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## Biological Products: Manufacturing, Handling, Packaging and Storage

Nahla S. Barakat  
King Saud University,  
College of Pharmacy,  
Dept. of Pharmaceutics,  
Saudi Arabia

### 1. Introduction

A biological product is defined as “a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or any other trivalent organic arsenic compound, applicable to the prevention, treatment or cure of a disease or condition of human beings”. Throughout the 20th century, the world witnessed great discoveries in the biological sciences. One of the earliest biological products introduced to the U.S. marketplace was a blood protein called Factor VIII first sold in 1966. The earliest FDA approval for a modern biotech product designed for human therapeutic use was given to human insulin in 1982, approval was given in 1985 to a human growth hormone (HGH) for the treatment of dwarfism. In the 1990s FDA granted approvals for vaccines against rabies, tetanus toxoids, and pertussis. The manufacturing process for a biological product usually different from the process for drugs. The manufacture of biological medicinal products involves certain specific considerations arising from the nature of the products and the processes. Persons responsible for production and quality control should have an adequate background in relevant scientific disciplines, such as bacteriology, biology, biometry, chemistry, medicine, pharmacy, pharmacology, virology, immunology and veterinary medicine. The degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the product and the production step. Animals are used for the manufacture of a number of biological products, in addition, animals may also be used in the quality control of most sera, antibiotics and vaccines. All biological products should be clearly identified by labels which should be approved by the national control authority. The evaluation of stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies.

Throughout the 20th century, the world witnessed great discoveries in the biological sciences, many of which led to the prevention or eradication of diseases that have devastated populations in the past. For 100 years, what is now known as FDA's Center for Biologics Evaluation and Research or "CBER," has played a significant role in ensuring the safety and efficacy of the fruits of these scientific discoveries. CBER is responsible for the regulation of "biologics," which are medical products such as vaccines, blood and blood

derivatives, allergenic patch tests and extracts, HIV and hepatitis tests, gene therapy products, cells and tissues for transplantation, and new treatments for cancers, arthritis, and other serious diseases. CBER reviewed the first vaccines to immunize persons against infectious diseases, such as polio, pertussis ("whooping cough"), and German measles. CBER research led to important discoveries to safely collect, prepare, and transfuse blood and blood plasma.

## 2. Biological products, industry history<sup>(1)</sup>

Biological products were created with biotechnology, the scientific and engineering procedures involved in manipulating organisms or biological components at the cellular, subcellular, or molecular level. These manipulations were carried out to make or modify plants and animals or other biological substances with desired traits. Although examples of primitive biotech processes dated back to ancient times (such as the use of fermentation in brewing and leavening agents in baking), their use in medical and pharmaceutical applications was an innovation of the latter decades of the twentieth century. Some analysts compared the biotech industry's impact on global medical care with the computer industry's impact on communication.

Biotech researchers produced products in essentially three ways: by developing ways to achieve commercial production of naturally occurring substances; by genetically altering naturally occurring substances; and by creating entirely new substances. Some of the tools used by biotech researchers included recombinant DNA and monoclonal antibodies. Recombinant DNA involved the ability to take the deoxyribonucleic acid (DNA) from one organism and combine it with the DNA from another organism thereby creating new products and processes. By using recombinant DNA techniques researchers were able to select specific genes and introduce them into other cells or living organisms to create products with specific attributes. Monoclonal antibodies were developed from cultures of single cells using cloning techniques. They were designed for use in attacking toxins, viruses, and cancer cells. Because the biological products presented for approval often involved new technologies or innovative therapies for diseases that had not been previously treated successfully, the approval process frequently proved to be long and costly. Many companies struggled financially through the 1980s waiting for an FDA determination. One of the earliest biological products introduced to the U.S. marketplace was a blood protein first sold in 1966. The blood protein, called Factor VIII, was used by patients with hemophilia A to control bleeding episodes. Factor VIII, the blood factor responsible for normal clotting action, was manufactured from human blood received from donors. It was followed by the development of Factor IX for patients with hemophilia B.

During the early 1980s, problems arose as a result of AIDS contamination in the blood supply used to produce blood clotting factors. In 1984 manufacturers began using a heat treatment process to guard against future contamination, but, according to a report in the *Wall Street Journal*, approximately half of the nation's 20,000 hemophiliacs contracted AIDS, primarily through the use of Factors VIII and IX.<sup>(2)</sup>

The earliest FDA approval for a modern biotech product designed for human therapeutic use was given to human insulin in 1982. Human insulin was used for treating patients with diabetes. In 1984 the FDA approved an agricultural vaccine against colibacillosis (a disease

commonly called scours, which causes diarrhea or dysentery in newborn animals). Approval was given in 1985 to a human growth hormone (HGH) for the treatment of dwarfism.

The first genetically engineered vaccine approved for use in the United States was a vaccine against hepatitis-B. It received approval in 1986. The vaccine had been created by inserting part of a hepatitis-B virus into yeast cells. Although the portion of the hepatitis-B virus used was not infectious, it caused an immune reaction against infection from the entire hepatitis-B virus.

Other firsts occurring in 1986 included the approval of therapeutic monoclonal antibodies (MABs) and alpha interferon. MABs were approved for use along with immunosuppressive drugs to help prevent kidney rejection in transplant patients. Alpha interferon's first approved use was in the treatment of hairy cell leukemia. Other approved uses for alpha interferon followed: for Kaposi's sarcoma in 1988, venereal warts in 1988, non-A/non-B hepatitis in 1991, and hepatitis-B in 1992. A product to dissolve blood clots in patients with acute myocardial infarction (heart attack) was approved in 1987. An agricultural vaccine to protect against pseudorabies won FDA approval the same year.

Erythropoietin (EPO), which was to become the largest single biotech product, received its first FDA approval in 1989. EPO, a protein that stimulates production of red blood cells, won initial approval for use with anemia associated with kidney disease. In the same year, the Health Care Financing Administration agreed to pay for EPO given to dialysis patients under the Medicare program. Within a few years, EPO was being used by approximately 82,000 dialysis patients in the United States. In 1991 the FDA gave additional approval for its use in treating AIDS-related anemia.

Advances continued during the 1990s. As the industry matured, cooperation between product developers and government regulators improved. The steps in the approval process became more predictable, and a shift in technology was also noted. The primary products of the 1980s had involved the use of recombinant DNA proteins without further alterations. During the early 1990s, researchers turned their attention to products requiring more extensive genetic modification and to more obscure applications.

In the 1990s FDA granted approvals for vaccines against rabies, tetanus toxoids, and pertussis. According to government statements, vaccines were one of the most effective and cheapest ways to eradicate some diseases. Accordingly, the National Institute of Health's Office of Financial Management reported that funding for vaccine research and development rose 65 percent from 1993 to 1999. Concern about health care costs during the early 1990s focused the national spotlight on the pharmaceutical industry and questions were raised about the high cost of biological products <sup>(3)</sup>.

## 2.1 Definition

The definition of a biologic has changed over time. In the U.S., a biological product is defined as "a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or arsphenanaine, or derivative of arsphenamine) or any other trivalent organic arsenic compound), applicable to the prevention, treatment or cure of a disease or condition of human beings" (Public Health

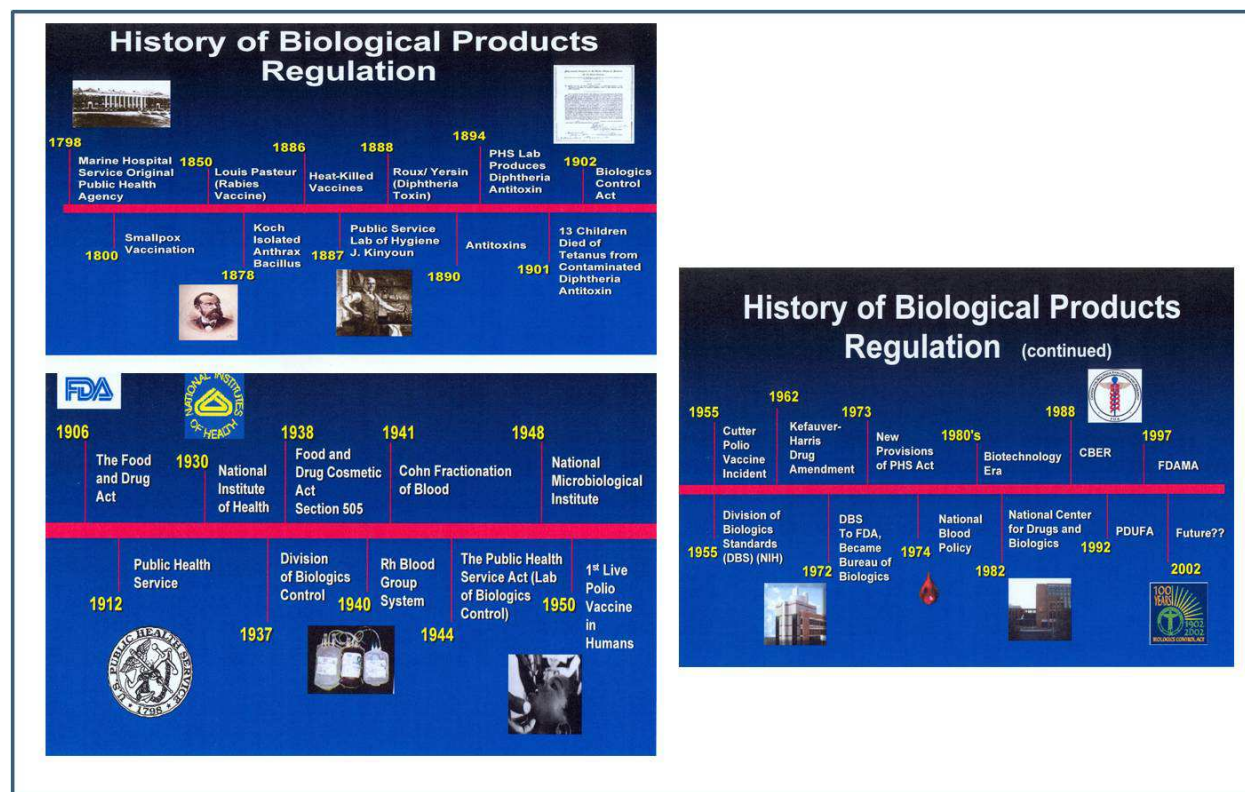


Chart 1. History of biological products regulation

Drug	Indication	Approval Date	2003 Sales \$ (million)
Humulin (human insulin)	Diabetes	October 1982	1,060
Intron A (interferon alfa-2b)	Cancer, infection	June 1986	1,851
Humatrope (somatropin)	Growth failure	March 1987	371
Infanrix (diphtheria-tetanus-pertussis vaccine)	Immunization against diphtheria, pertussis, and tetanus	March 1987	551
Epogen (epoetin alfa)	Anemia	June 1989	2,435
Enerix-B (hepatitis B vaccine)	Immunization against hepatitis B	August 1989	684
Botox (botulinum toxin type A)	Cervical dystonia	December 1989	564
Epogin (epoetin beta)	Anemia	April 1990	551
Procrit (epoetin alfa)	Anemia	December 1990	3,984
Neupogen (filgrastim)	Neutropenia	January 1991	1,267
Cerezyme (imiglucerase)	Gaucher's disease	April 1991	739
NovoSeven (recombinant factor VII)	Hemophilia	April 1992	589

\* The patents on these products expire after 20 years; most patents are applied for during the drug-development stage. Data are from *MedAdNews*, "Top 200 World's Best Selling Medicines" (2004;23(5):60-4).

Table 1. Top selling Biopharmaceuticals approved before 1993

Services Act 42 U.S.C. § 262(i)). By statute, biological products include viruses, therapeutic sera, toxins and antitoxins, vaccines, blood, blood components or derivatives, allergenic products, any analogous products, and arsphenamines used for treating disease. The statute does not offer a definition of "biologic," but is fairly broad. The inclusion of the term "analogous products" makes the definition particularly broad since the basis for determining analogous products is not provided by the statute.

1. A virus is interpreted to be a product containing the minute living cause of an infectious disease and includes but is not limited to filterable viruses, bacteria, rickettsia, fungi, and protozoa.
2. A therapeutic serum is a product obtained from blood by removing the clot or clot components and the blood cells.
3. A toxin is a product containing a soluble substance poisonous to laboratory animals or to man in doses of 1 milliliter or less (or equivalent in weight) of the product, and having the property, following the injection of non-fatal doses into an animal, of causing to be produced therein another soluble substance which specifically neutralizes the poisonous substance and which is demonstrable in the serum of the animal thus immunized.
4. An antitoxin is a product containing the soluble substance in serum or other body fluid of an immunized animal which specifically neutralizes the toxin against which the animal is immune.

Biological products, like other drugs, are used for the treatment, prevention or cure of disease in humans. In contrast to chemically synthesized small molecular weight drugs, which have a well-defined structure and can be thoroughly characterized, biological products are generally derived from living material--human, animal, or microorganism--are complex in structure, and thus are usually not fully characterized.

Biological products can be composed of sugars, proteins, or nucleic acids, or a combination of these substances. They may also be living entities, such as cells and tissues. Biologics are made from a variety of natural resources--human, animal, and microorganism--and may be produced by biotechnology methods. Most biologics, however, are complex mixtures that are not easily identified or characterized. Biological products differ from conventional drugs in that they tend to be heat-sensitive and susceptible to microbial contamination. This requires sterile processes to be applied from initial manufacturing steps.

The categories of therapeutic biological products regulated by Center for Drug Evaluation and Research (CDER) (under the Federal Food Drug and Cosmetics Act (FDCA) and/or the Public Health Service Act (PHSA), as appropriate include the following:

- Monoclonal antibodies for in vivo use.
- Most proteins intended for therapeutic use, including cytokines (e.g., interferons), enzymes (e.g. thrombolytics), and other novel proteins, except for those that are specifically assigned to the Center for Biologics Evaluation and Research (CBER) (e.g., vaccines and blood products). This category includes therapeutic proteins derived from plants, animals, humans, or microorganisms, and recombinant versions of these products. Exceptions to this rule are coagulation factors (both recombinant and human-plasma derived).
- Immunomodulators (non-vaccine and non-allergenic products intended to treat disease by inhibiting or down-regulating a pre-existing, pathological immune response).

- Growth factors, cytokines, and monoclonal antibodies intended to mobilize, stimulate, decrease or otherwise alter the production of hematopoietic cells in vivo.

### **3. Good manufacturing practices for biological products** <sup>(3, 4)</sup>

The manufacturing process for a biological product usually different from the process for drugs because, in many cases, there is limited ability to identify the identity of the clinically active component(s) of a complex biological product, such products are often defined by their manufacturing processes. Changes in the manufacturing process, equipment or facilities could result in changes in the biological product itself and sometimes require additional clinical studies to demonstrate the product's safety, identity, purity and potency. Traditional drug products usually consist of pure chemical substances that are easily analyzed after manufacture. Since there is a significant difference in how biological products are made, the production is monitored by the agency from the early stages to make sure the final product turns out as expected. For this reason, in the manufacture of biological products full adherence to GMP is necessary for all production steps, beginning with those from which the active ingredients are produced.

## **4. Manufacture of biological medicinal products for human use**

### **4.1 Principle**

The manufacture of biological medicinal products involves certain specific considerations arising from the nature of the products and the processes. The ways in which therapeutic biological products are produced, controlled and administered make some particular precautions necessary.

Unlike conventional medicinal products, which are reproduced using chemical and physical techniques capable of a high degree of consistency, the production of therapeutic biological products involves biological processes and materials, such as cultivation of cells or extraction of substances from living organisms, including human, animal and plant tissues. Propagation of microorganisms in embryos or animals, growth of strains of microorganism and eukaryotic cells, hybridoma techniques are also involved. These biological processes may display inherent variability, so that the range and nature of by-products are variable. Moreover, the materials used in these cultivation processes provide good substrates for growth of microbial contaminants.

Control of therapeutic biological products usually involves biological analytical techniques which have a greater variability than physico-chemical determinations. In-process controls therefore take on a great importance in the manufacture of therapeutic biological products.

Therapeutic biological products manufactured by these methods include: vaccines, immune sera, immunoglobulins (including monoclonal antibodies), antigens, hormones, cytokines, allergens, enzymes and other products of fermentation (including products derived from r-DNA).

### **4.2 Personnel**

All personnel (including those concerned with cleaning, maintenance or quality control) employed in areas where biological medicinal products are manufactured should receive

additional training specific to the products manufactured and to their work. Personnel should be given relevant information and training in hygiene and microbiology.

Persons responsible for production and quality control should have an adequate background in relevant scientific disciplines, such as bacteriology, biology, biometry, chemistry, medicine, pharmacy, pharmacology, virology, immunology and veterinary medicine, together with sufficient practical experience to enable them to exercise their management function for the process concerned.

The immunological status of personnel may have to be taken into consideration for product safety. All personnel engaged in production, maintenance, testing and animal care (and inspectors) should be vaccinated where necessary with appropriate specific vaccines and have regular health checks. Apart from the obvious problem of exposure of staff to infectious agents, potent toxins or allergens, it is necessary to avoid the risk of contamination of a production batch with infectious agents. Visitors should generally be excluded from production areas.

Any changes in the immunological status of personnel which could adversely affect the quality of the product should preclude work in the production area.

Production of BCG vaccine and tuberculin products should be restricted to staff who are carefully monitored by regular checks of immunological status or chest X-ray. In the case of manufacture of products derived from human blood or plasma, vaccination of workers against hepatitis B is recommended.

During the working day, personnel should not pass from areas where exposure to live organisms or animals is possible to areas where other products or different organisms are handled. If such passage is unavoidable, clearly defined decontamination measures, including change of clothing and shoes and, where necessary, showering should be followed by staff involved in any such production.

The names and qualifications of those responsible for approving lot processing records (protocols) should be registered with the national control authority

#### **4.3 Premises and equipment <sup>(5)</sup>**

The degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the product and the production step, bearing in mind the level of contamination of the starting materials and the risk to the finished product.

The risk of cross-contamination between biological medicinal products, especially during those stages of the manufacturing process in which live organisms are used, may require additional precautions with respect to facilities and equipment, such as the use of dedicated facilities and equipment, production on a campaign basis and the use of closed systems. The nature of the product as well as the equipment used will determine the level of segregation needed to avoid cross-contamination.

In principle, dedicated facilities should be used for the production of BCG vaccine and for the handling of live organisms used in production of tuberculin products. Dedicated



facilities should be used for the handling of *Bacillus anthracis*, of *Clostridium botulinum* and of *Clostridium tetani* until the inactivation process is accomplished.

Production on a campaign basis may be acceptable for other spore forming organisms provided that the facilities are dedicated to this group of products and not more than one product is processed at any one time.

Simultaneous production in the same area using closed systems of biofermenters may be acceptable for products such as monoclonal antibodies and products prepared by DNA techniques.

Processing steps after harvesting may be carried out simultaneously in the same production area provided that adequate precautions are taken to prevent cross contamination. For killed vaccines and toxoids, such parallel processing should only be performed after inactivation of the culture or after detoxification.

Positive pressure areas should be used to process sterile products but negative pressure in specific areas at point of exposure of pathogens is acceptable for containment reasons. Where negative pressure areas or safety cabinets are used for aseptic processing of pathogens, they should be surrounded by a positive pressure sterile zone.

Air filtration units should be specific to the processing area concerned and recirculation of air should not occur from areas handling live pathogenic organisms.

The layout and design of production areas and equipment should permit effective cleaning and decontamination (e.g. by fumigation). The adequacy of cleaning and decontamination procedures should be validated.

Equipment used during handling of live organisms should be designed to maintain cultures in a pure state and uncontaminated by external sources during processing. Pipework systems, valves and vent filters should be properly designed to facilitate cleaning and sterilization. The use of 'clean in place' and 'sterilize in place' systems should be encouraged. Valves on fermentation vessels should be completely steam sterilizable. Air vent filters should be hydrophobic and validated for their scheduled life span.

Primary containment should be designed and tested to demonstrate freedom from leakage risk.

Effluents which may contain pathogenic micro-organisms should be effectively decontaminated.

Due to the variability of biological products or processes, some additives or ingredients have to be measured or weighed during the production process (e.g. buffers). In these cases, small stocks of these substances may be kept in the production area.

Seed lots and cell banks used for the production of biological products should be stored separately from other materials. Access should be restricted to authorized personnel.

#### **4.4 Animal cell substrates for biological products**

The selection of an appropriate cell substrate for use in the production of biological products has been a recurring focus of attention and anxiety for at least the past 50 years. The reasons

for that are not difficult to understand because the central issue has always been “Is the product manufactured in a given cell substrate going to be safe to use in humans?”

#### 4.4.1 Phenotypic characteristics of animal cells grown in vitro

A large number of phenotypic characteristics of animal cells have been described in the literature. Of those, three characteristics have been particularly important in the assessment of cells grown in vitro that might be considered as substrates for the production of biological products. These include: (1) life potential; (2) tumorigenic potential; and (3) chromosomal complement.

With regard to life potential, cells grown in vitro may be divided into two large general classes: those with a finite life potential such as human diploid cells; and those with an apparent infinite life potential such as cells derived from tumor tissue.

When cells grown in vitro are assessed for their ability to produce tumors in animal test systems, they again may be divided into two general classes: those that have the ability to produce tumors; and those that do not display the characteristic.

However, it is important to note that the results of any tumorigenicity assay depend very heavily on the sensitivity of the assay system itself. A variety of such assays have been developed over the past 50 years, and a number of the more recent systems are able to detect the tumorigenic potential of inoculated cells that had been scored as negative in earlier systems. The chromosomal complement of cells grown in vitro also may be divided into two general classes: diploid cells and heteroploid cells. Diploid cells of those that contain the normal number of chromosomes for the species from which the cells were derived; whereas heteroploid cells contain an abnormal number of chromosomes that also usually have numerous structural abnormalities.

#### 4.4.2 Animal quarters and care <sup>(6)</sup>

Animals are used for the manufacture of a number of biological products (Table 2), for example polio vaccine (monkeys), snake antivenoms (horses and goats), rabies vaccine (rabbits, mice and hamsters) and serum gonadotropin (horses). In addition, animals may also be used in the quality control of most sera and vaccines, e.g. pertussis vaccine (mice), pyrogenicity (rabbits), BCG vaccine (guinea-pigs). Antibodies are often generated using animals as hosts for an antigen towards which an antibody is needed. There are, however, ways of generating antibodies or molecules showing similar properties but using fewer and sometimes no live animals. These include phage, yeast and ribosomal display methods and using egg yolk IgY instead of animal-derived IgG or IgM antibodies.

Other biological products include botulinum toxin, insulin and other hormones and vaccines. Each of these products are produced in batches and some products are still produced in animals.

General requirements for animal quarters, care and quarantine are laid down in Directive 86/609/EEC2. Quarters for animals used in production and control of biological products should be separated from production and control areas. The health status of animals from

which some starting materials are derived and of those used for quality control and safety testing should be monitored and recorded. Staff employed in such areas must be provided with special clothing and changing facilities. Where monkeys are used for the production or quality control of biological medicinal products, special consideration is required as laid down in the current WHO Requirements for Biological Substances n° 7

Animal	vaccine
Hamster	SPF Chicken Embryo Measles Vaccine, Live
Rabbit	Attenuated Rubella Vaccine, Live
Monkey	Attenuated Poliomyelitis Vaccine, Live
Gerbil	Attenuated Hemorrhagic Fever with Renal Syndrome Vaccine, Live
Specific-pathogen -free (SPF) Chicken Embryo	Measles Vaccine

Table 2. Animals used in vaccine preparation

## 5. Documentation

Specifications for biological starting materials may need additional documentation on the source, origin, method of manufacture and controls applied particularly microbiological controls. Specifications are routinely required for intermediate and bulk biological medicinal products.

## 6. Production

Standard operating procedures should be available and maintained up to date for all manufacturing operations.

The source of cells (laboratory or culture collection) from which the cell substrate was derived should be stated, and relevant references from the scientific literature should be cited. Information obtained directly from the source laboratory is preferred. When this is not available, literature references may be utilized.

### 6.1 Starting materials

The source, origin and suitability of starting materials for biological products should be clearly defined. Where the necessary tests take a long time, it may be permissible to process starting materials before the results of the tests are available. In such cases, release of a finished product is conditional on satisfactory results of these tests.

Where sterilization of starting materials is required, it should be carried out where possible by heat. Where necessary, other appropriate methods may also be used for inactivation of biological materials (e.g. irradiation).

### 6.2 Seed lot and cell bank system

In order to prevent the unwanted drift of properties which might ensue from repeated subcultures or multiple generations, the production of biological medicinal products

obtained by microbial culture, cell culture or propagation in embryos and animals should be based on a system of master and working seed lots and/or cell banks (Chart. 2).

### Block flow diagram of a typical production process

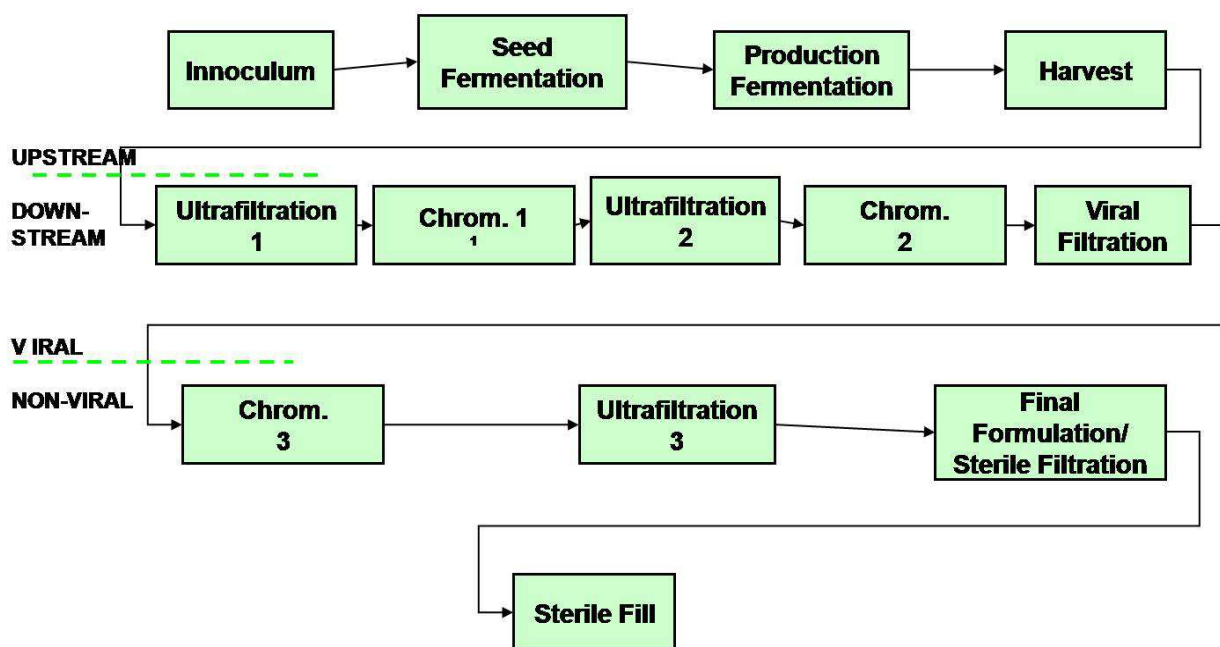


Chart 2. Block flow diagram of a typical production process

The number of generations (doublings, passages) between the seed lot or cell bank and the finished product should be consistent with the marketing authorization dossier. Scaling up of the process should not change this fundamental relationship.

Seed lots and cell banks should be adequately characterized and tested for contaminants. Their suitability for use should be further demonstrated by the consistency of the characteristics and quality of the successive batches of product. Seed lots and cell banks should be established, stored and used in such a way as to minimize the risks of contamination or alteration.

Establishment of the seed lot and cell bank should be performed in a suitably controlled environment to protect the seed lot and the cell bank and, if applicable, the personnel handling it.

During the establishment of the seed lot and cell bank, no other living or infectious material (e.g. virus, cell lines or cell strains) should be handled simultaneously in the same area or by the same persons.

Evidence of the stability and recovery of the seeds and banks should be documented. Storage containers should be hermetically sealed, clearly labelled and kept at an appropriate

temperature. An inventory should be meticulously kept. Storage temperature should be recorded continuously for freezers and properly monitored for liquid nitrogen. Any deviation from set limits and any corrective action taken should be recorded.

Only authorized personnel should be allowed to handle the material and this handling should be done under the supervision of a responsible person. Access to stored material should be controlled. Different seed lots or cell banks should be stored in such a way to avoid confusion or cross-contamination. It is desirable to split the seed lots and cell banks and to store the parts at different locations so as to minimize the risks of total loss.

All containers of master or working cell banks and seed lots should be treated identically during storage. Once removed from storage, the containers should not be returned to the stock.

## **7. Operating principles**

The growth promoting properties of culture media should be demonstrated. Addition of materials or cultures to fermenters and other vessels and the taking of samples should be carried out under carefully controlled conditions to ensure that absence of contamination is maintained. Care should be taken to ensure that vessels are correctly connected when addition or sampling take place.

Centrifugation and blending of products can lead to aerosol formation, and containment of such activities to prevent transfer of live micro-organisms is necessary.

If possible, media should be sterilized in situ. In-line sterilising filters for routine addition of gases, media, acids or alkalis, defoaming agents etc. to fermenters should be used where possible.

Careful consideration should be given to the validation of any necessary virus removal or inactivation undertaken. In cases where a virus inactivation or removal process is performed during manufacture, measures should be taken to avoid the risk of recontamination of treated products by nontreated products.

A wide variety of equipment is used for chromatography, and in general such equipment should be dedicated to the purification of one product and should be sterilized or sanitised between batches. The use of the same equipment at different stages of processing should be discouraged. Acceptance criteria, life span and sanitation or sterilization method of columns should be defined.

## **8. Antibiotics as preservatives <sup>(7)</sup>**

Antibiotics are authorized for use as preservatives for biological products if used within the limitations as to kinds and amounts prescribed in this section.

When an antibiotic or combination of antibiotics, with or without a fungistat is to be used in the preparation of a biological product, the kind(s) and amount(s) of each shall be specified in the outline for such product in such a way that the concentration in the final product may be calculated. Except as may be approved by the Administrator, only those individual antibiotics or combinations of antibiotics listed this section shall be used.

### 8.1 Permitted individual antibiotics

- a. The antibiotic level of a specified individual antibiotic in one ml. of a biological product, when prepared as recommended for use, shall not exceed the amounts listed in this paragraph: *Provided*, That in the case a desiccated biological product is to be used with an indefinite quantity of water or other menstruum, the determination shall be based on 30 ml. per 1,000 dose vial or equivalent.
- b. When only one antibiotic be used as a preservative in a biological product, the kind and maximum amount per ml. of such antibiotic shall be restricted to: Amphotericin B 2.5 µg; Nystatin 30 units; Mycostatin 30µg; Penicillin 30 units; Streptomycin 30 µg; polymixin 30µg ; neomycin 30 µg; Gentamycin 30 µg

### 8.2 Permitted combinations

1. Penicillin and streptomycin.
2. Either amphotericin B or nystatin, but not both, may be used with one of the other antibiotics listed in paragraph (b) of this section, or with a combination of penicillin and streptomycin, or with a combination of polymyxin B and neomycin.
3. The maximum amount of each antibiotic in a combination shall be the amount prescribed for such antibiotic in paragraph (b) of this section.
- c. Antibiotics used in virus seed stock purification are not restricted as to kind or amounts provided carryover into the final product is controlled and specified in outlines of production.

## 9. Labeling

All biological products should be clearly identified by labels. The labels used must remain permanently attached to the containers under all storage conditions and an area of the container should be left uncovered to allow inspection of the contents. If the final container is not suitable for labeling (for example a capillary tube), it should be in a labeled package.

The information given on the label on the container and the label on the package should be approved by the national control authority.

#### The label on the container should show:

- the name of the drug product
- a list of active ingredients and the amount of each present
- the batch or final lot number assigned by the manufacturer
- the expiration date
- recommended storage conditions
- direction for use and warning and precautions that may be necessary
- the name and address of the manufacturer or the company

The label on the package should show at least the nature and amount of any preservative or additive in the product. The leaflet in the package should provide instructions for the use of the product, and mention and contraindications or potential adverse reactions.

## 10. Storage and handling

Biological products at licensed establishments should be protected at all times against improper storage and handling. Completed product should be kept under refrigeration at 35 °to 45 °F. (2 °to 7 °C.) unless the inherent nature of the product makes storage at a different temperature advisable, in which case, the proper storage temperature shall be specified in the filed Outline of Production. All biological products to be shipped or delivered should be securely packed.

## 11. Expiration date determination

Unless otherwise provided for in a Standard Requirement of filed Outline of Production, the expiration date for each product shall be computed from the date of the initiation of the potency test. Prior to licensure, stability of each fraction shall be determined by methods acceptable to Animal and Plant Health Inspection Service. Expiration dates based on this stability data shall be confirmed as follows:

- a. *Products consisting of viable organisms.* Each serial shall be tested for potency at release and at the approximate expiration date until a statistically valid stability record has been established.
- b. *Nonviable biological products.* Each serial presented in support of licensure shall be tested for potency at release and at or after the dating requested.
- c. Subsequent changes in the dating period for a product may be granted, based on statistically valid data submitted to support a revision of the Outline of Production.

## 12. Quality of biotechnological products <sup>(8)</sup>

### 12.1 Stability testing of biotechnological products

The evaluation of stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies.

Appropriate physico-chemical, biochemical and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program whenever purity and molecular characteristics of the product permit use of these methodologies.

During manufacture of biotechnological/biological products, the quality and control of certain intermediates may be critical to the production of the final product. In general, the manufacturer should identify intermediates and generate in-house data and process limits that assure their stability within the bounds of the developed process. While the use of pilot plant-scale data is permissible, the manufacturer should establish the suitability of such data using the manufacturing-scale process.

Stability information should be provided on at least three batches of final container product representative of that which will be used at manufacturing scale. Where possible, batches of final container product included in stability testing should be derived from different batches of bulk material. A minimum of six months data at the time of submission should be submitted in cases where storage periods greater than six months are requested. For

medicinal products with storage periods of less than six months, the minimum amount of stability data in the initial submission should be determined on a case by case basis.

On the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity and potency of the product will be detected.

At the time of submission, applicants should have validated the methods that comprise the stability-indicating profile and the data should be available for review.

When the intended use of a product is linked to a definable and measurable biological activity, testing for potency should be part of the stability studies. For the purpose of stability testing of the products described in this guideline, potency is the specific ability or capacity of a product to achieve its intended effect. It is based on the measurement of some attribute of the product and is determined by a suitable quantitative method.

In some biotechnological/biological products, potency is dependent upon the conjugation of the active substance(s) to a second moiety or binding to an adjuvant. Dissociation of the active substance(s) from the carrier used in conjugates or adjuvants should be examined in real-time/real-temperature studies (including conditions encountered during shipment).

The following product characteristics, though not specifically relating to biotechnological/biological products, should be monitored and reported for the medicinal product in its final container:

- Visual appearance of the product (colour and opacity for solutions/suspensions; colour, texture and dissolution time for powders), visible particulates in solutions or after the reconstitution of powders or lyophilised cakes, pH, and moisture level of powders and lyophilised products.
- Sterility testing or alternatives (e.g. container/closure integrity testing) should be performed at a minimum initially and at the end of the proposed shelf life.
- Additives (e.g. stabilisers, preservatives) or excipients may degrade during the dating period of the medicinal product. If there is any indication during preliminary stability studies that reaction or degradation of such materials adversely affect the quality of the medicinal product, these items may need to be monitored during the stability program.
- The container/closure has the potential to adversely affect the product and should be carefully evaluated.

## 12.2 FDA's role regarding biological products

FDA's regulatory authority for the approval of biologics resides in the Public Health Service Act (PHSA). However, biologics are also subject to regulation under the Federal Food, Drug, and Cosmetic Act (FD&C Act) because most biological products also meet the definition of "drugs" cited within this Act.

Similarly, some medical devices used to produce biologics are regulated by Center for Biologics Evaluation and Research (CBER) under the FD&C Act's Medical Device Amendments of 1976.



FDA also

- reviews new biological products and new indications and usage for already approved products in order to get biological products on the market for the treatment of known diseases
- helps protect against threats of emerging infectious diseases
- helps provide the public with information to promote the safe and appropriate use of biological products
- conducts inspections of plants that manufacture biologics before product approval is granted, and thereafter, on a regular basis
- monitors the safety of biological products after they are marketed

The PHS Act also

- allows FDA to approve biological products and immediately suspend licenses where there exists a danger to public health
- allows the agency to prepare or procure products in the event of shortages and critical public health needs
- enforces regulations to prevent the introduction or spread of communicable diseases within the country and between states

### **13. The responsibilities of a licensed biologics manufacturer**

The PHS Act requires individuals or companies who manufacture biologics for introduction into interstate commerce to hold a license for the products. These licenses are issued by FDA

Responsibilities of a licensed biologics manufacturer include

- complying with the appropriate laws and regulations relevant to their biologics license and identifying any changes needed to help ensure product quality
- reporting certain problems to FDA's Biological Product Deviation Reporting System
- reporting and correcting product problems within established timeframes
- recalling or stopping the manufacture of a product if a significant problem is detected

#### **13.1 Regulation and licensing of biological products**

The licensing of a vaccine or other biological product requires the issue of licenses for both the manufacturing establishment and the product. The approval or licensing of a manufacturing establishment for the production of biological products should be granted only if the manufacture complies with the relevant international or equivalent national standards for good manufacturing practice.

The normal procedure for the issue of a product licence consists of the following three stages:

- a. the manufacturing establishment and product licence applications are received from the manufacturer, screened for completeness, and then reviewed for evidence of compliance with good manufacturing practices and for safety, quality and efficacy by the authority's technical staff;

- b. the authority may perform laboratory tests, review reports of or perform pre-licensing inspections, and seek the advice of external experts on specific technical questions when deciding whether or not to authorize the marketing of the product;
- c. the formal administrative action to grant or refuse a licence is then taken by the designated authorized person.

The assessment of the product must be based on its safety, quality and efficacy when used as intended. However, the availability of the product may be dictated by national policy considerations, such as the national need for comparative efficacy and /or safety, or cost-effectiveness.

### **13.2 Renewal and variation of licences <sup>(9)</sup>**

The precise circumstances under which licence-holders are required to apply for a renewal or variation in a product licence differ from country to country and should be clearly defined by the national authority. In general, if a manufacturer wishes to vary the conditions of the approved licence to any significant extent, the variations must be submitted to the authority for approval. Significant changes might include changes in aspects of the manufacturing procedures or the facility, or in the product specifications, dosage forms or labeling. In many countries, re-registration, but not licence renewal, is required annually. In others, licences must be renewed every 5 or 7 years.

### **13.3 Post-licensing monitoring**

#### **13.3.1 Product release**

At the time a product is approved, the national control authority should decide what controls are to be applied to the release of batches of the product. This decision will be influenced by the nature of the product and the resources available for laboratory testing. Controls will usually be imposed on complex products, e.g. vaccines, and on those obtained by complex manufacturing procedures.

The *testing of samples of intermediate, bulk or final product* should confirm compliance with the requirements and agreed specifications. The nature and frequency of the tests to be carried out are decided by the national control authority.

The *evaluation of the manufacturer's protocols* for the manufacture and control of each batch will be undertaken by the national control authority. The critical review of batch protocols by the authority is a most important part of the control of biological products. The information provided should make it possible to review the manufacture and testing of each batch of a particular product, including all required in-process control tests on final products to confirm compliance with the approved specifications.

#### **13.3.2 Inspections**

Periodic inspection of the manufacturing facility should be carried out on behalf of the national control authority to assure continued compliance with good manufacturing practices and with the specifications established for the product at the time of approval. Records of complaints and reports of adverse reactions should be examined.

### 13.3.3 Post-licensing surveillance

Countries should establish a national system for post-licensing surveillance of biological products. Clinicians and other health workers should be encouraged to report to national control authorities and manufacturers any unexpected adverse events occurring after the administration of biological products.

## 14. Existing legal basis for approval of biologics

Two U.S. statutes apply to the regulation of biological products, the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 *et seq*) (FFDCA), and the Public Health Services Act (42 U.S.C. § 262) (PHSA). The U.S. regulations charge the FDA with the protection of public health in part by ensuring that human drugs and biological products are safe and effective. The FDA administers the FFDCA and PHSA (among other statutes). FFDCA applies to all drugs and medical devices, and PHSA applies to “biological products.” Marketing approval under the FFDCA is by means of a New Drug Application (NDA) while approval under the PHSA is by means of a Biologics License Application (BLA). Both drugs and biologics are subject to Investigational New Drug Application (INDA) regulations. Pre-clinical research on new compounds is carried out in a laboratory, using a wide variety of techniques. Promising candidates are then studied in animals, and, subsequently, various clinical studies in humans are carried out following strict guidelines:

**Phase I:** A small number of healthy volunteers is given the compound to determine mainly that the drug is safe for human use.

**Phase II:** A small number of patients is given the medicine to assess its efficacy and safety and to ensure that there are no unacceptable side-effects.

**Phase III:** A large number of patients, usually thousands, take the medicine under supervision over a defined period of time, with the results used to establish efficacy.

If the results show the drug to be efficacious and safe, the data are presented to the FDA. The FDA reviews the data, and if the data is acceptable, a marketing authorization is issued. Alternatively, the FDA may request additional studies or reject the application.

Following the grant of marketing authorization, the drug product is studied in large numbers of patients in hospitals and clinics to further assess its clinical effectiveness. This stage is called Phase IV or post-marketing study. Safety Assessment of Marketed Medicines (SAMM) studies help identify any unforeseen side effects.

In order to be marketed, a biologic requires only proper labeling and an approved BLA that indicates the product has been determined safe, pure, and potent, and that the manufacturing facilities meet the requirements to ensure safety, purity, and potency. Though biologics have traditionally been subject to much more scrutiny in manufacturing than drugs, those differences are being eroded.

Biologics have been approved under FFDCA and PHSA, thus, both NDA and BLA applications have been submitted for biologics. The exceptions are glucagon and follistim that were approved under § 505(b)(2), and insulin, which was approved under its own statute for a time. The default

approval pathway for biologics now is a BLA, unless the product is a hormone, in which case §505(b) is used.

## 15. Challenges for the coming years

Biological products often represent the cutting edge of medical science and research. Also known as biologics, these products replicate natural substances such as enzymes, antibodies, or hormones in our bodies.

Biologics are made from a variety of natural resources-human, animal, and microorganism-and may be produced by biotechnology methods.

Gene-based and cellular biologics, at the forefront of biomedical research today, may make it possible to treat a variety of medical conditions, including illnesses for which no other treatments are available. Research continues to develop more biologics that will help treat medical conditions or add to existing treatment options.

New therapies such as xenotransplantation (the transplantation of animal cells, tissues or organs into a human) offer hope for an added source of organs. "One of the challenges in using animal tissues or organs is how do you test for what's infectious? Our biggest challenge over the next century or maybe even less than a century is going to really be to understand this, and how can we make sure that when we repair, replace, restore, regenerate, that it's done in a safe manner?"

With continued advancements in medical research and medical technology, CBER will face new challenges - not just scientific and regulatory, but legal and ethical. In the 21st Century, CBER will continue its rich tradition of melding strong scientific research with innovative regulations that ensure timely access to safe and effective biological products.

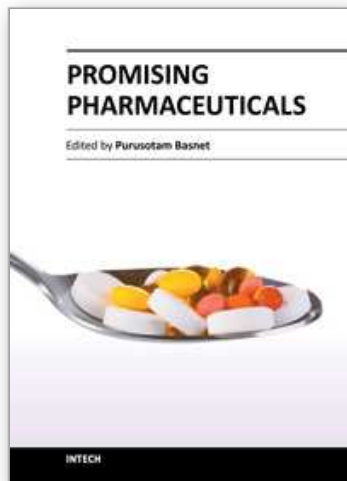
CBER's major challenge for the 21st Century is to expedite approval of biological products for use by the public while, at the same time, maintain high levels of safety and quality. CBER's careful risk management of approved products already in the market also plays an important and essential role in protecting the public health.

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From the dawn of civilization, humans have been dreaming of happy, healthy and long-life. Our life expectancy is twice longer than 100 years ago. We know more about the diseases. Therefore we have developed new drugs to fight against them. The demand for drugs was so high that we developed Pharma industries. Although Pharma industries took responsibility of producing the needed drugs and gave us a quality of life, misuse of drugs brought further complication. Therefore, discovery, production, distribution, and the phase of administration of patients' quality assurance has to be controlled with a technological procedure and tight regulations to make the system as effective as possible for the benefit of human health. Our book provides selected but vital information on the sources, tools, technologies and regulations regarding the current status of medicine development.

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### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
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### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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