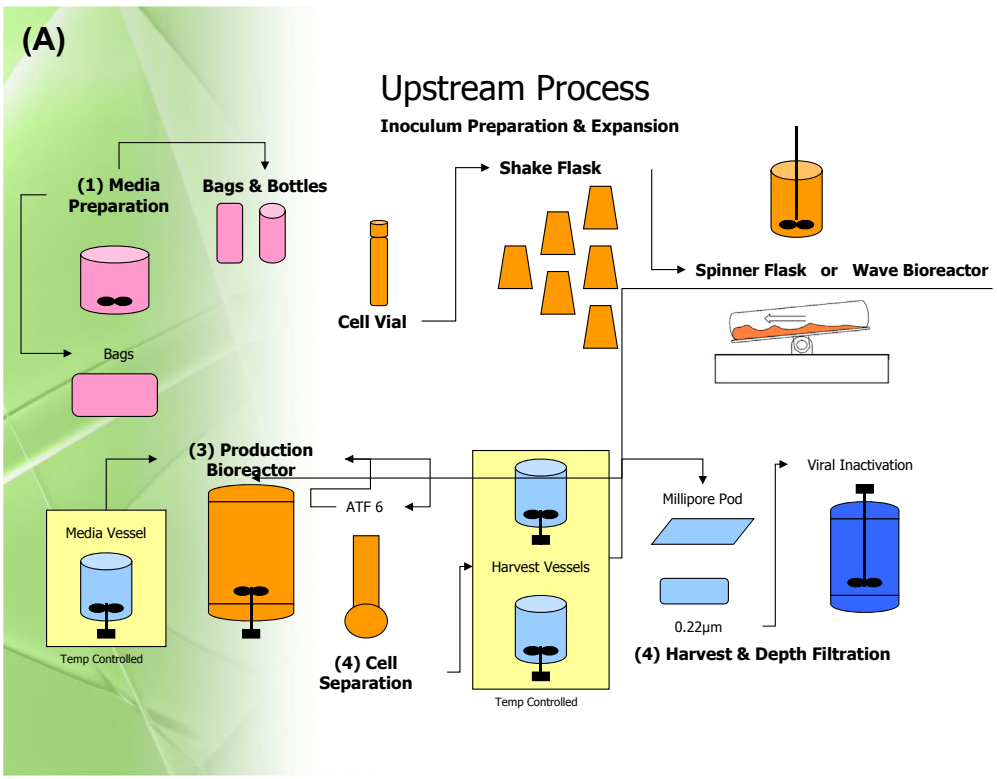

FIGURE 26.16 Biologics manufacturing process. (A) Upstream process, (B) Downstream processes and (C) Fill finish processes. *(Source: Cytovance Biologics Inc. John Conner 2013.)*

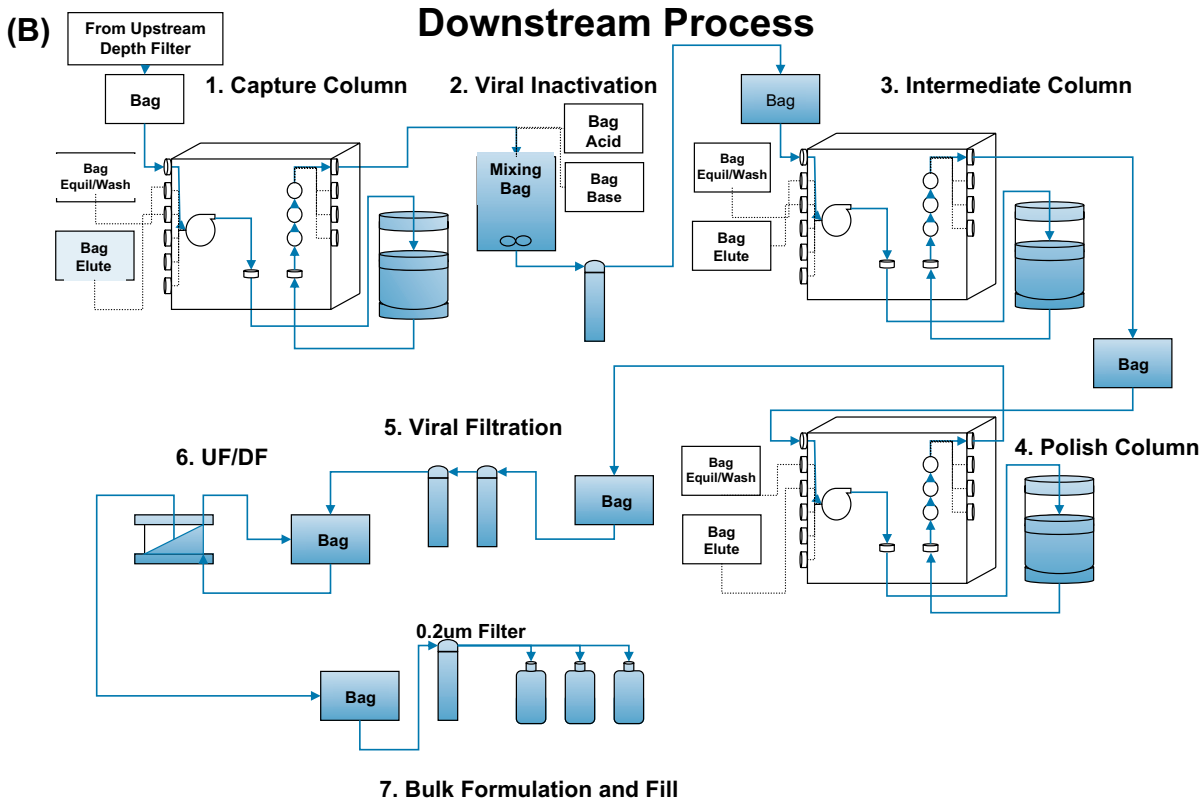

FIGURE 26.16B

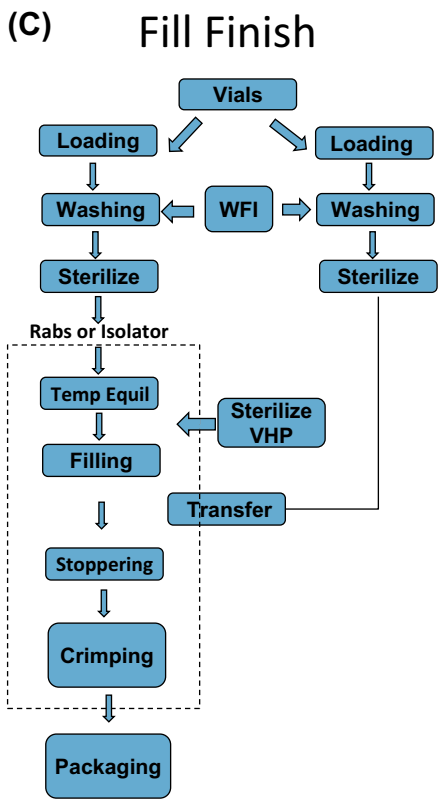

FIGURE 26.16C

Fed-batch bioreactor processes are the most common bioreactor processes used. This process starts with a lower starting volume and feeds nutrients and medium on a set schedule without a contentious removal of harvest material. Once the process has finished, the material is harvested for downstream processing.

Microbial Upstream Operations

Microbial fermentation involves the growth of a specific microorganism that has been programed to produce a specific protein. An example of a host organism used for this purpose is *Escherichia coli.* Production of the target substance begins with the thawing of the microbial cell bank. This cell bank is resuspended in growth medium and incubated within an incubator/shaker that controls the temperature and agitation rate of the culture. The culture is incubated to allow growth to the proper optical density for inoculation of the production fermenter. The production fermenter is designed to control parameters including dissolved oxygen, pH, temperature, and gas flows. This control allows for an optimized environment to be created for the growth of the microorganism. The fermentation process usually utilizes a fed-batch process which allows for feeding of additional specialized medium and supplements designed to support growth of the microorganism. (Figure 26.20)

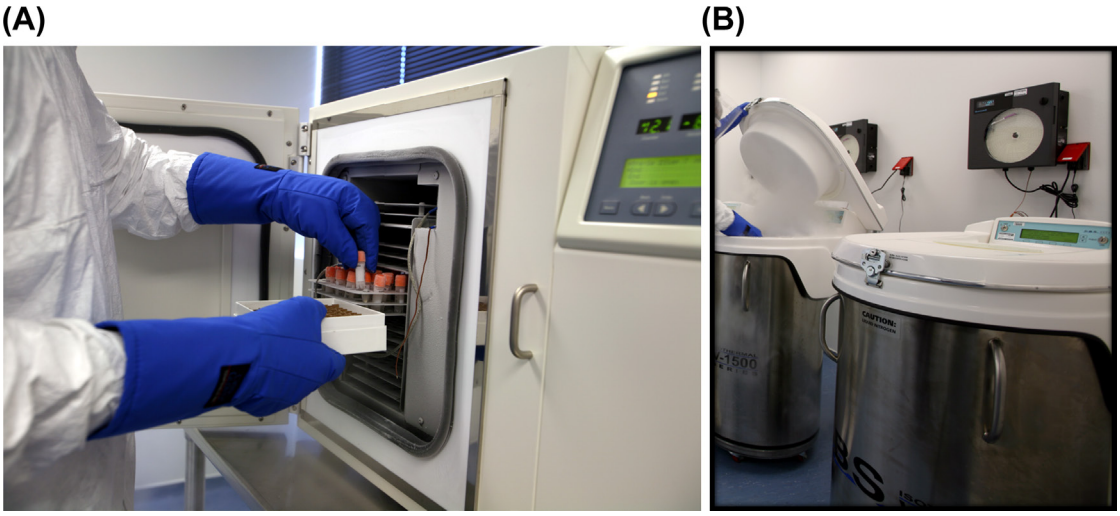

FIGURE 26.17 A and B. (A) Manufacturing cell bank production. (B) Cryopreservation and cryostorage. *(Source: Cytovance Biologics Inc., 2013.)*

FIGURE 26.18 Mammalian upstream bioreactor operations. *(Source: Cytovance Biologics Inc., 2013.)*

FIGURE 26.19 Mammalian upstream single-use bioreactor (SUB) operations. *(Source: Cytovance Biologics Inc., 2013.)*

Once the fermentation process is completed (approximately 48 hours) the microorganisms are harvested by centrifugation. This is important as the targeted substances are intracellular (inside the host cell). The centrifugation step allows for the removal of the growth medium. Being as the targeted substance is intracellular, the host cells must be disrupted to allow the extraction of the product. A common practice to accomplish this is the use of a high-pressure homogenizer. Once the targeted substance has been released for the cell, the resulting lysate must be centrifuged. This last centrifugation step results in the separation of the inclusion bodies from the remaining cell debris (a result of the homogenization process). The isolated inclusion bodies may then be frozen and stored before further downstream purification.

Downstream Bioprocessing Operations

Downstream processing, as it applies to biomanufacturing, refers to the separation, purification, and modification of macromolecules from complex biological feedstocks. Most commonly the feedstock is a cell suspension containing billions of "host cells" that synthesized the macromolecule of interest. The ultimate goal of a pharmaceutical downstream processing operation is to prepare a drug product for safe and effective delivery into humans or animals. The delivery method is a primary focus of fill/finish operations and can be parenteral, oral, or topical.

Pharmaceutical macromolecules are used in a vast array of applications including:

1. Cancer therapy
2. Enzyme replacement for enzyme-deficiency syndromes
3. Immune system suppression for autoimmune disorders
4. Elimination of infectious agents

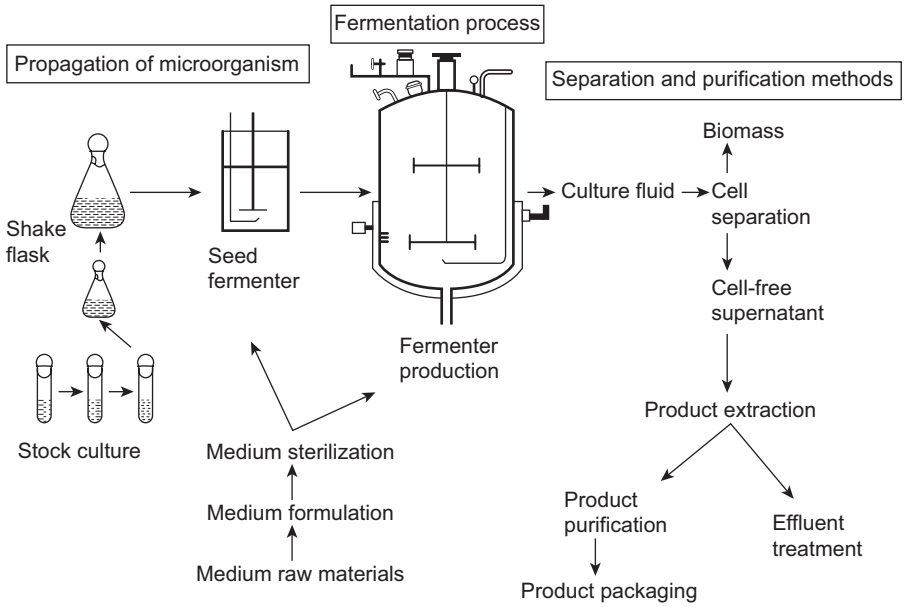

FIGURE 26.20 Microbial fermentation operations. *(Source: Intech.* http://www.intechopen.com/books/biomass-now-sustainable-growth-and-use/continuous-agave-juice-fermentation-for-producing-bioethanol.*)*

5. Anemia
6. Diabetes
7. Gene therapy

Downstream processing has undergone drastic advances in the last 30 years as new strategies have emerged to increase throughput, purity, and process yield. The recent technology advances in downstream processing have driven operating margins upward and have broken down costly barriers to entry into the biologics market. Start-up companies who are mindful of the recent cost-saving and process-optimization technologies in downstream processing are now more able than ever to bring life-saving biologic therapies to market, often tapping into the expertise of contract manufacturers and clinical research firms. Furthermore, regulatory agencies around the globe have established robust guidelines to ensure the new strategies being implemented in downstream processing keep the safety of the patient as a top priority. Due to the close proximity of downstream process operations to the final drug product, patient safety considerations are absolutely critical.

Downstream Process Flow

A downstream unit operation, a single step in the downstream process, can be categorized into a mechanical separation, chemical separation, or dual mechanical/chemical separation step. In other words, the molecule of interest is separated from the remaining impurities mechanically, by its dimensional (size, shape) characteristics, or chemically, by its biochemical (electrical charge, interaction with other macromolecules, oiliness) properties. Several downstream processing techniques apply both mechanical and chemical separations simultaneously, and can be highly selective for the molecule of interest. Product separation and purification is accomplished through a series of process steps including, but not limited to: filtration, chromatography, precipitation, and centrifugation. As a general rule of thumb, product purity increases and volume decreases through each unit operation of the downstream process (see Figure 26.21).

In addition to the separation and purification of the target drug molecule, downstream processes modify the drug molecule and its environment. These modifications can be minor or extensive, depending on the ability of the expression system to produce the molecule. Some examples of product modification in downstream processing include:

1. Complete reconstruction of the product in solution (protein refolding).
2. Increasing the concentration of product in solution.
3. Attaching synthetic molecules to the product to enhance immune response or product stability.
4. Splitting of the product into multiple subunits.
5. Adding excipients (salts, amino acids, detergents, emulsifiers) to enhance product stability.

Harvest and Clarification

Downstream processing begins with the separation of large insoluble contaminants from the feedstock or "harvest" solution, usually whole cells and cell debris. This mechanical separation process is referred to as clarification. For expression systems in which the molecule of interest is secreted out of the cell into the surrounding solution (mammalian cell culture) and a relatively low density of cell debris is present,

depth filtration is a common clarification technique. Depth filtration is a 3-D filter matrix that serves to remove the bulk of large particulates from the feedstock, analogous to a sand bank at the foot of a river. Water and very small particles pass through the sand while large debris cannot.

The advantage of depth filtration is low equipment cost and seamless transfer from bench scale to production scale. Several varieties of depth filters are readily available with some acting as both mechanical and chemical separators that bind charged contaminants from the host cell such as DNA and proteins (see Figures 26.22A and B).

Certain feedstocks with a high density of cell debris (microbial fermentation broth) require a primary clarification with a centrifuge prior to, or in lieu of, depth filtration. In contrast to traditional laboratory centrifuges that require multiple batches to process large volumes, continuous flow centrifuges mechanically separate the product from the feedstock in a single batch, taking advantage of density differences between liquids and solids. The feedstock is split into two or more portions: product stream(s) and a waste stream. The waste stream is discarded and the product stream(s) captured for further downstream processing. (See Figure 26.23.)

After the large insoluble particulates have been removed via depth filtration and/or continuous flow centrifugation, the harvest solution is passed through a fine filter that ensures all living cells are removed. This final filtration step ensures further downstream processing steps are protected from unwanted contaminants and debris.

Chromatography

Chromatography is a general term that refers to the separation of molecules that exist together in a solution. Chromatography is the primary tool used in downstream processing, enabling biologics manufacturers to separate

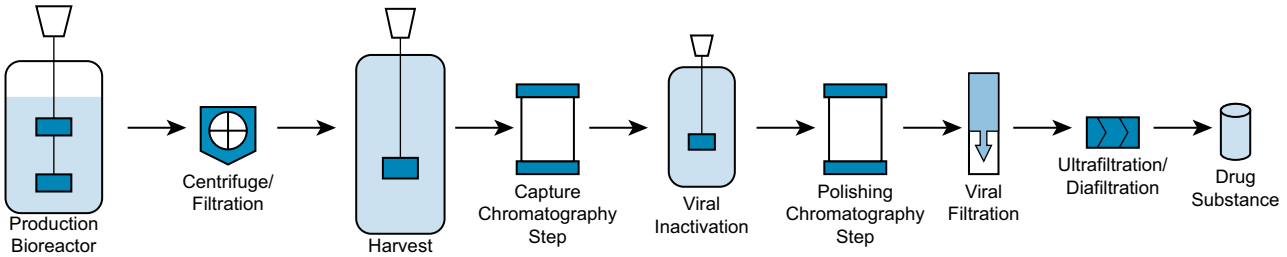

FIGURE 26.21 Typical downstream processing flow of a monoclonal antibody (mAb). The arrows represent the flow of the antibody product between unit operations. Each unit operation mechanically or chemically separates the antibody from host cell contaminants. *(Source: Figure modified from: Ahmed, I., B. Kaspar, and U. Sharma. (February 2012). "Biosimilars: Impact of Biologics Product Life Cycle and European Experience on the Regulatory Trajectory in the United States."* Clinical Therapeutics.*)*

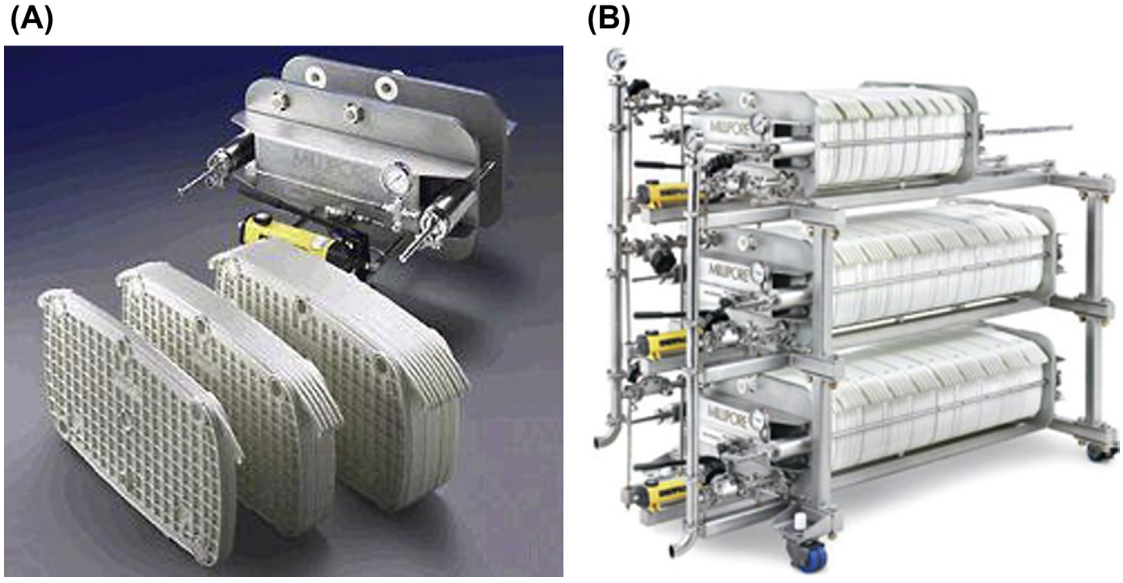

FIGURE 26.22 A and B. Typical depth filtration used in cell culture harvest clarification. Example of a small (A) and large (B) production-scale disposable depth filtration system. These units are Millipore Millistak+®POD disposable depth filters manufactured by EMD Millipore. *(Source:* www.millipore.com.*)*

a product molecule from thousands of others in solution. Column chromatography is by far the most common form of chromatography in biomanufacturing, in which a liquid "mobile phase" containing the molecule of interest passes through a solid "stationary phase." Columns are essentially hollow tubes made of glass, plastic, or steel with nets on both ends that contain the stationary phase within the column. A pump system with an array of monitoring devices pushes the mobile phase through the column and directs the product stream away from the waste stream. Based on the scale of the operation, columns can vary in size from 1 centimeter in diameter to 200 centimeters in diameter and greater. Regardless of size, the principle of column chromatography remains the same. (See Figures 26.24A and B.)

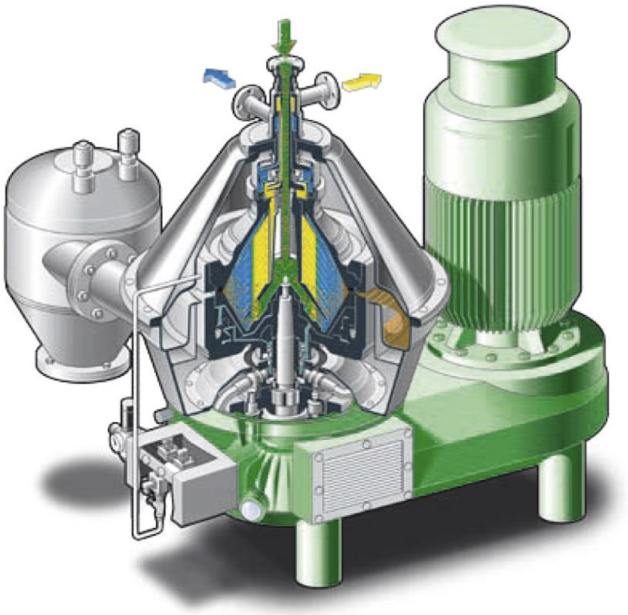

FIGURE 26.23 A typical centrifuge used in downstream operations to separate product from feedstock. A specialized centrifuge from GEA/Westfalia for the separation of a feedstock into low-density liquid, high-density liquid, and solid components. *(Source: GEA Westfalia Separator Group.* www.westfalia-separator.com.*)*

The stationary phase, commonly referred to as chromatography resin or media, contains immobilized chemicals called "ligands" and can operate in different ways depending on the downstream process operation. The ligands can bind to the product molecule, allowing other unwanted molecules to pass through the column and be discarded. This strategy is referred to as "bind-and-elute" chromatography. The exact opposite occurs in "flow through" chromatography, in which the product molecule passes through the column and is captured while impurities bind to the ligands. Lastly, all molecules pass through the column in size-exclusion chromatography, with separation being achieved because molecules travel at different speeds through the column. (See Figure 26.25.)

Only a small portion of a chromatography column is actually ligand, with most of the column volume consisting of a polymer that holds the ligands upright and typically does not interact with macromolecules in solution. The polymer backbone is a highly porous spherical bead in column chromatography, or in a 3-D matrix in the case of membrane chromatography. In both cases the goal is to maximize the exposure of ligands to the surrounding solution so that chromatographic separation can occur. (See Figure 26.26.)

Column packing refers to the various strategies used to optimally place chromatography media within a column tube. A delicate balance exists with packing a column with

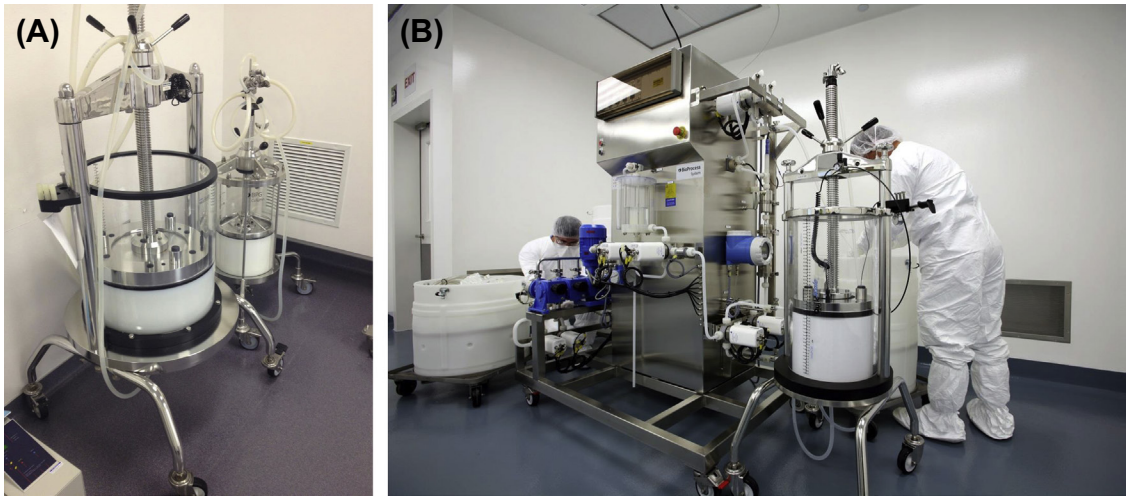

FIGURE 26.24 A and B. (A) Typical Chromatography columns packed with various chromatography resins. (B) Downstream purification Technician using a Chromatography Skid connected to buffer and chromatography column to purify protein therapeutics. Note the white resin matrix used to separate the macromolecules in solution. *(Source: Cytovance Biologics Inc., 2013.)*

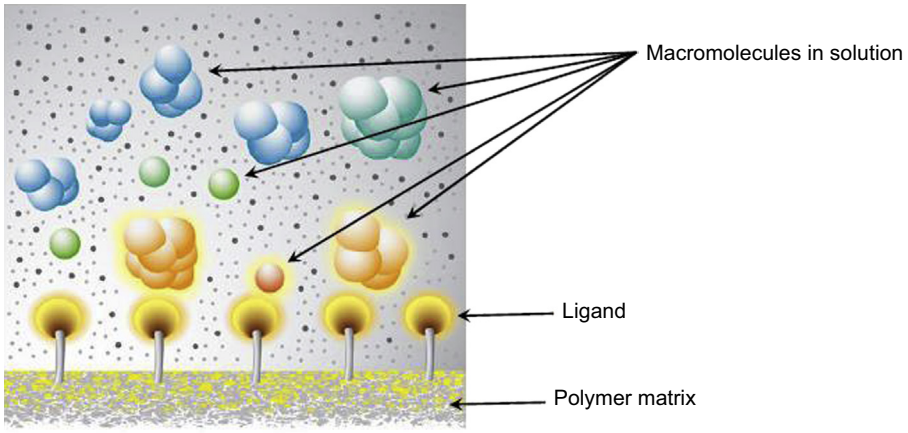

FIGURE 26.25 Chromatography Resin in Action: Highly magnified artist's model of a chromatography resin in action. Blue and green macromolecules do not interact with the ligands, while the orange and red macromolecules are bound to the ligands. *(Source: http://microsite.sartorius.com/sartobind-phenyl/hic.html.)*

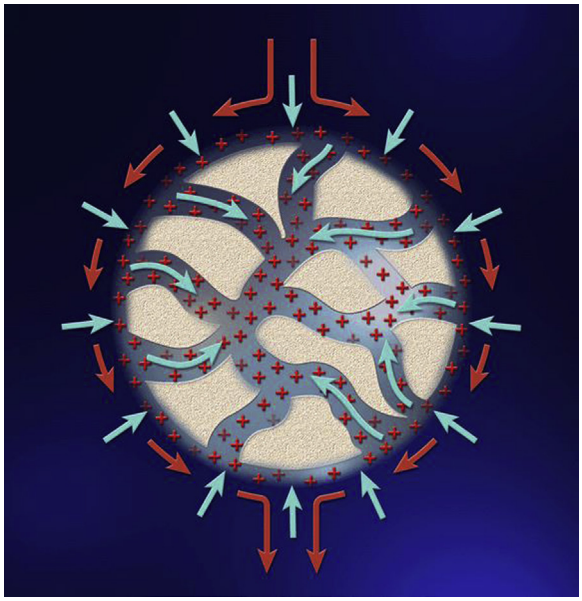

FIGURE 26.26 Positively charged chromatography resin bead. Note the porous nature of the bead, with channels throughout to allow access to the surrounding solution. Light gray arrows represent macromolecules that pass through the interior of the bead, while dark gray arrows represent macromolecules that are excluded from the resin bead. Plus signs (+) represent ligands affixed to the polymer matrix. *(Source: Pall Corporation.* www.pall.com.*)*

media: chromatography. Media should be installed into a column tube to ensure it remains stationary (hence the "stationary phase"), but does not become damaged or compressed to the extent that ligands cannot be accessed by the molecules in solution. A variety of strategies exist to pack columns and the appropriate method should be selected based on the resin's properties and the type of column hardware used in the manufacturing facility. In recent years, automated column packing methods (GE AxiChrom) have been developed to improve the reproducibility and robustness of column chromatography unit operations.

Capture Chromatography

The capture chromatography step is the first chromatography step of a downstream process and is the "workhorse" of the entire process. Imagine a gold prospector on an average day in the field who pans for several hours, ending up with a few small gold flakes at the end of the day. Hundreds of pounds of silt, water, and microscopic gold dust pass through the pan for every visible gold flake. Even the visible gold flakes are compounded with other metal impurities. The gold gathered is not the final product yet, but the prospector is much closer to the end result than he or she was several hours ago. Capture chromatography follows the same principle as gold panning; a relatively small loss of product yields huge dividends with at least a 100-fold increase in purity. Capture chromatography is typically the most efficient downstream process step.

Capture chromatography usually involves the use of an affinity ligand, a molecule that strongly attracts the product's macromolecule. A common affinity ligand is Protein A, a naturally occurring bacterial protein that "locks on" to human antibodies. Protein A-based resins are widely used in biomanufacturing to separate monoclonal antibody products from mammalian host-cell impurities. For nonantibody products, most chromatography resins can be used as the initial capture step. Fine tuning is often required to capture nonantibody products from a feedstock solution, and precise conditions must exist to bind the macromolecule product and allow others to pass through.

Polishing Chromatography

Polishing chromatography is the general term that refers to additional chromatography unit operations after the capture step. Polishing chromatography further enhances the purity of the target macromolecule to greater than 95 percent, in preparation for delivery of the drug to the patient. If applied

FIGURE 26.27 A membrane chromatography column. The steel cylinder contains a 3-D matrix with bound ligands rather than spherical beads. (Family of CIM® Monolithic Columns from BIA Separations.) *(Source: BIA Separations.* http://www.biaseparations.com.*)*

to the gold panning analogy, polishing chromatography is the metallurgist that transforms the dull gold flakes into pure 24K gold bars. Hundreds of resin types for polishing chromatography are available in varying bead sizes, ligand types, and polymer matrices. The vast diversity of biological macromolecules is a reflection of the numerous types of polishing chromatography resins and strategies which contract manufacturers can expect to work with.

Membrane Chromatography

Membrane chromatography is an alternative to traditional column chromatography, as ligands are attached to a 3-D matrix rather than a spherical bead. Membrane chromatography exists in most of the same ligand types as traditional resin. The use of membrane chromatography is advantageous for some downstream processes as higher flow rates through the chromatography matrix can be achieved as compared to most traditional resin types, and capital costs are lower without the need for expensive column hardware. However, membrane chromatography can be cumbersome to use at commercial production scales, where the material costs of the membranes can outweigh the capital cost savings of opting out of column chromatography.

A small subset of membrane chromatography systems combines the flow rate advantages of membrane chromatography with the reusability of traditional column chromatography (see Figure 26.27). BIA Separations has developed a membrane chromatography column for production use. The steel cylinder contains a 3-D matrix with bound ligands rather than spherical beads.

Buffer Preparation

While choosing the proper stationary phase is critical to a downstream processing operation, selecting the right mobile phase is equally as important. The salt solutions passed through chromatography media that establish the proper mobile phase conditions are called buffers. A great deal of attention is directed at buffer preparation to ensure the chemical components meet rigorous regulatory standards for pharmaceutical use and the buffers are prepared correctly. A buffer that does not meet the requirements for its downstream unit operation can mean the difference between the product macromolecule binding to the resin or being discarded to waste. Advances in disposable technology are particularly applicable to the buffer preparation process. A capital cost to store large volumes of buffer solutions in stainless steel tanks is not feasible for most biomanufacturers, so disposable plastic bags are preferred.

Tangential Flow Filtration

Tangential flow filtration (TFF) is a technique widely adopted in downstream processing and is similar in principle to dialysis. Tangential flow filtration is used to remove the buffer surrounding the macromolecule product and add another buffer to the product that is more suitable for the next process step. The pores in a tangential flow filter are small enough that the drug product does not pass through—it moves parallel to the filter surface. Impurities, salts, and water pass through the filter and are discarded. TFF can be utilized as a preparative step between chromatography steps or to formulate the product of interest with the optimal salts and excipients. The exact formulation delivered by a TFF system varies greatly from product-to-product. Salts, amino acids, sugars, and surfactants are common additives. (See Figure 26.28A and B.)

Viral Reduction Techniques

Several mammalian expression systems contain viruses that are intentionally present to manufacture the drug macromolecule. Foreign viruses can also contaminate the cell culture and can be difficult to detect. To protect patients from harmful viral agents, downstream processes have built-in safeguards to eliminate viral contamination, known as "viral clearance."

The primary viral clearance operation in most downstream processes is known as viral filtration. Many types of viral filters are available, they are all highly specialized filters with precise pore sizes that allow the product molecule to pass through but trap viruses. The challenge with viral filtration is some viruses, especially parvoviruses, are

FIGURE 26.28 A and B. (A) Tangential flow filtration model. The feed stream containing the product molecule, travels in parallel to the filter surface. Only impurities such as salts and smaller molecules pass through the filter. (*Source:* Spectrum Labs. *www.spectrumlabs.c.*) (B) An example of a production-scale tangential flow filtration system. The stainless steel plates on the right are used to hold the tangential flow filters (held by the technician) in place. *(Source: Sartorius. www.sartorius.com.)*

incredibly small and similar in size to product molecules. The precision to which these filters are made is critical for patient safety. To ensure the filter performs properly, an air test is performed to detect microscopic leaks that could have allowed a virus through.

Viruses are often susceptible to acid and detergents, while many biologic drugs are not as sensitive. Acid treatment is a common tactic for viral reduction in a monoclonal antibody downstream process, while detergent treatment is sometimes used to reduce viral contamination for enzyme products. Chromatography often doubles as a viral clearance tool. Electrically charged chromatography resins are particularly effective at removing viral contaminants as the ligands attract viruses like a magnet.

Bulk Filtration and Fill

Bulk fill is the final step in the downstream process after the product has been formulated appropriately. A sterile filter is used to ensure any potential contaminants that may have been inadvertently introduced into the product are removed. Downstream processing specialists that perform a bulk fill operation must be highly trained and are monitored for contaminants throughout the process. Once the formulated drug product passes through the filter, it is delivered into sterile containers that are stored in highly controlled areas.

Equipment Cleaning

All equipment that has direct contact with products in downstream processing must be vigorously cleaned between uses to remove soilants that remain bound to the equipment surface. The cleaning agents used are either acidic or alkaline, often contain detergents, and can be heated to increase cleaning potency. Applications with difficult-to-clean soilants or applications using large equipment often necessitate automated clean-in-place systems that distribute cleaning solutions to the equipment surfaces. Whether automated clean-in-place or manual cleaning is performed, all cleaning solution residues must be rinsed away with medical-grade water before using the equipment again. Highly sensitive analytical assays, such as the total organic carbon (TOC) assay, are industry standard for the detection of residual soilants and cleaning agents. (See Figure 26.29.)

Drug Product Manufacturing

For biological drug products, having a stability and robust final product formulation buffer is critical to the drug product manufacturing process. Developing a drug product formulation involves the characterization of a drug's physical, chemical, and biological properties in order to identify those ingredients that can be used in the drug manufacturing process to aid in the processing, storage, and handling of the drug product. Evaluating the drug substance under a variety of stress conditions such as freeze/thaw, temperature, and shear stress to identify mechanisms of degradation is extremely important.

Formulation studies should consider such factors as particle size, polymorphism, pH, and solubility that can influence the bioavailability and the activity of a drug. The drug in combination with inactive additives must ensure that the quality of drug is consistent in each dosage unit throughout the manufacture, storage, and handling to the point of use. It is essential that these initial studies be conducted using drug samples of known purity. The presence of impurities can lead to erroneous conclusions. Stability testing during each stage of drug development is a critical

FIGURE 26.29 A typical clean-in-place system used to clean upstream and downstream process equipment. The tanks in the background are used to mix and heat up the cleaning solutions before delivery of the cleaning solutions to the downstream process equipment through a network of piping. *(Source: Turn-Key Modular Systems.* http://www.tkmodular.com/StandardProducts/CIPSystems.aspx.*)*

facet to ensuring product quality. Drug stability testing is important during preclinical testing and clinical trials to establish an accurate assessment of the product being evaluated. Stability data is required at each of the various stages of development to demonstrate and document the product's stability profile. A product's stability must be assessed with regard to its formulation; the influence of its pharmaceutical ingredients; the influence of the container and closure; the manufacturing and processing conditions; packaging components; storage conditions; anticipated conditions of shipping, temperature, light, and humidity; and the anticipated duration and conditions of pharmacy shelf-life and patient use. Holding process bulk intermediates or product components for long periods before processing into finished drug products can also affect the stability of both the intermediate component and the finished product. Therefore, in-process stability testing, including testing of intermediate components, is essential. The following harmonized guidelines provide an outline of the regulatory requirements for drug substance and drug product stability testing to aid in formulation development:

1. Stability testing of new drug substances and products.
2. Quality of biotechnological products: stability testing of biotechnology/biological.
3. Drug products.
4. Photo-stability testing of new drug substances and products.
5. Stability testing of new dosage forms.

A drug's kinetic and shelf-life stability profile is the extent to which a product remains within specification limits through its period of storage and use while maintaining the same properties and characteristics that it possessed at the time of manufacture. Stability studies should address several key drug stability concerns:

1. Active ingredients retain their chemical integrity within the specified limits.
2. Drug physical properties are retained.
3. Sterility and/or container integrity is maintained.
4. Potency/therapeutic effect of the drug remain unchanged.
5. No increase in toxicity occurs.

Drug products should be subjected to long-term stability studies under the conditions of transport and storage expected during product distribution. In conducting these studies, the different climate zones to which the product may be subjected must be evaluated for expected variances in conditions of temperature and humidity. A drug product may encounter more than a single zone of temperature and humidity variations during its production and shelf-life. In general, long-term testing of new drug entities should be conducted at 25°C ± 2°C and at a relative humidity of 60 percent ± 5 percent.

There are many agents and ingredients that can be used to prepare the final formulation buffer of a drug substance to enhance the stability profile of the drug. These ingredients may be used to achieve the desired physical and chemical characteristics of the product or to enhance the stability of the drug substance, particularly against hydrolysis and oxidation. In each instance, the added agent or ingredient must be compatible with and must not detract from the stability or potency of the drug substance.

Hydrolysis is the most important cause of drug decomposition primarily because of the number of active agents that are susceptible to the hydrolytic process. Hydrolysis is a process in which drug molecules interact with water molecules to yield breakdown byproducts. There are several approaches to the stabilization of drugs subject to hydrolysis. The most obvious is the reduction or elimination of water from the system. In some liquid drug products, water can be replaced or reduced in the formulation through the use of glycerin, propylene glycol, and alcohol. In certain injectable products, anhydrous vegetable oils may be used as the drug's solvent to reduce the chance of hydrolytic decomposition. Decomposition by hydrolysis may also be prevented in other liquid drug formulations by suspending them in a nonaqueous vehicle. For certain unstable active agents, when an aqueous preparation is desired, the drug may be supplied in a dry form for reconstitution by adding a specified volume of purified water

just before dispensing to a patient. Refrigeration is generally required for most drugs subject to hydrolysis. In addition to temperature, pH is also a factor that affects the stability of a drug prone to hydrolytic decomposition. Drug stability can frequently be improved though the use of buffering agents between pH 5 and pH 6.

Oxidation is another destructive process that produces instability in drug products. Oxidation is the loss of electrons from an atom or a molecule. Each electron lost is accepted by some other molecule, reducing the recipient. Oxidation frequently involves free chemical radicals, which are molecules containing one or more unpaired electrons such as oxygen and free hydroxyl. These radicals tend to take electrons from other chemicals, thereby oxidizing the donor. Oxidation of a drug substance is most likely to occur when it is not kept dry in the presence of oxygen, when it is exposed to light, or combined with other chemical agents. Oxidation of a chemical in a drug substance is usually accompanied by an alteration in the color of that drug and may also result in precipitation or a change in odor. The oxidative process can be controlled with the use of antioxidants that react with one or more compounds in the drug to prevent the oxidation progress. Antioxidants act by providing electrons and hydrogen atoms that are accepted more readily by the free radicals rather than those present in the drug. Among those most frequently used in aqueous preparations are sodium sulfite, sodium bisulfite, sodium metabisulfite, hypophosphorous acid, and ascorbic acid. The FDA labeling regulations require a warning about possible allergic-type reactions, including anaphylaxis, in the package insert for prescription drugs that contain sulfites in the final dosage form.

Because oxygen can adversely affect their stability, certain drugs require an oxygen-free atmosphere during processing and storage. Oxygen is present in the airspace within the storage container or may be dissolved in the liquid vehicle. Oxygen-sensitive drugs must be prepared in the dry state and packaged in sealed containers with the air replaced by an inert gas such as nitrogen. Light can also act as a catalyst to oxidation reactions by transferring energy to drug molecules making them more reactive. As a precaution against light-induced oxidation, sensitive drugs must be packaged in light-resistant or opaque containers. Because most drug degradations proceed more rapidly as the temperature increases, it is also advisable to store oxidizable drugs under refrigerated temperatures. Another factor that can affect the stability of an oxidizable drug in solution is the pH of the formulation buffer. Each drug must be maintained in solution at the pH most favorable to its stability. This varies from preparation-to-preparation and must be determined on an individual basis for the drug.

Formulated drug substances and drug products are stored in container closure systems for extended periods of time. For biological drugs, container closures typically include plastic bags/containers, bottles, vials, and syringes. The containers are typically made from glass or plastic. It is important to determine that there are no interactions between the drug and the container. When a plastic container is used, tests should be conducted to determine if any of the ingredients become adsorbed by the plastic or whether any plasticizers, lubricants, pigments, or stabilizers leach out of the plastic into the drug. The adhesives for the container label need to be tested to ensure they do not leach through the plastic container into the drug. Trace metals originating from the chemical in the drug, solvent, container, or stopper may also be a source of concern in preparing stable solutions of oxidizable drugs.

Freezing and thawing of bulk protein solutions are common practices in bulk intermediate, drug substance, and drug-product manufacturing. Freezing-induced aggregation and denaturation caused by cryopreservation or a pH shift due to crystallization of buffer components can lead to a significant loss in biological activity of the drug. Formulation development and analysis of the impact of freezing on proteins are a significant part of optimizing biological drug storage systems involving cryoprotectants, stabilizing excipients, freezing process parameters, and cryocontainers. Cryoprotectants function by lowering the glass transition temperature of a solution. The cryoprotectant prevents freezing, and the solution maintains some flexibility during the freezing process. Some cryoprotectants also function by forming hydrogen bonds with biological molecules displacing water molecules. Hydrogen bonding in aqueous solutions is important for proper protein and DNA function. As the cryoprotectant replaces the water molecules, the biological material retains its native physiological structure and function. Conventional cryoprotectants, such as glycerol and dimethyl sulfoxide (DMSO), have been used to reduce ice formation in biological material stored in liquid nitrogen. For some biological material, mixtures of cryoprotectants have less toxicity and are more effective than single-agent cryoprotectants. Cryoprotectant mixtures have also been used for vitrification (solidification without crystal ice formation). Vitrification has important applications in preserving embryos, biological tissues, and organs for transplantation.

Similar to cryoprotectants, lyoprotectants are molecules that protect material during lyophilization. Lyoprotectants are typically polyhydroxy compounds such as sugars (mono-, di-, and polysaccharides), polyalcohols, and their derivatives. Lyophilization is frequently used for biological drugs to increase the shelf-life of products, such as vaccines and other injectables that are subject to hydrolysis degradation. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection. The development of freeze-dried formulations involves selecting a suitable lyoprotectant that stabilizes the drug within a defined amorphous matrix and control key drug process parameters: freeze concentration, solution-phase

concentration, product appearance, minimizing reactive products, increasing the surface area, and decreasing vapor pressure of solvents. During the lyophilization process, the freezing phases are the most critical. Amorphous materials do not have a eutectic point but instead have a critical temperature typically between −50°C and −80°C, below which the product must be maintained to prevent melt-back or the collapse of the biological material during the lyophilization primary and secondary drying steps.

During the primary freeze-drying phase, the pressure is lowered to the range of a few millibars and the temperature controlled based on the molecule's latent heat of sublimation. It is important to cool the material below its triple point, the lowest temperature at which the solid and liquid phases of the material can coexist. During this initial drying phase, approximately 95 percent of the water in the form of ice is removed from the product by sublimation. This phase is typically a slow process to avoid altering the molecular structure of the biological material. During this phase, pressure is controlled through the application of partial vacuum. The vacuum speeds up the sublimation during the drying process.

The secondary drying phase removes the remaining unfrozen water molecules from the primary phase. This part of the freeze-drying process is governed by the material's adsorption isotherms. In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0°C to break any physicochemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage, typically in the range of microbars or fractions of a Pascal, to encourage desorption. After the freeze-drying process is complete, the vacuum is broken with an inert gas, such as nitrogen, before the container is sealed. At the end of the lyophilization process, the final residual water content in the drug product is typically around 1 to 4 percent.

The lyophilization process includes the transfer of aseptically filled product in partially sealed containers. To prevent contamination of a partially closed sterile product, an aseptic process must be designed to minimize exposure of sterile articles to the potential contamination hazards of the manufacturing operation. Limiting the duration of exposure of sterile product components, providing the highest possible environmental control, optimizing process flow, and designing equipment to prevent the introduction of lower-quality air into the Class 100 (ISO 5) clean area are essential to achieving a high assurance of final product sterility. In an aseptic process, the drug product, container, and closure are first subjected to separate sterilization methods before being assembled. Because there is not a terminal sterilization process for biological drug products, it is critical that containers be filled and sealed in an extremely high-quality environment. Before the aseptic assembly of a final product, the individual parts of the final drug product are sterilized by separate processes: glass containers by dry heat; rubber closures by moist heat; and liquid dosage forms are subjected to filtration. Each of these manufacturing processes requires validation and control to eliminate the risk of product contamination. Both personnel and material flow must also be optimized to prevent unnecessary activities that could increase the potential for introducing contaminants to exposed product, container closures, or the surrounding environment. The number of personnel in an aseptic processing room should be minimized. The flow of personnel should be designed to limit the frequency of entries and exits into and from an aseptic processing room. The number of transfers into the critical area of a cleanroom, or an isolator, must be minimized, and movement adjacent to the critical area should be restricted. Any intervention or stoppage during an aseptic process can increase the risk of contamination. The design of equipment used in aseptic processing should limit the number and complexity of aseptic interventions by personnel. Personnel interventions should be reduced by integrating an on-line weight check device that eliminates a repetitive manual activity within the critical area, sterilizing preassembled connections using sterilize-in-place (SIP) technology eliminating significant aseptic manipulations, and the use of automation technologies such as robotics to further reduce contamination risks to the product (See Figure 26.30 A and B.).

Product transfers should occur under appropriate cleanroom conditions. Carefully designed curtains, rigid plastic shields, and the use of isolator systems can be used to achieve segregation of the aseptic processing line. If stoppered vials exit an aseptic processing zone prior to capping, appropriate controls should be in place to safeguard the product until completion of the crimping step. Use of on-line detection devices to identify improperly seated stoppers provides additional sterility assurance. Aseptic processing operations must be validated using microbiological growth media fill process simulations. Media fill process simulations should incorporate risk factors that occur during normal product manufacturing such as exposure to product contact surfaces of equipment, container closure systems, critical environments, and process manipulations. Media fills should closely simulate aseptic manufacturing operations incorporating worst-case activities and conditions that may occur during aseptic operations.

MANUFACTURING SUPPORT FUNCTIONS

Quality Assurance (QA)

Good manufacturing practices require that all persons involved in manufacturing be responsible for quality and that the manufacturers implement an effective system for managing quality. This system should require that the quality unit be independent of the manufacturing unit and that the quality unit be involved in all quality-related matters.

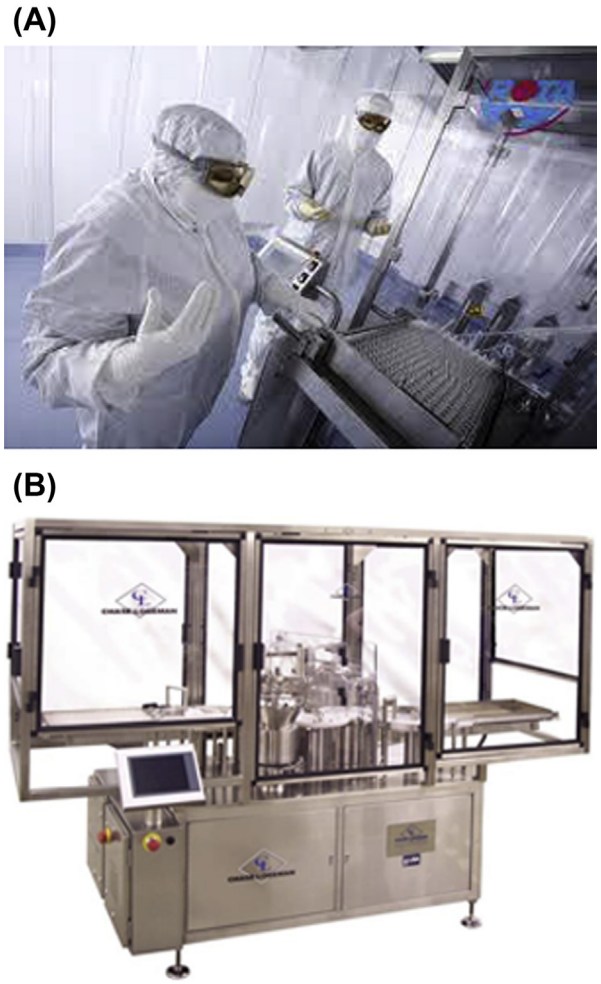

FIGURE 26.30 A and B. (A) Drug product manufacturing: formulation and final container fill finish. An example of a typical glass vial filling operations. (*Source*: Google Images.) (B) Cytovance biologics chase-Logeman Vial Fill Finish System. *(Source: Cytovance Biologics Inc. 2013.)*

Most manufacturers establish the quality unit by developing subteams that support the various manufacturing functions.

Incoming Quality Control/Quality Assurance (QC/QA)

This group performs functions related to assessment, testing, and disposition of materials that are designated to be utilized in the manufacture of intermediates and final products. They ensure proper storage, labeling, and segregation of materials is maintained and also document disposition of incoming material.

Operations Quality Assurance

This group performs functions throughout the actual manufacture of intermediates and final product. They are the independent "eyes" on the floor that provide quality oversight to the manufacturing activities. Their specific activities include but are not limited to verification of critical process steps, line releases, the audit of production areas, and perform in-process inspections.

Document Control

This group performs functions related to the control of all quality records. They ensure that revision history is maintained for documents, that processes for the distribution and reconciliation are maintained, and that change notifications are processed and maintained. Some of the types of quality records that are maintained are:

1. **Standard Operating Procedures (SOPs)—**Contains written instructions on how to perform manufacturing processes and support functions.
2. **Batch records—**Provides a record of actual executed manufacturing steps.
3. **Material specs—**Contains requirements for materials utilized in the manufacture of intermediates and final products.
4. **Training documents—**Provides objective evidence of personnel qualifications and completed training on required processes that are part of or support the manufacture of intermediates and final products.

Quality Assurance for Batch Disposition

This group performs an independent review of product batch records, associated investigations, and test results to verify that all production and testing requirements have been met and/or resolved before determining the final disposition. This group has the authority to determine whether a product will be accepted or rejected.

Quality Compliance

This group provides oversight to the entire quality system and manufacturing functions. They perform roles in quality management by ensuring that internal and external audits are conducted, establish and implement systems that govern quality system monitoring, and are the hosts to inspections and audits from regulatory agencies, customers, and clients.

Qualification/Validation

Good manufacturing practices require that manufacturers establish systems to ensure that their facilities, equipment, processes, test methods, and automated systems are adequate for the support of intermediate and final product manufacture. This system requires that critical product and process attributes be identified and measures implemented to control, test, and monitor these attributes. These systems require that documented qualifications and validations be executed and documented in order to provide evidence of suitability. These systems are driven and overseen by

personnel not involved in the manufacture of product and are made up of personnel with engineering, facility, and quality backgrounds. These personnel perform multiple yet segregated roles in writing, executing, reviewing, and approving the documents that are utilized in qualifications and validations.

Facility and Equipment

When new facilities are built or reconfigured, building utilities (water, electrical, and HVAC) must be qualified to ensure that the facility can meet the requirements of the manufacturing process.

Process

As manufacturing processes are defined and critical parameters that include ranges and boundaries are established, objective evidence through validation protocols must be executed to ensure that the manufacturing process is robust and controlled at a level that can repeatedly produce the same product that meets the same specifications.

Analytical Methods

During the manufacture of intermediates and products, the verification of critical steps can be determined through sample testing. The test utilized must be qualified or validated. The qualification and/or validation of test methods ensure that the defined method can detect the required component or product ingredient. Depending on the test material, standard pharmacopoeia methods can be implemented or new methods can be developed. These methods must meet the requirements of analytical method validation and show specificity, accuracy, precision, detection limits, quantitation limits, linearity, range, and robustness.

Quality Control

Analytical Methods

Suitable analytical test methods are extremely critical components for establishing identity, quality, purity, and strength/potency of a drug product. The current good manufacturing practice regulations [21 CFR 211.194 (a)] require that test methods used for assessing compliance of pharmaceutical products with established specifications must meet proper standards of accuracy and reliability. All test methods are established as standard operating procedures (SOP) in the quality control (QC) laboratory. While it is not necessary to have analytical methods being qualified for testing process development (PD) demonstration run materials or scale-up engineering run material, all test methods need to be at least qualified for any GMP lot material during early stages of the drug development and manufacturing program. The need and scope of analytical method qualification/validation should be defined under the master validation plan for the organization. This should be established in the form of an approved SOP.

It is necessary to perform qualification/validation of the test methods according to the International Conference on Harmonisation (ICH) Tripartite Guideline "Validation of Analytical Procedures: Text and Methodology, Q2 (R1)." Based on the ICH guideline, certain key parameters from the full assay validation program are addressed during the assay qualification. The analytical methods qualification/validation package should include (1) SOP, (2) assay qualification/validation protocol, (3) assay qualification/validation report, and (4) relevant analytical data. Method qualification and validation can be performed in a QC laboratory and/or analytical science laboratory (under R&D unit) of the organization. However, the release tests for the GMP manufactured products must be performed in the QC laboratory. Analytical method qualification/validation needs to be performed with qualified and calibrated instruments/equipment including the material storage units. While noncompendial test methods need to be qualified/validated based on ICH guidelines, according to the regulations [21 CFR 211.194 (a) (2)] all compendial methods as described in the United States Pharmacopeia (USP) and the National Formulary (NF) are not required to validate accuracy and reliability of these methods. These methods merely need to be verified for their suitability under actual conditions of use.

Quality Control Laboratory

The quality control laboratory is set up to support the manufacturing department for the production of drug substances and drug products. As defined in 21 CFR Part 211.22 the responsibility and authority of the quality control unit includes testing and approval (or rejection) of all components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products. According to the regulations, a QC unit also has the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product. It also requires that the responsibilities and procedures applicable to the QC unit shall be in writing and such written procedures shall be followed.

A Description of In-Process and Release Tests Performed in a QC Laboratory for the Product Manufactory Process

The scope of this section is to provide a list of tests generally performed for protein-based drug manufacturing platforms. For nonprotein-based (biologics) drug development

program testing, requirements can be different which are highly dependent on the nature and characteristics of the product. The production process of protein drugs (e.g., antibody and other proteins) is a two-stage process. The first stage is a growth and production phase which begins with different protein expression platforms like a mammalian expression system (e.g., Chinese hamster ovary [CHO] cell lines) or a microbial expression system (e.g., *E. coli*). The second stage is a downstream purification and formulation process. In general, the required in-process tests while manufacturing a product is defined by the product development phase of the project. All the specifications are developed and established during the process validation in order to define various process control steps. This activity is in turn guided by the process development activities of product development. Release tests for a drug substance (DS) and drug product (DP) are established based on the DS and DP in question in addition to certain regulatory guidelines, and the tests should demonstrate/establish identity, strength/potency, quality, and purity of a drug product. Several of the release test methods also serve as stability-indicating test methods during the required stability study program for DS and DP.

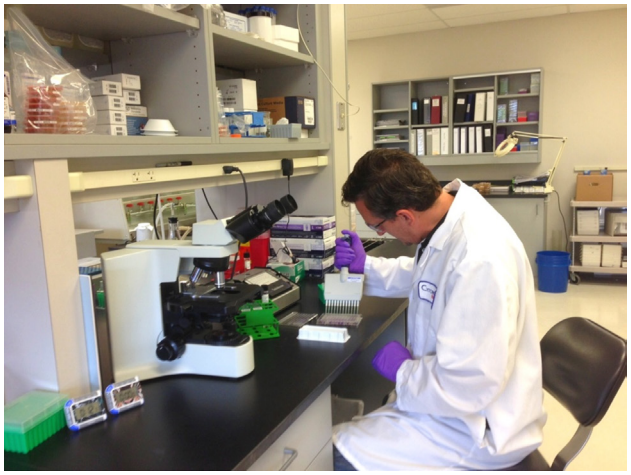

FIGURE 26.31 Quality Control Microbiology. *(Source: Cytovance Biologics Inc., 2013.)*

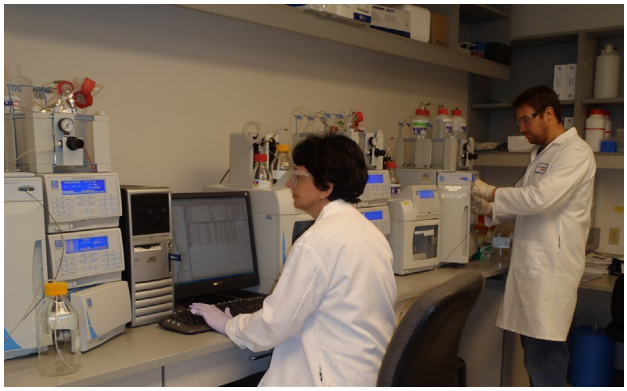

FIGURE 26.32 Quality control analytics. *(Source: Cytovance Biologics Inc., 2013.)*

Microbiology (See Figure 26.31)

In-Process Tests For unprocessed bulk harvest common in-process tests are:

- Bioburden
- Mycoplasma detection with mycoplasmastasis
- *In vitro* assay for nonendogenous or adventitious viruses
- Detection of Mouse Minute Virus (MMV) DNA by quantitative polymerase chain reaction (qPCR),
- Transmission electron microscopy (TEM) of supernatant

Endotoxin testing is performed on the clarified bulk harvest.

Release Tests for Drug Substance

- Bioburden
- Endotoxin

Chemistry (See Figure 26.32)

In-Process Tests

- pH
- Conductivity
- Protein content by UV A280
- Titer analysis (e.g. Protein A-HPLC [high-performance liquid chromatography] for antibody, or enzyme-linked immunosorbent assay- [ELISA] based test method for other protein products)
- Sometimes size exclusion-HPLC (SE-HPLC), reverse-phase-HPLC (RP-HPLC), and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (reduced/nonreduced) methods are also used as in-process tests to monitor the efficiency of purification steps, state of aggregation of the product, and step-wise improvement in the product quality. Occasionally suitable potency or activity assay is also performed as an in-process test to monitor product quality as one moves through the manufacturing process. (See Table 26.3.)

Environmental Tests

For a compliant biomanufacturing facility, it is essential to develop a contamination control program. This program needs to address three basic tasks:

1. Control (minimization) of bioburden throughout the process of product manufacturing.
2. Control (minimization) of cross-over contamination of residuals from batch-to-batch production.

TABLE 26.3 Quality Control Release Tests for Drug Substance

Attribute	Drug Substance	Drug Product
Safety	Endotoxin	Endotoxin
Safety	Bioburden	Sterility
Impurities	Host cell protein (HCP)	N/A
Impurities	Residual DNA	N/A
Impurities	Tween 20 or Tween 80 or Triton X 100 or any other excipient (if added during formulation)	N/A
Impurities	Residual Protein A (if Protein A was used during purification process, e.g., for antibody product)	N/A
Quality	pH	pH
Quality	Osmolality	Osmolality
Quality	Appearance (color, visible particulate matter)	Appearance (color, visible particulate matter)
Quality	N/A	Subvisible particulate matter (10 μm, 25 μm), (2 μm, 5 μm, 8 μm for information only)
Quality	N/A	Volume in container
Quality	N/A	Container closure integrity
Strength/content	Protein concentration by A280 (or any other assay such as Bradford, BCA, etc.)	Protein concentration by A280 (or any other assay such as Bradford, BCA, etc.)
Purity	SE-HPLC	
Purity	SDS-PAGE (reduced and nonreduced), CE-SDS	SDS-PAGE (reduced and nonreduced), CE-SDS
Purity	RP-HPLC	RP-HPLC
Identity and charge heterogeneity	IEF, cIEF	IEF, cIEF
Identity and charge heterogeneity	CEX, CZE	CEX, CZE
Identity	Peptide map	Peptide map
Potency	Binding ELISA	Binding ELISA
Potency	Bioassay	Bioassay

Source: *Cytovance Biologics Inc., 2013.*

3. Control (minimization) of cross-over contamination of residuals from cleaning materials during the production period.

In order to achieve these goals it is critical to establish suitable laboratory test methods and operational practices. Appropriate gowning procedures, personnel monitoring, environmental monitoring (EM), air quality monitoring with settling plates, facility surface cleaning and disinfection monitoring, and purified water monitoring (by total organic carbon [TOC] analysis) are key programs that need to be established in the form of SOPs and implemented for a compliant biomanufacturing facility which is engaged in the production of quality drug products. (See Figure 26.33A and B.)

CONTRACT (CMO) VERSUS IN-HOUSE MANUFACTURING

Traditionally in the pharmaceutical industry the paradigm was to build small molecule manufacturing facilities that could manufacture large quantities of product reproducibly. These plants had enormous amounts of stainless steel and required a large facility footprint as well as a large workforce. With the discovery of therapeutic biologics or (large molecules), biopharmaceutical and biomanufacturing facilities were both newly constructed and others were converted into biomanufacturing facilities. These early in-house facilities were expensive to construct and at times retrofits were not right for the technology or difficult to

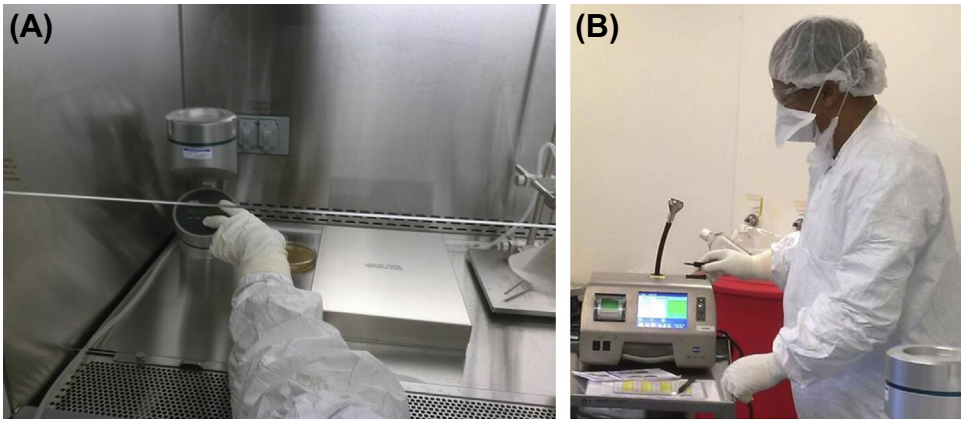

FIGURE 26.33 A and B. (A) Environmental Monitoring (EM) Technician setting up test equipment to monitor viable air particles (microbials) during processing. (B) the EM Technician setting up a Particle Counter to monitor particles in the air of the production suite. Quality control environmental microbiology/environmental monitoring. *(Source: Cytovance Biologics Inc., 2013).*

scale and reproduce. Thus many companies outsourced development and manufacturing of their biologic products to contract development organizations (CDOs) or contract manufacturing organizations (CMOs) or contract development and manufacturing organizations (CDMOs). Most CMO facilities were designed to have a scaled process to support preclinical through Phases 1, 2, and 3 and some that could support the commercial launch of complex biologic products.

Academic facilities, small biotech companies, medium seasoned biotech companies, large biopharmaceutical companies, and virtual companies have varying abilities to financially support and build a facility for multiple biologic platforms in-house. Most CMOs have already built manufacturing capabilities to produce and purify biologic drugs at a certain scale to accommodate their scale or "niche" market. These capabilities are large capital expenditures such as stainless steel bioreactors, fermentors, mixing tanks, water systems, chromatography skids, chromatography columnsclean roomsms, QC labs, EM Labs, process development labs as well as Quality systems. The advantage of a CMO facility is that it is ready for manufacturing the developed product. Although, today there are a variety of disposable options in the small-scale manufacture of biologics which would allow a small company to consider biomanufacturing in-house. There are single-use bioreactors (SUBs) that range from a 25 liter to a 2000 liter scale as well as single-use mixing units (SUMs) in the same scale. Some biotech companies are using disposables to perform small-scale studies to support some preclinical or pilot-scale work and then transfer the process and further scale-up to a CMO. This allows the biotech company more control and hands on during early-process development.

A major disadvantage for the in-house model is the capital outlay for an in-house facility. In terms of time and equipment, an in-house facility would be very costly in respect to supporting in-house expertise to manage the design, fabrication, installation and qualification of the facility, process, and support equipment. There are other considerations such as cleaning and maintenance of all product contact surfaces such as bioreactors and mixing tanks. These require studies and validation of the cleaning processes. Samples would have to be taken and tested creating additional in-house expertise, time, and resources and this would be contingent on suite availability to develop and manufacture the product. Whereas outsourcing these activities to a contract manufacturing organization (CMO) would shorten the timeline significantly because the CMO would already have the expertise, process, and facility equipment in place to support the development and manufacturing activities. Other aspects that need to be considered is the support for biomanufacturing with QA and QC groups that are necessary for the GMP documentation, in-process testing, and release testing. The Chemistry, Manufacturing and Control (CMC) section of a company's investigational new drug (IND) could be completed by the CMO and would be support for the biotech company as one of the CMO's regulatory offerings.

The availability of reliable and functional disposable equipment at small scales such as SUBs and SUMs mentioned previously might be reasonable for some small companies that may have a modular (factory-built panels delivered and assembled on-site with a factory-impervious finish) or stick-built (onsite piece-by-piece construction such as framing, sheet rock, and epoxy-type finishes) facility with proper cleaning and HVAC (that can deliver a classified or controlled environment) to bring in a SUB or SUM. The disposables or single use equipment offers quick installation, flexibility and no cleaning validation as compared to it's stainless steel bioreactor, buffer tanks and mixer predecessors. However, a company would still

have to invest in clean rooms and infrastructure to support biologic GMP production. At the end of the day it would be more cost and time line effective to enlist a CMO to carry the "heavy lifting" of the drug development process. As a result, many biotechnology and biopharmaceutical companies are more commonly outsourcing some or all of their biomanufacturing needs. Outsourcing allows a company to remain focused on the crucial components in-house while taking advantage of a CMO to supply their resources for process development, analytical development, manufacturing, quality and regulatory support. Utilizing a CMO for outsourcing can help reduce or eliminate the need to build, manage, and maintain a facility, or it allows a company time to grow until it can justify a permanent facility.

SUMMARY

The incredible amount of recent biologic therapeutic discoveries has led to an increased need for scientific and engineering knowledge available to characterize and biomanufacture these large and complex molecules. In this chapter we have followed what role biomanufacturing has in the development of a biotherapeutic product. We have followed the biologic product's development from "proof-of-concept," examined the history of these large molecules, and developed a knowledge-base that we would use in developing a scaled process that would be tech-transferred into manufacturing. Within this chapter we also discussed key partners in the biomanufacturing process such as raw material suppliers, outsource testing vendors, as well as key support teams such as facilities, engineering, process development, quality assurance, quality control, environmental monitoring, manufacturing, and manufacturing science and technology teams required to fully develop and manufacture a biologic that can meet all of the critical safety, quality, and regulatory parameters.

It is essential that the biomanufacturing process be able to deliver and demonstrate a safe therapeutic drug at each step of the clinical trial, be economically able to reproducibly manufacture sufficient inventory with a stable shelf-life, and to successfully traverse the regulatory and efficacy hurdles in order to obtain regulatory approval. In biomanufacturing "the process is the product" is an old paradigm, but it is still relevant in the development and manufacture of a biotherapeutic.