BIOTECHNOLOGY INSPECTION GUIDE REFERENCE MATERIALS AND TRAINING AIDS

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BIOTECHNOLOGY INSPECTION GUIDE FOR INVESTIGATORS

INTRODUCTION

Biotechnology, defined as "the application of biological systems and organisms to technical and industrial processes", is not new. The use of yeast to ferment grain into alcohol has been ongoing for centuries. Likewise, farmers and breeders use a form of "genetic engineering" to produce improved crops and stock by selecting for desirable characteristics in plants and animals. Only recently have "new" biotechnology techniques enabled scientists to modify an organism's genetic material at the cellular or molecular level. These methods are more precise, but the results are similar to those produced with classical genetic techniques involving whole organisms. Biotechnology - derived products (BDP) used in this Guide refers to those products derived from the new biotechnology techniques.

The development of BDP and the inspection of the manufacture and control of these products offer many challenges. Because of the diversified manufacturing and control processes that are continuously being developed, considerable effort is required to achieve a level of technical competence to inspect these operations. Although the level of technology is increasing, it must be recognized that the same basic regulations and requirements are applicable to the manufacture and control of biotechnically- derived substances and devices as for "conventionally" manufactured products.

The same criteria have been used for many years in the inspection of manufacturers of antibiotics, enzymes and other high molecular weight substances including insulin, heparin, and albumin. This Guide will address some of the basic problems identified during inspections of manufacturers of BDP. Production systems may include animals, cell clones (e.g. hybridomas), mammalian and insect cell cultures, yeast, and bacteria or combinations of these systems.

A. Objective

The major objective of an inspection is to determine whether the manufacturer is operating in a state of control and in compliance with the laws and regulations. The firm's commitment to quality is vital, regardless of the type of company or product that is being manufactured.

One important aspect of an inspection is to identify defective product, non-conforming product and system failures. The way in which companies investigate and correct

objectionable conditions and deficient manufacturing and control systems is an important part of an inspection and typically illustrates the level of quality within a firm.

B. Inspection Team

As with pre-approval inspections of human and veterinary drugs and devices, it is recommended that inspections of biotech firms be conducted by teams, with the lead Investigator being responsible for the overall conduct of the inspection. Analysts (Chemists and/or Microbiologists), Computer Specialists, and Engineers can participate in all or parts of the inspection. Prior to the inspection, the "team" should discuss the duties of the particular team members.

C. Inspection Approach

The biotech inspection is also a product-specific inspection. As with any inspection, coverage is generally an audit and is not all inclusive. Thus, validation data for all systems, processes, controls and test procedures cannot be reviewed. However, specific detailed coverage should be given to a few systems or controls. A flow chart from the application document or from the firm should be obtained prior to or early in the inspection and the specific manufacturing steps should be reviewed with the manufacturer's responsible personnel.

CELL CULTURE AND FERMENTATION

A. Master Cell Bank and Working Cell Bank

The starting material for manufacturing BDP includes the bacterial, yeast, insect or mammalian cell culture which expresses the protein product or monoclonal antibody of interest. The cell seed lot system is used by manufacturers to assure identity and purity of the starting raw material. A cell seed lot consists of aliquots of a single culture. The master cell bank (MCB) is derived from a single colony (bacteria, yeast) or a single eukaryotic cell, stored cryogenically to assure genetic stability and is composed of sufficient ampules of culture to provide source material for the working cell bank (WCB). The WCB is defined as a quantity of cells derived from one or more ampules of the MCB, stored cryogenically and used to initiate the production batch.

1. Origin and History

Because genetic stability of the cell bank during storage and propagation is a major concern, it is important to know the origin and history (number of passages) of both the MCB and WCB. A MCB ampule is kept frozen or lyophilized and only used once. Occasionally, a new MCB may be generated from a WCB. The new MCB should be tested and properly characterized. For biological products, a product license application or amendment must be submitted and approved before a new MCB can be generated from a WCB.

2. Characterization and Qualifying Tests

Information about the construction of the expression vector, the fragment containing the genetic material that encodes the desired product, and the relevant genotype and phenotype of the host cell(s) are submitted as part of a product application. The major concerns of biological systems are genetic stability of cell banks during production and

storage, contaminating microorganisms, and the presence of endogenous viruses in some mammalian cell lines. As part of the application document, manufacturers will submit a description of all tests performed to characterize and qualify a cell bank.

It must be emphasized that the tests required to characterize a cell bank will depend on the intended use of the final product, the host/expression system and the method of production including the techniques employed for purification of the product. In addition, the types of tests may change as technology advances.

The MCB is rigorously tested. The following tests are generally performed, but are not limited to:

- a. Genotypic characterization by DNA fingerprinting
- b. Phenotypic characterization by nutrient requirements, isoenzyme analysis, growth and morphological characteristics
- c. Reproducible production of desired product
- d. Molecular characterization of vector/cloned fragment by restriction enzyme mapping, sequence analysis
- e. Assays to detect viral contamination
- f. Reverse transcriptase assay to detect retroviruses
- g. Sterility test and mycoplasma test to detect other microbial contaminants

It is not necessary to test the WCB as extensively as the MCB; however, limited characterization of a WCB is necessary. The following tests are generally performed on the WCB, but this list is not inclusive:

- a. Phenotypic characterization
- b. Restriction enzyme mapping
- c. Sterility and mycoplasma testing
- d. Testing the reproducible production of desired product

3. Storage Conditions and Maintenance

The MCB and WCB must be stored in conditions that assure genetic stability. Generally, cells stored in liquid nitrogen or its vapor phase are stable longer than cells stored at -70 C. In addition, it is recommended that the MCB and WCB be stored in more than one location in the event that a freezer malfunctions.

4. Inspection Approach

a. Verify that the written procedures reflect accurately what is submitted in the application document. b. Determine that batch records follow written procedures. c. Determine the identity and traceability of the MCB/WCB. d. Check the conditions of storage at each location. e. Check the accessability to MCB and WCB. Determine if there are security measures and accountability logs. f. Document any samples of the MCB/WCB that failed to meet all specifications, especially if they have been released for use.

B. Media

1. Raw Materials

Raw materials used to prepare the media must be carefully selected to provide the

proper rate of growth and the essential nutrients for the organisms producing the desired product. Raw materials should not contain any undesirable and toxic components that may be carried through the cell culture, fermentation and the purification process to the finished product. Water is an important component of the media and the quality of the water will depend on the recombinant system used, the phase of manufacture and intended use of the product. Raw materials considered to be similar when supplied by a different vendor should meet acceptance criteria before use. In addition, a small scale pilot run followed by a full-scale production run is recommended when raw materials from a different vendor are used, to assure that growth parameters, yield, and final product purification remain the same.

2. Bovine Serum

Most mammalian cell cultures require serum for growth. Frequently, serum is a source of contamination by adventitious organisms, especially mycoplasma, and firms must take precautions to assure sterility of the serum. Some Brazilian bovine serum (BBS) have been contaminated with hoof and mouth disease. Also make sure that the serum is indeed bovine serum and not derived from human sources.

There is an additional concern that bovine serum may be contaminated with bovine spongiform encephalopathy (BSE) agent. BSE is a slow disease which has been detected in herds from the United Kingdom. Because there is no sensitive in vitro assay to detect the presence of this agent, it is essential that the manufacturers know the source of the serum and request certification that the serum does not come from areas where BSE is endemic. Other potential sources of BSE may be proteases and other enzymes derived from bovine sources. Biological product manufacturers have been requested to determine the origin of these materials used in manufacturing.

3. Sterilization

The media used must be sterilized. A sterilized in place (SIP) or a continuous sterilizing system (CSS) process is usually used. Any nutrients or chemicals added beyond this point must be sterile. Air lines must include sterile filters.

4. Inspection Approach

- a. Determine the source of serum.
- b. Confirm that the sterilization cycle has been properly validated to ensure that the media will be sterile.
- c. Verify that all raw materials have been tested by quality control. Determine the origin of all bovine material.
- d. Document instances where the media failed to meet all specifications.
- e. Verify that expired raw materials have not been used in manufacture.
- f. Check that media and other additives have been properly stored.

C. Culture Growth

1. Inoculation and Aseptic Transfer

Bioreactor inoculation, transfer, and harvesting operations must be done using

validated aseptic techniques. Additions or withdrawals from industrial bioreactors are generally done through steam sterilized lines and steam-lock assemblies. Steam may be left on in situations for which the heating of the line or bioreactor vessel wall would not be harmful to the culture.

2. Monitoring of Growth Parameters and Control

It is important for a bioreactor system to be closely monitored and tightly controlled to achieve the proper and efficient expression of the desired product. The parameters for the fermentation process must be specified and monitored. These may include: growth rate, pH, waste byproduct level, viscosity, addition of chemicals, density, mixing, aeration, foaming, etc. Other factors which may affect the finished product include shear forces, process-generated heat, and effectiveness of seals and gaskets.

Many growth parameters can influence protein production. Some of these factors may affect deamidation, isopeptide formation, or host cell proteolytic processing. Although nutrient-deficient media are used as a selection mechanism in certain cases, media deficient in certain amino acids may cause substitutions. For example, when E. coli is starved of methionine and/or leucine while growing, the organism will synthesize norleucine and incorporate it in a position normally occupied by methionine, yielding an analogue of the wild-type protein. The presence of these closely related products will be difficult to separate chromatographically; this may have implications both for the application of release specifications and for the effectiveness of the product purification process.

Computer programs used to control the course of fermentation, data logging, and data reduction and analysis should be validated.

3. Containment

Bioreactor systems designed for recombinant microorganisms require not only that a pure culture is maintained, but also that the culture be contained within the systems. The containment can be achieved by the proper choice of a host-vector system that is less capable of surviving outside a laboratory environment and by physical means, when this is considered necessary.

Revision of Appendix K of the NIH Guidelines (1991) reflects a formalization of suitable containment practices and facilities for the conduct of large-scale experiments involving recombinant DNA-derived industrial microorganisms. Appendix K replaces portions of Appendix G when quantities in excess of 10 liters of culture are involved in research or production. For large-scale research or production, four physical containment levels are established: GLSP, BL1-LS,BL2-LSand BL3-LS.

GLSP

(Good Large-ScalePractice) level of physical containment is recommended for large-scale research of production involving viable, nonpathogenic and nontoxigenic recombinant strains derived from host organisms that have an extended history or safe large scale use. The GLSP level of physical containment is recommended for organisms such as those that have built-in environmental limitations that permit optimum growth in the large scale setting but limited

survival without adverse consequences in the environment.

BL1-LS

(Biosafety Level 1 -Large Scale) level of physical containment is recommended for large-scaleresearch or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale.

BL2-LS

Level of physical containment is required for large-scaleresearch or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale.

BL3-LS

Level of physical containment is required for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale.

No provisions are made at this time for large-scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale.

4. Contamination Control

There should be no adventitious organisms in the system during cell growth. Contaminating organisms in the bioreactor may adversely affect both the product yield and the ability of the downstream process to correctly separate and purify the desired protein. The presence or effects of contaminating organisms in the bioreactor can be detected in a number of ways -growth rate, culture purity, bacteriophage assay, and fatty acid profile.

5. Inspection Approach:

- a. Verify that there are written procedures to assure absence of adventitious agents and criteria established to reject contaminated runs.
- b. Review cell growth records and verify that the production run parameters are consistent with the established pattern. c. Review written procedures to determine what investigations and corrective actions will be performed in the event that growth parameters exceed established limits.
- c. Review written procedures to determine what investigations and corrective actions will be performed in the event that growth parameters exceed established limits.
- d. Assure proper aseptic techniques during cell in5. Inspection Approach:
- e. Determine that appropriate in-processcontrols are utilized prior to further processing.

ASCITES PRODUCTION

Monoclonal antibodies can be produced in cell culture or in the abdomen of a mouse. There are unique critical points in ascites production that should be examined.

A. Mouse Colony

1. Characterization and Control of the Mouse Colony

   Characterization and control of the mouse colony used to produce ascites are critical. The type of mouse, source, vendor, and certification that the colony is free from viral disease should be recorded. Animals used in production should be quarantined and inspected daily for a period of a week to assure that mice remain in good health and meet all acceptance criteria. Mice should be observed daily during production. There should be strict SOPs to remove any mouse that does not remain in overt good health during quarantine and production.

2. Animal Quarters/Environmental Controls

   Strict attention to the animal quarters is necessary to assure that mice remain free from disease, especially viruses that commonly infect colonies. To prevent contamination of colonies housed in different rooms, it is a good idea for people to wear disposable gloves, lab coats, head coverings and booties so that these items can be changed before entering another room. Animal quarters and cages must be kept in sanitary condition.

B. Manufacturing Processes

1. Animal Identification

   Individual mice must be identified so that a record of the number of times a mouse has been tapped and the amount of fluid obtained from each tap can be accurately maintained.

2. Tapping Procedure

   Injection of mice and removal of ascites fluid should be done in a clean environment such as under a unidirectional hood or at a station that will protect the mice from infectious agents. There should be written procedures that describes the tapping process. A different needle for each mouse is recommended to prevent the possibility of transmitting infections from other mice. There should also be written procedures for handling needles with strict adherence to biohazardous containment to prevent cross contamination.

3. Storage and Pooling of Ascites

   In addition, there should be written procedures that describes the storage temperatures and conditions before processing. This will include establishing a time limitation on collection and processing. Pooling of ascites is acceptable, but there should be written procedures that describes how a pool is made (how many and which mice make up a pool) and records must accurately reflect what makes up the pool. Thus, if it is discovered that an animal is infected, the records will reflect which pool contains the infected animal's ascites fluid.

4. Purification

   Pristane is sometimes used to prime mice and enhance ascites production. For parenteral products, the firm must demonstrate that the purification process will

remove pristane. This should not be a concern for products used in in vitro diagnostic devices.

5. Inspection Approach

- a. Review SOPs to assure adequate controls for quarantining and accepting mice, housing and caring for mice, mice identification, maintaining a clean environment to prevent viral infection of colony, disposing unhealthy mice, and processing of ascites fluid.
- b. Review records to assure that animals are in good health and are observed daily during the quarantine period and production.
- c. Verify the presence of a qualified animal care staff.

EXTRACTION, ISOLATION AND PURIFICATION

A. Introduction

Once the fermentation process is completed, the desired product is separated, and if necessary, refolded to restore configurational integrity, and purified. For recovery of intracellular proteins, cells must be disrupted after fermentation. This is done by chemical, enzymatic or physical methods. Following disruption, cellular debris can be removed by centrifugation or filtration. For recovery of extracellular protein, the primary separation of product from producing organisms is accomplished by centrifugation or membrane filtration. Initial separation methods, such as ammonium sulfate precipitation and aqueous two-phase separation, can be employed following centrifugation to concentrate the products. Further purification steps primarily involve chromatographic methods to remove impurities and bring the product closer to final specifications.

B. Process Types

- 1. Extraction and Isolation
  - a. Filtration -Ultrafiltration is commonly used to remove the desired product from the cell debris. The porosity of the membrane filter is calibrated to a specific molecular weight, allowing molecules below that weight to pass through while retaining molecules above that weight.
  - b. Centrifugation -Centrifugation can be open or closed. The adequacy of the environment must be evaluated for open centrifugation.
- 2. Purification -The purification process is primarily achieved by one or more column chromatography techniques.
  - a. Affinity Chromatography
  - b. Ion-Exchange Chromatography (IEC)
  - c. Gel filtration
  - d. Hydrophobic Interaction Chromatography (HIC)
  - e. Reverse- Phase HPLC

C. Description/Written Procedures

All separation and purification steps should be described in detail and presented with flow charts. Adequate descriptions and specifications should be provided for all equipment, columns, reagents, buffers and expected yields. When applicable, written procedures should be compared with the application documents submitted to the Agency. In-process storage conditions and quality control assays should be reviewed.

D. Process Validation

FDA defined process validation in the May 1987 "Guideline on General Principles of Process Validation" as follows:

Validation -establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.

1. Documentation

   We expect to see documentation that justifies the process and demonstrates that the process works consistently. For biological products, all validation data are submitted and reviewed and the specifications are established and approved as part of the product licensing application (PLA).

   Manufacturers should have validation reports for the various key process steps. For example, if an ion-exchange column is used to remove endotoxins, there should be data documenting that this process is consistently effective. By determining endotoxin levels before and after processing, a manufacturer should be able to demonstrate the validity of this process. It is important to monitor the process before, during, and after to determine the efficiency of each key purification step. "Spiking" the preparation with a known amount of a contaminant to demonstrate its removal may be a useful method to validate the procedure.

2. Validation

   Typically, manufacturers develop purification processes on a small scale and determine the effectiveness of the particular processing step. When scale-up is performed, allowances must be made for several differences when compared with the laboratory-scale operation. Longer processing times can affect product quality adversely, since the product is exposed to conditions of buffer and temperature for longer periods. Product stability, under purification conditions, must be carefully defined. Manufacturers should define the limitations and effectiveness of the particular step. Process validation on the production size batch will then compare the effect of scale-up.Manufacturers may sometimes use development data on the small scale for validation. However, it is important that validation be performed on the production size batches.

   Process validation and/or reports for the validation of some of the purification processes should be reviewed. Additionally, the controls and tests used to assure the consistency of the process should also be reviewed.

   Often columns are regenerated to allow repeated use. Proper validation procedures should be performed and the process should be periodically monitored for chemical and microbial contamination.

3. Follow Up Investigations

   Manufacturers occasionally reject the product following the purification

process. As with other regulated products, it is expected that reports of investigations be complete and relate to other batches. For example, during one inspection it was noted that approximately six batches of a BDP were rejected because of low potency and high levels of impurities. The problem was attributed to a column and all of the batches processed on the column were rejected. It should be pointed out that any batch failing specifications should be investigated.

It is, therefore, important to identify defective product so that the specific manufacturing and control systems can be given more detailed inspectional coverage.

E. Process Water/Buffers/WFI

The quality of water should depend on the intended use of the finished product. For example, CBER requires Water for Injection (WFI) quality for process water. On the other hand, for in-vitro diagnostics purified water may suffice. For drugs, the quality of water required depends on the process. Also, because processing usually occurs cold or at room temperature, the self-sanitization of a hot WFI system at 75 to 80 C is lost.

For economic reasons, many of the biotech companies manufacture WFI by reverse osmosis rather than by distillation. Most of these systems have been found to be contaminated. Typically, they employ plastic pipe (PVC) and non- sealed storage tanks, which are difficult to sanitize. Any threads or drops in a cold system provide an area where microorganisms can lodge and multiply. Some of the systems employ a terminal sterilizing filter. However, the primary concern is endotoxins, and the terminal filter may merely serve to mask the true quality of the WFI used. The limitations of relying on a 0.1 ml sample of WFI for endotoxins from a system should also be recognized. The system should be designed to deliver high purity water, with the sample merely serving to assure that it is operating adequately. As with other WFI systems, if cold WFI water is needed, point-of-use heat exchangers can be used.

Buffers can be manufactured as sterile, non-pyrogenic solutions and stored in sterile containers. Some of the smaller facilities have purchased commercial sterile, non-pyrogenic buffer solutions.

The production and/or storage of non-sterile water that may be of reagent grade or used as a buffer should be evaluated from both a stability and microbiological aspect.

WFI systems for BDP are the same as WFI systems for other regulated products. As with other heat sensitive products, cold WFI is used for formulation. Cold systems are prone to contamination. The cold WFI should be monitored both for endotoxins and microorganisms. Validation data and reports of monitoring should be reviewed.

F. Plant Environment

Microbiological quality of the environment during various processing steps is a concern. As the process continues downstream, increased consideration should be given to environmental controls and monitoring. The environment and areas used for the isolation of the BDP should also be controlled to minimize microbiological and other foreign contaminants. The typical isolation of BDP should be of the same control as the environment used for the formulation of the solution prior to sterilization and filling.

CLEANING PROCEDURE

Validation of the cleaning procedures for the processing of equipment, including columns, should be carried out. This is especially critical for a multi-product facility. The manufacturer should have determined the degree of effectiveness of the cleaning procedure for each BDP or intermediate used in that particular piece of equipment.

Validation data should verify that the cleaning process will reduce the specific residues to an acceptable level. However, it may not be possible to remove absolutely every trace of material, even with a reasonable number of cleaning cycles. The permissible residue level, generally expressed in parts per million (ppm), should be justified by the manufacturer. Cleaning should remove endotoxins, bacteria, toxic elements, and contaminating proteins, while not adversely affecting the performance of the column. Specific inspectional coverage for cleaning should include:

A. Detailed Cleaning Procedure

There should be a written equipment cleaning procedure that provides details of what should be done and the materials to be utilized. Some manufacturers list the specific solvent for each BDP and intermediate.

For stationary vessels, often clean-in-place (CIP) apparatus may be encountered. For evaluation of these systems, diagrams will be necessary, along with identification of specific valves.

B. Sampling Plan

After cleaning, there should be some routine testing to assure that the surface has been cleaned to the validated level. One common method is the analysis of the final rinse water or solvent for the presence of the cleaning agents last used in that piece of equipment. There should always be direct determination of the residual substance.

C. Analytical Method/Cleaning Limits

Part of the answer to the question, "how clean is clean?", is, "how good is your analytical system?" The sensitivity of modern analytical apparatus has lowered some detection thresholds below parts per million (ppm), down to parts per billion (ppb).

The residue limits established for each piece of apparatus should be practical, achievable, and verifiable. When reviewing these limits, ascertain the rationale for establishment at that level. The manufacturer should be able to document, by means of data, that the residual level permitted has a scientifically sound basis.

Another factor to consider is the possible non-uniform distribution of the residue on a piece of equipment. The actual average residue concentration may be more than the level detected.

PROCESSING AND FILLING

A. Processing

Most BDP cannot be terminally sterilized and must be manufactured by aseptic processing. The presence of process related contaminants in a product or device is chiefly a safety issue. The sources of contaminants are primarily the cell substrate (DNA, host cell proteins, and other cellular constituents, viruses), the media (proteins, sera, and additives) and the purification process (process related chemicals, and product related impurities).

Because of stability considerations, most BDP are either refrigerated or lyophilized. Low temperatures and low moisture content are also deterrents to microbiological proliferation. For the validation of aseptic processing of the non- preserved single dose biopharmaceutical (that is aseptically filled) stored at room temperature as a solution, the limitations of 0.1% media fill contamination rate should be recognized.

Media fill data and validation of the aseptic manufacturing process should be reviewed during an inspection. Some BDP may not be very stable and may require gentle mixing and processing. Whereas double filtrations are relatively common for aseptically filled parenterals, single filtration at low pressures are usually performed for BDP. It is for this reason that manufacturing directions be specific, with maximum filtration pressures given.

The inspection should include a review of manufacturing directions in batch records to assure that they are complete and specific.

The environment and accessibility for the batching of the non-sterile BDP should be controlled. Because many of these products lack preservatives, inherent bacteriostatic, or fungistatic activity, bioburden before sterilization should be low and-the bioburden should be determined prior to sterilization of these bulk solutions and before filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). One concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of these products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be stored prior to sterilization. Good practice would also include limitations on the length of manufacturing time between formulation and sterilization.

B. In-Process Quality Control

In-process testing is an essential part of quality control and ensures that the actual, real-time performance of an operation is acceptable. Examples of in-process controls are: stream parameters, chromatography profiles, protein species and protein concentrations, bioactivity, bioburden, and endotoxin levels. This set of in-process controls and the selection of acceptance criteria require coordination with the results from the validation program.

C. Filling

The filling of BDP into ampules or vials presents many of the same problems as with the processing of conventional products. In established companies these issues are relatively routine. However, for the new BDP facility, attempting to develop and prove clinical effectiveness and safety along with validation of sterile operations, equipment and systems, can be a lengthy process, particularly if requirements are not clearly understood.

The batch size of a BDP, at least when initially produced, likely will be small. Because of the small batch size, filling lines may not be as automated as for other products typically filled in larger quantities. Thus, there is more involvement of people filling these products, particularly at some of the smaller, newer companies.

Problems that have been identified during filling include inadequate attire; deficient environmental monitoring programs; hand-stoppering of vials, particularly those that are to be lyophilized; and failure to validate some of the basic sterilization processes. Because of the active involvement of people in filling and aseptic manipulations, the number of persons involved in these operations should be minimized, and an environmental program should include an evaluation of microbiological samples taken from people working in aseptic processing areas. This program along with data should be reviewed during the inspection.

Another concern about product stability is the use of inert gas to displace oxygen during both the processing and filling of the solution. As with other products that may be sensitive to oxidation, limits for dissolved oxygen levels for the solution should be established. Likewise, validation of the filling operation should include parameters such as line speed and location of filling syringes with respect to closure, to assure minimal exposure to air (oxygen) for oxygen-sensitive products. In the absence of inert gas displacement, the manufacturer should be able to demonstrate that the product is not affected by oxygen. These data may be reviewed during an inspection (These data are evaluated as part of a Product Licensing Application (PLA) review).

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been observed that utilize an operator to place each stopper on top of the vial by hand. The concern is the immediate avenue of contamination offered by the operator. The observation of operators and active review of filling operations should be performed.

Another major concern with the filling operation of a lyophilized product is assurance of fill volumes. Obviously, a low fill would represent a subpotency in the vial. Unlike a powder or liquid fill, a low fill would not be readily apparent after lyophilization, particularly for a product where the active ingredient may be only a milligram. Because of the clinical significance, subpotency in a vial potentially can be a very serious situation, clinically.

Again, the inspection should include the observation and the review of filling operations, not only regarding aseptic practices, but also for fill uniformity.

D. Lyophilization

Many products are lyophilized for stability concerns. Unfortunately, GMP aspects of the design of lyophilizers have lagged behind the sterilization and control technology employed for other processing equipment. It is not surprising that many problems with the lyophilization process have been identified.

These problems are not limited to BDP but generally pertain to lyophilization of all products including BDP. A detailed discussion of lyophilization and controls can be found in Inspection Technical Guide No. 43, issued 4/18/86.

LABORATORY CONTROLS

During the inspection of the firm's laboratory facility, the following areas should be reviewed and any deficiencies should be documented:

A. Training

Laboratory personnel should be adequately trained for the jobs they are performing.

B. Equipment Maintenance/Calibration/Monitoring

Firms should have documentation and schedules for maintenance, calibration, and monitoring of laboratory equipment involved in the measurement, testing and storage of raw materials, product, samples, and reference reagents.

All laboratory methods should be validated with the equipment and reagents specified in the test methods. Changes in vendor and/or specifications of major equipment/reagents would require revalidation.

C. Method Validation

Firms should have raw data to support validation parameters in submitted applications.

D. Standard/Reference Material

Reference standards should be well characterized and documented, properly stored, secured, and utilized during testing.

E. Storage of Labile Components

Laboratory cultures and reagents, such as enzymes, antibodies, test reagents, etc., may degrade if not held under proper storage conditions.

F. Laboratory SOPs

Procedures should be written, applicable and followed. Quality control samples should be properly segregated and stored.

TESTING

The following tests may be applicable to component, in process, bulk and/or final product testing. The tests that are needed will depend on the process and the intended use of the product.

A. Quality

1. Color/Appearance/Clarity
2. Particulate Analysis
3. pH Determination
4. Moisture Content
5. Host Cell DNA

B. Identity

A single test for identity may not be sufficient. Confirmation is needed that the methods employed are validated. Availability of reference material should be checked. A comparison of the product to the reference preparation in a suitable bioassay will provide additional evidence relating to the identity and potency of the product.

Tests that may be encountered:

1. Peptide Mapping (reduced/non-reduced)
2. Gel Electrophoresis
   - SDS PAGE
   - Isoelectric Focusing (IEF)
   - Immunoelectrophoresis
3. 2-Dimensional Electrophoresis
4. Capillary Electrophoresis
5. HPLC (Chromographic Retention)
   - Immunosassay
   - ELISA
   - Western Blot
   - Radioimmunoassay
6. Amino Acid Analysis
7. Amino Acid Sequencing
8. Mass Spectroscopy
9. Molecular Weight (SDS PAGE)
10. Carbohydrate Composition Analysis (glycosylation)

C. Protein Concentration/Content

Tests that may be encountered:

1. Protein Quantitations
   - Lowry
   - Biuret Method
2. UV Spectrophotometry
3. HPLC
4. Amino Acid Analysis
   - *Partial Sequence Analysis

D. Purity

"Purity" means relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient or deleterious to the product. Purity includes, but is not limited to, relative freedom from residual moisture or other volatile substances and pyrogenic substances. Protein impurities are the most common contaminants. These may arise from the fermentation process, media or the host organism. Endogenous retroviruses may be present in hybridomas used for monoclonal antibody production. Specific testing for these constituents is imperative in in vivo products. Removal of extraneous antigenic proteins is essential to assure the safety and the effectiveness of the product.

Tests that may be encountered:

- 1. Tests for Protein Impurities:
  - a. Electrophoresis

        - SDS PAGE
        - IEF
        - 2-Dimensional Electrophoresis
    - b. Peptide Mapping
    - c. Multiantigen ELISA
    - d. HPLC Size Exclusion HPLC Reverse Phase HPLC
- 2. Tests for Non-Protein Impurities:
    - a. DNA Hybridization
    - b. HPLC
    - c. Pyrogen/Endotoxin Testing
        - U.S.P. Rabbit Pyrogen Test
        - Limulus Amebocyte Lysate (LAL) E
        - ndogenous Pyrogen Assay

Pyrogen Contamination - Pyrogenicity testing should be conducted by injection of rabbits with the final product or by the limulus amebocyte lysate (LAL) assay. The same criteria used for acceptance of the natural product should be used for the biotech product.

The presence of endotoxins in some in vitro diagnostic products may interfere with the performance of the device. Also, it is essential that in vivo products be tested for pyrogens. Certain biological pharmaceuticals are pyrogenic in humans despite having passed the LAL test and the rabbit pyrogen test. This phenomenon may be due to materials that appear to be pyrogenic only in humans. To attempt to predict whether human subjects will experience a pyrogenic response, an endogenous pyrogen assay is used. Human blood mononuclear cells are cultured in vitro with the final product, and the cell culture fluid is injected into rabbits. A fever in the rabbits indicates the product contains a substance that may be pyrogenic in humans.

Tests that may be encountered:

1. U.S.P. Rabbit Pyrogen Test
2. Limulus Amebocyte Lysate (LAL)
3. Assay Endogenous Pyrogen Assay

Viral Contamination - Tests for viral contamination should be appropriate to the cell substrate and culture conditions employed. Absence of detectable adventitious viruses contaminating the final product should be demonstrated.

Tests that may be encountered:

1. Cytopathic effect in several cell types
2. Hemabsorption Embryonated Egg Testing
3. Polymerase Chain Reaction (PCR)
4. Viral Antigen and Antibody Immunoassay
5. Mouse Antibody Production (MAP)

Nucleic Acid Contamination - Concern about nucleic acid impurities arises from the possibility of cellular transformation events in a recipient. Removal of nucleic acid at each step in the purification process may be demonstrated in pilot experiments by examining the extent of elimination of added host cell DNA. Such an analysis would provide the theoretical extent of the removal of nucleic acid during purification.

Direct analyses of nucleic acid in several production lots of the final product should be performed by hybridization analysis of immobilized contaminating nucleic acid utilizing appropriate probes, such as nick-translated host cell and vector DNA. Theoretical concerns regarding transforming DNA derived from the cell substrate will be minimized by the general reduction of contaminating nucleic acid.

Tests that may be encountered:

1. DNA Hybridization (Dot Blot)
2. Polymerase Chain Reaction (PCR)

Protein Contamination

Tests that may be encountered for product-related proteins:

1. SDS PAGE
2. PLC
3. IEF

Tests that may be encountered for foreign proteins:

1. Immunoassays
2. Radioimmunoassays
3. ELISA
4. Western Blot
5. SDS Page
6. 2-Dimensional Electrophoresis

Microbial Contamination - Appropriate tests should be conducted for microbial contamination that demonstrate the absence of detectable bacteria (aerobes and anaerobes), fungi, yeast, and mycoplasma, when applicable.

Tests that may be encountered:

1. U.S.P. Sterility Test
2. Heterotrophic Plate Count and Total Yeasts and Molds
3. Total Plate Count
4. Mycoplasma Test
5. LAL/Pyrogen

Chemical Contaminants - Other sources of contamination must be considered, e.g., allergens, petroleum oils, residual solvents, cleaning materials, column leachable materials, etc.

E. Potency (Activity)

"Potency" is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to produce a given result. Tests for potency should consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency. A reference preparation for biological activity should be established and used to determine the bioactivity of the final product. Note: Where applicable, in-house

biological potency standards should be cross-referenced against international (World Health Organization (WHO), National Institute of Biological Standards and Control (NIBSC)) or national (National Institutes of Health (NIH), National Cancer Institute (NCI), Food and Drug Administration (FDA)) reference standard preparations, or USP standards.

Tests that may be encountered:

1. Validated method of potency determination
   - Whole Animal Bioassays
   - Cell Culture Bioassays
   - Biochemical/Biophysical Assays
   - Receptor Based Immunoassays
2. Potency Limits
3. Identification of agents that may adversely affect potency
4. Evaluation of functional activity and antigen/antibody specificity
   - Various immunodiffusion methods (single/double)
   - Immunoblotting/Radio-or Enzyme-linked Immunoassays
5. HPLC-validated to correlate certain peaks to biological activity

F. Stability

"Stability" is the capacity of a product to remain within specifications established to ensure its identity, strength, quality, purity, safety, and effectiveness as a function of time. Studies to support the proposed dating period should be performed on the final product. Real-time stability data would be essential to support the proposed dating period. Testing might include stability of potency, pH, clarity, color, particulates, physiochemical stability, moisture and preservatives. Accelerated stability testing data may be used as supportive data. Accelerated testing or stress tests are studies designed to increase the ratio of chemical or physical degradation of a substance or product by using exaggerated storage conditions. The purpose is to determine kinetic parameters to predict the tentative expiration dating period. Stress testing of the product is frequently used to identify potential problems that may be encountered during storage and transportation and to provide an estimate of the expiration dating period. This should include a study of the effects of temperature fluctuations as appropriate for shipping and storage conditions. These tests should establish a valid dating period under realistic field conditions with the containers and closures intended for the marketed product.

Some relatively fragile biotechnically-derived proteins may require gentle mixing and processing and only a single filtration at low pressure. The manufacturing directions must be specific with maximum filtration pressures given in order to maintain stability in the final product. Products containing preservatives to control microbial contamination should have the preservative content monitored. This can be accomplished by performing microbial challenge tests (i.e. U.S.P. Antimicrobial Preservative Effectiveness Test) or by performing chemical assays for the preservative. Areas that should be addressed are:

- Effective monitoring of the stability test environment (i.e. light, temperature, humidity, residual moisture);

- Container/closure system used for bulk storage (i.e. extractables, chemical modification of protein, change in stopper formulations that may change extractable

profile);

- Identify materials that would cause product instability and test for presence of aggregation, denaturation, fragmentation, deamination, photolysis, and oxidation;

- Tests to determine aggregates or degradation products.

Tests that may be encountered:

1. SDS PAGE
2. IEF
3. HPLC
4. Ion Exchange Chromatography
5. Gel Filtration
6. Peptide Mapping
7. Spectrophotometric Methods
8. Potency Assays
9. Performance Testing
10. 2-Dimensional Electrophoresis

G. Batch To Batch Consistency

The basic criterion for determining that a manufacturer is producing a standardized and reliable product is the demonstration of lot-to-lot consistency with respect to certain predetermined release specifications. -

Uniformity: identity, purity, functional activity

- Stability: acceptable performance during shelf life, precision, sensitivity, specificity

ENVIRONMENTAL COVERAGE

Environmental/biocontainment coverage for biotechnology facilities should be conducted as part of regular GMP inspections, particularly pre-approval or pre-licensing inspections. FDA is responsible under the National Environmental Policy Act (NEPA) for ascertaining the environmental impact that may occur due to the manufacture, use, and disposal of FDA regulated products. No other federal or state regulatory agency can be informed by FDA of the existence of an unapproved product application. Consequently, FDA must also make sure that the product sponsor is conducting investigations safely.

A. Environmental Assessments

Typically, a product sponsor describes environmental control measures in environmental assessments (EAs) that are part of the product application. When the product is approved, the EA is released to the public. FDA must be able to verify the accuracy and the appropriateness of the information contained in the EA. The Investigator should have a copy of the firm's environmental assessment, addressing the manufacture of the product that is the subject of the GMP inspection. The EA should be requested from the originating office if it has not been provided.

B. Inspection Approach

1. Review the NIH Guidelines for Recombinant DNA Research (1987, 1988, 1991). Pay particular attention to Appendix K (1991), regarding the establishment of guidelines for the level of containment appropriate to Good Industrial Large Scale Practices (see references).

2. Determine that the equipment and controls described in the EA as part of the biocontainment and waste processing systems are validated to operate to the standards; the equipment is in place, is operating, and is properly maintained. Such equipment may include, for example, HEPA filters, spill collection tanks with heat or hypochlorite treatment, and diking around bioreactors and associated drains. SOPs should be in use for the cleanup of spills, for actions to be taken in the case of accidental exposure of personnel, for opening and closing of vessels, for sampling and sample handling, and for other procedures which involve breaching containment or where exposure to living cells may occur.

3. Determine if there is a workplace and/or environmental monitoring program designed to verify that organisms are subject to appropriate biocontainment practices. Review SOPs for the sampling, isolation, counting, and reporting of results. Obtain copies of relevant SOPs and monitoring data for inclusion in reports to headquarters.

4. Ask for and obtain copies of all federal, state and local permits governing emissions and occupational safety for the facility being inspected. Determine whether any of the permits have expired and whether there is any action pending relating to violations of the permits.

5. For facilities in foreign countries, the same procedures should be followed. Compliance with the requirement of the foreign country should be demonstrated.

APPENDIX:

A. FLOW CHART

[Graphic: Flow Chart]

B. TEST METHODS

Affinity Chromatography - A chromatography separation method based on a chemical interaction specific to the target species. Types of affinity methods are: biosorption -site recognition (e.g., monoclonal antibody, protein A); hydrophobic interaction -contacts between non-polar regions in aqueous solutions; dye-ligand specific binding of macromolecules to triazine and triphenylmethane dyes; metal chelate - matrix bound chelate complexes with target molecule by exchanging low melecular weight metal bound ligands; and covalent - disulfide bonding reversible under mild conditions.

Amino Acid Composition Analysis - Used to determine the amino acid composition and/or the protein quantity. A two step process involving a complete hydrolysis (chemical or enzymatic) of the protein into its component amino acids followed by chromatographic separation and quantitation via HPLC. The complete amino acid composition of the peptide or protein should include accurate values for methionine, cysteine, and tryptophan. The amino acid composition presented should be the average of at least three (3) separate hydrolysates of each lot number. Integral values for those

amino acid residues generally found in low quantities, such as tryptophan and/or methionine, could be obtained and used to support arguments of purity.

Amino Acid Sequencing - A partial sequencing (8- 15 residues) of amino acids within a protein or polypeptide by either amino- terminal or carboxy-terminal sequencing. This method is done to obtain information about the primary structure of the protein, its homogeneity, and the presence or absence of polypeptide cleavages. The sequence data determined by HPLC analysis is presented in tabular form and should include the total yield for every amino acid at each sequential cleavage cycle. Full sequence is often done by sequencing the peptide fragments isolated from HPLC fractionation.

Capillary Electrophoresis - Used as a complement to HPLC, particularly for peptide mapping. This technique is faster and will often separate peptides that coelute using HPLC. Separation is accomplished by relative mobility of the peptides in a buffer in response to an electrical current.

Carbohydrate Analysis - Used to determine the consistency of the composition of the covalently bound monosaccharides in glycoproteins. Unlike the polypeptide chain of the glycoprotein where production is controlled by the genetic code, the oligosaccharides are synthesized by posttranslational enzymes. Microheterogeneity of the carbohydrate chains is common. Determination can be accomplished on underivatized sugars after hydrolysis by HPLC separation with pulsed amperometric detection or by gas chromatography after derivatization.

Circular Dichroism - With optical rotary dispersion, one of the optical spectrophotometric methods used to determine secondary structure and to quantitate the specific structure forms (a- helix, B-pleated sheet, and random coil) within a protein. The resultant spectra are compared to that of the natural protein form or to the reference standard for the recombinant.

DNA Hybridization (Dot Blot) Analysis - Detection of DNA to the nanogram level using hybridization of cellular DNA with specific DNA probes. Manifestation can be by 32P- labeling, chemiluminescence, chromogenic or avidin- biotin assays.

Edman Degradation - A type of protein sequencing from the amino-terminus.

Electrophoresis - Methods in which molecules or molecular complexes are separated on the basis of their relative ability to migrate when placed in an electric field. An analyte is placed on an electrophoretic support, then separated by charge (isoelectric focusing) or by molecular weight (SDS-PAGE). Visualization is accomplished by staining of the protein with nonselective (Coomassie Blue) or selective (silver) staining techniques.

The dye-binding method using Coomassie blue is a quantifiable technique when a laser densitometer is used to read the gels. The silver stain method is much more sensitive and therefore used for detection of low levels of protein impurities, but due to variability of staining from protein to protein, it cannot be used for quantitation.

Two-dimensional Gel Electrophoresis - A type of electrophoresis in which proteins are separated first in one direction by charge followed by a size separation in the perpendicular direction.

Enzyme-linked Immunosorbent Assay (ELISA) - A multiantigen test for unknown

residual (host) cellular protein and confirmation of desired protein. It may be used to determine the potency of a product. It is extremely specific and sensitive, basically simple, and inexpensive. It requires a reference standard preparation of host cell protein impurities to serve as an immunogen for preparation of polyclonal antibodies used for the assay.

Endogenous Pyrogen Assay - An in vitro assay based on the release of endogenous pyrogen produced by endotoxin from human monocytes. This assay appears to be more sensitive than the USP Rabbit Pyrogen Test, but is much less sensitive than the LAL assay. It does have the advantage that it can detect all substances that cause a pyrogenic response from human monocytes.

Gel Permeation or Filtration (Size Exclusion) Chromatography (GPC, GFC or SEC) - A separation method based on the molecular size or the hydrodynamic volume of the components being separated. This can be accomplished with the proteins in their natural state or denatured with detergents.

High Performance Liquid Chromatography (HPLC) - An instrumental separation technique used to characterize or to determine the purity of a BDP by passing the product (or its component peptides or amino acids) in liquid form over a chromatographic column containing a solid support matrix. The mode of separation, i.e. reversed phase, ion exchange, gel filtration, or hydrophobic interaction, is determined by the column matrix and the mobile phase. Detection is usually by UV absorbance or by electrochemical means.

Hydrophobic Interaction Chromatography (HIC) - HIC is accomplished in high salt medium by binding the hydrophobic portions of a protein to a slightly hydrophobic surface containing such entities as phenyl, or short- chain hydrocarbons. The protein can be eluted in a decreasing salt gradient, with the most hydrophobic proteins eluting from the column last.

Immunoassay - A qualitative or quantitative assay technique based on the measure of interaction of high affinity antibody with antigen used to identify and quantify proteins.

Immunoblotting - A technique for transferring antibody/antigen from a gel to a nitrocellulose filter on which they can be complexed with their complementary antigen/antibody.

Immunodiffusion (single) - An identity diffusion technique whereby the product (antigen) is placed in a well cut into a medium such as agar containing its complementary antibody. The product diffuses into the medium forming a ring shaped precipitate whose density is a function of antigen concentration.

Immunodiffusion (double, Ouchterlony technique) - A technique in which an antigen and antibody are placed in two adjacent wells cut into a medium such as agar. As they diffuse through the medium, they form visible precipitation lines of antigen/antibody complexes at the point where the respective concentrations are at the optimum ratio for latice formation.

Ion Exchange Chromatography (IEC) - A gradient driven separation based on the charge of the protein and its relative affinity for the chemical backbone of the column. Anion/cation exchange is commonly used for proteins.

Isoelectric Focusing (IEF) - An electrophoretic method which separates proteins by their pI. They move through a pH gradient medium in an electric field until they are located at their isoelectric point where they carry no net charge. Prior to reaching their pI, protein mobility also depends upon size, conformation, steepness of pH gradient, and the voltage gradient. This method is used to detect incorrect or altered forms of a protein as well as protein impurities.

Limulus Amoebocyte Lysate Test (LAL) - A sensitive test for the presence of endotoxins using the ability of the endotoxin to cause a coagulation reaction in the blood of a horseshoe crab. The LAL test is easier, quicker, less costly and much more sensitive that the rabbit test, but it can detect only endotoxins and not all types of pyrogens and must therefore be thoroughly validated before being used to replace the USP Rabbit Pyrogen test. Various forms of the LAL test include a gel clot test, a colormetric test, a chromogenic test, and a turbidimetric test.

Mass Spectrometry - A technique useful in primary structure analysis by determining the molecular mass of peptides and small proteins. Often used with peptide mapping to identify variants in the peptide composition. Useful to locate disulfide bonds and to identify post- translational modifications.

Northern Blot - Technique for transferring RNA fragments from an agarose gel to a nitrocellulose filter on which they can be hybridized to a complementary DNA.

Peptide Mapping - A powerful technique which involves the breakdown of proteins into peptides using highly specific enzymes. The enzymes cleave the proteins at predictable and reproducible amino acid sites and the resultant peptides are separated via HPLC or electrophoresis. A sample peptide map is compared to a map done on a reference sample as a confirmational step in the identity profiling of a product. It is also used for confirmation of disulfide bonds, location of carbohydrate attachment, sequence analysis, and for identification of impurities and protein degradation.

Polymerase Chain Reaction (PCR) - In vitro technique for amplifying nucleic acid. The technique involves a series of repeated cycles of high temperature denaturation, low temperature oligonucleotide primer annealing and intermediate temperature chain extension. Nucleic acid can be amplified a million- fold after 25- 30 cycles.

Protein Quantification - Quantitation of the total amount of protein can be done by a number of assays. There is no one method that is better than the rest; each has its own disadvantages ranging from the amount of protein required to do the test to a problem with variability between proteins. Some of the types include Lowry, Bicinchonic Acid (BCA), Bradford, Biuret, Kjeldahl, Ultraviolet spectroscopy.

Protein Sequencing - (See Amino Acid Sequencing).

Rabbit Pyrogen Test. U.S.P. - An assay for the presence of pyrogens (not restricted to endotoxins as is the LAL test) involving the injection of the test material into rabbits that are well controlled and of known history. The rabbits are then monitored for a rise in temperature over a period of three hours.

Radioimmunoassay (RIA) - A generic term for immunoassays having a radioactive label (tag) on either the antigen or antibody. Common labels include I125 and H3 which are used for assay detection and quantitation. Classical RIA's are competitive binding assays where the antigen and tagged antigen compete for a limited fixed

number of binding sites on the antibody. The antibody bound tagged complex is inversely proportional to the concentration of the antigen.

Reverse Phase Chromatography - A chromatographical separation method based on a column stationary phase coated to give non- polar hydrophobic surface. Analyte retention is proportional to hydrophobic reactions between solute and surface. Retention is roughly proportional to the length of the bonded carbon chain.

SDS PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) - An electrophoretic separation of proteins based on their molecular weights. A uniform net negative charge is imposed on the molecules by the addition of SDS. Under these conditions, migration toward the anode through a gel matrix allows separation via size, not charge, with the smaller molecules migrating the longest distance. This technique is not reliable for sizes below a MW of ca. 8000.

Proteins are observed via Coomassie blue or silver staining or can be further transferred to membranes for antigen/antibody specificity testing.

Southern Blot - Technique for transferring DNA fragments from an agarose gel to a nitrocellulose filter on which they can be hybridized to a complementary DNA.

UV Spectroscopy - A quantitation technique for proteins using their distinctive absorption spectra due to the presence of side- chain chromophores (phenylalanine, tryptophan, and tyrosine). Since this absorbance is linear, highly purified proteins can be quantitated by calculations using their molar extinction coefficient.

Western Blot - This test is used to detect contaminating cell substrates and to evaluate recombinant polypeptides. After electrophoretic separation, the negatively charged proteins (the antigens) are electrophoretically transferred from the polyacrylamide gel onto a nitrocellulose membrane positioned on the anode side of the gel. Following incubation of the membrane with a specific antibody, they are labeled with another anti- antibody for detection.

C. GLOSSARY

ADVENTITIOUS ORGANISM - Bacteria, yeast, mold, mycoplasma or viruses that can potentially contaminate prokaryote or eukaryote cells used in production. Potential sources of adventitious organisms include the serum used in cell culture media, persistently or latently infected cells, or the environment.

AFFINITY - The thermodynamic quantity defining the energy interaction or binding of two molecules, usually that of antibody with its corresponding antigenic determinant.

ANTIBODY (IMMUNOGLOBULIN) - A protein molecule having a characteristic structure consisting of two types of peptide chains: heavy (H) and light (L). Antibodies contain areas (binding sites) that specifically fit to and can bind to its corresponding determinant site on an antigen, which has induced the production of that antibody by the B- lymphocytes and plasma cells in a living species.

ANTIGEN - Substance, usually a foreign protein or carbohydrate, which when introduced into a organism, activates specific receptors on the surface immunocompetent T and B lymphocytes. After interaction between antigen and receptors, there usually will be induction of an immune response, i.e. production of

antibodies capable of reacting specifically with determinant sites on the antigen.

ANTIGENIC DETERMINANT - The specific part of a structure of an antigen which will induce an immune response, i.e. will fit to the receptors on T and B lymphocytes and will also be able to react with the antibodies produced.

ANTISERUM - Blood serum which contains antibodies against a particular antigen (or immunogen). This frequently means serum from an animal that has been inoculated with the antigen.

ASCITES - Liquid accumulations in the peritoneal cavity. Monoclonal antibodies can be purified from the ascites of mice that carry a transplanted hybridoma.

ASSOCIATION CONSTANT - A reaction between antibody and its determinant which comprises a measure of affinity. The constant is quantitated by mass action law rate constants for association and for dissociation. AUTORADIOGRAPHY - Detection of radioactively labelled molecules on X- ray film.

AVIDITY - The total binding strength between all available binding sites of an antibody molecule and the corresponding determinants present on antigen.

BACTERIOPHAGE - A virus that attacks bacteria. The lambda bacteriophage is frequently used as a vector in recombinant gene experiments.

BINDING SITE - The part of the antibody molecule that will specifically bind antigen.

BIOACTIVITY - The level of specific activity or potency as determined by animal model, cell culture, or in vitro biochemical assay.

BIOLOGICAL CONTAINMENT - Charhcteristics of an organism that limit its survival and/or multiplication in the environment.

BIOLOGICAL RESPONSE MODIFIER - Generic term for hormones, neuroactive compounds, and immunoreactive compounds that act at the cellular level; many are possible candidates for biotechnological production.

BIOREACTOR - A vessel in which the central reactions of a biotechnological process takes place. Typically the vessel contains microbes grown under controlled conditions of temperature, aeration, mixing, acidity and sterility.

BIOSENSORS - The powerful recognition systems of biological chemicals (enzymes, antibodies, DNA) are coupled to microelectronics to enable rapid, accurate low- level detection of such substances as sugars and proteins (such as hormones) in body fluids, pollutants in water and gases in air.

CALIBRATOR - A term in clinical chemistry commonly referring to the standard used to "calibrate" an instrument or used in construction of a standard (calibrator) curve.

CELL CULTURE - The in- vitro growth of cells isolated from multicellular organisms. These cells are usually of one type.

CELL DIFFERENTIATION - The process whereby descendants of a common parental cell achieve and maintain specialization of structure and function.

CELL FUSION - The formation of a hybrid cell with nuclei and cytoplasm from different cells, produced by fusing two cells of the same or different species.

CELL LINE - Cells that acquire the ability to multiply indefinitely in- vitro.

CHEMOTAXIS - Net oriented movement in a concentration gradient of certain compounds. Various sugars and amino acids can serve as attractants while some substances such as acid or alkali serve as repellants in microbial chemotaxis. White blood cells and macrophages demonstrate chemotactic movement in the presence of bacterial products, complement proteins and antigen activated T cells to contribute to the local inflammatory reaction and resistance to pathogens.

CISTRON - The smallest unit of genetic material which is responsible for the synthesis of a specific polypeptide.

CLONE - A cell line stemming from a single ancestral cell and normally expressing all the same genes. If this is a B lymphocyte clone, they will normally produce identical antibodies, i.e. monoclonal antibodies.

CODON - Group of three nucleotide bases in DNA or RNA that determines the composition of one amino acid in "building" a protein and also can code for chain termination.

COHESIVE TERMINI - DNA molecule with single- stranded ends with exposed (cohesive) complementary bases.

COMPLEMENTARY DNA (cDNA) - DNA that is complementary to messenger RNA; used for cloning or as a probe in DNA hybridization studies.

COSMID - A vector that is similar to a plasmid but it also contains the cohesive sites (cos site) of bacteriophage lambda to permit insertion of large fragments of DNA and in vitro packaging into a phage.

CROSS REACTION - Antibodies against an antigen A can react with other antigens if the latter has one or more determinants in common with the determinants present on the antigen A or carry one or more determinants that are structurally very similar to the determinants present on antigen A.

CYTOKINE - Small, non- immunoglobulin proteins produced by monocytes and lymphocytes that serve as intercellular communicators after binding to specific receptors on the responding cells. Cytokines regulate a variety of biological activities.

CYTOPATHIC EFFECT - Morphological alterations of cell lines produced when cells are infected with a virus. Examples of cytopathic effects include cell rounding and clumping, fusion of cell membranes, enlargement or elongation of cells, or lysis of cells.

CYTOTOXIC - Damaging to cells.

DENATURATION - Unfolding of a protein molecule into a generally bio- inactive form. Also the disruption of DNA duplex into two separate strands.

DNA (DEOXYRIBONUCLEIC ACID) - The basic biochemical component of the chromosomes and the support of heredity. DNA contains the sugar deoxyribose and is the nucleic acid in which genetic information is stored (apart from some viruses).

DNA CLONING - Production of many identical copies of a defined DNA fragment.

DNA LIBRARY - Set of cloned DNA fragments which together represent the entire genome or the transcription of a particular tissue.

DNA POLYMERASE - An enzyme which catalyses the synthesis of double- stranded DNA from single- stranded DNA.

DNA SYNTHESIS - The formation of DNA by the sequential addition of nucleotide bases.

DNase - An enzyme which produces single- stranded nicks in DNA. DNase is used in nick translation.

ELUTION - The removal of adsorbed material from an adsorbent such as the removal of a product from an enzyme bound on a column.

ENDONULEASES - Enzymes which cleave bonds within nucleic acid molecules.

ENDOTOXIN - A heat- stable lipopolysaccharide associated with the outer membrane of certain gram- negative bacteria. It is not secreted and is released only when the cells are disrupted. When injected into humans, endotoxins produce a febrile response, leading to severe clinical problems, including death. An endotoxin unit (EU) is defined in comparison to the current USP Reference Standard Lot EC- 5. One vial of lot EC- 5 contains 10,000 EU. The official test for endotoxin is found in the USP.

ENZYMES - Proteins that act as a catalyst in biochemical reactions.

EXONUCLEASES - Enzymes that catalyze the removal of nucleotides from the ends of a DNA molecule.

FERMENTATION - An anaerobic bioprocess. Fermentation is used in various industrial processes for the manufacture of products such as alcohols, acids, and cheese by the action of yeasts, molds, and bacteria. The fermentation process is used also in the production of monoclonal antibodies.

FUSION OF PROTOPLASTS - Fusion of two cells whose walls have been eliminated, making it possible to redistribute the genetic heritage of micro- organisms.

GENE - The basic unit of heredity, which plays a part in the expression of a specific characteristic. The expression of a gene is the mechanism by which the genetic information that it contains is transcribed and translated to obtain a protein. A gene is a part of the DNA molecule that directs the synthesis of a specific polypeptide chain. It is composed of many codons. When the gene is considered as a unit of function in this way, the term cistron is often used.

GENE TRANSFER - The use of genetic or physical manipulation to introduce foreign genes into a host cells to achieve desired characteristics in progeny.

GENETIC ENGINEERING - A technique used to modify the genetic information in a living cell, reprogramming it for a desired purpose (such as the production of a substance it would not naturally produce).

GENOME - All the genes carried by a cell.

GLYCOPROTEIN - Protein to which groups of sugars become attached. Human blood group proteins, cell wall proteins and some hormones are examples of glycoproteins.

GLYCOSYLATION - The covalent attachment of sugars to an amino acid in the protein portion of a glycoprotein.

HAPTEN - A low molecular weight substance that alone can react with its corresponding antibody. In order to be immunogenic, haptens are bonded to molecules having molecular weights greater than 5000. An example would be the hapten digoxin covalently bonded to bovine serum albumin, forming the digoxin- BSA immunogen.

HIGH AFFINITY ANTIBODY - Antibodies with a high affinity for antigen. These antibodies are predominantly IgG, and produced during a secondary response to antigen. Cells producing a high affinity antibody can be triggered by low concentration of antigen.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC OR LC) - (See Test Methods)

HOST - A cell whose metabolism is used for the growth and reproduction of a virus, plasmid, or other form of foreign DNA.

HYBRIDOMA TECHNOLOGY - Fusion between an antibody forming cell (lymphocyte) and a malignant myeloma cell ("immortal"), which will result in a continuously growing cell clone (hybridoma), that can produce antibodies of a single specificity.

IMMUNOASSAY SPECIFICITY - A performance characteristic determined by conducting cross- reactivity studies with structually similar substances that may be present in the analyte matrix. Specificity studies are determined with each new lot of polyclonal antibodies used in the immunoassay. For monoclonal antibody, each subsequent new lot is usually characterized by biochemical and biophysical techniques in lieu of comprehensive specificity studies.

IMMUNOELECTROPHORESIS (IEP) - (See Test Methods - Immunodiffusion (double, Ouchterlony techiques))

IMMUNOTOXIN - Monoclonal antibodies coupled with toxins that are capable of delivering the toxin moiety to a target cell.

IN SITU HYBRIDIZATION - Hybridization with an appropriate probe carried out directly on a chromosome preparation or histological section.

IN VITRO - Biological reactions taking place outside the body in an artificial system.

IN VIVO - Biological reaction taking place inside a living cell or organism.

INDUCER - A chemical or conditional change that activates the expression leading to the production of a desired product. A small molecule which interacts with a regulator protein and triggers gene transcription.

LIGASE - Enzyme used to join DNA molecules.

LOCUS - The site of a gene on a chromosome.

LYMPHOKINES - Substances released predominantly from T- lymphocytes after reaction with the specific antigen. Lymphokines are biologically highly active and will cause chemotaxis and activation of macrophages and other cell mediated immune reactions. Gamma- interferon is a lymphokine.

LYSIS - The process whereby a cell wall breakdown occurs releasing cellular content into the surrounding environment. Destruction of bacteria by infective phage.

MASTER CELL BANK (MCB) - A cell seed lot consisting of aliquots of a single culture (in most cases, expanded from a single cell) and stored cryogenically to assure genetic stability. There should be sufficient ampules of the MCB to provide the source material for a working seed bank.

MESSENGER RNA (mRNA) - RNA that serves as the template for protein synthesis; it carries the transcribed genetic code from the DNA to the protein synthesizing complex to direct protein synthesis.

MICROHETEROGENEITY - Slight differences in large, complex macromolecules that result in a population of closely related but not identical structures. Protein microheterogeneity can arise from many sources: genetic variants, proteolytic activity in cells, during translation into protein, during attachment of sugars and during commercial production.

MONOCLONAL ANTIBODIES - Antibodies that are produced by a cellular clone and are all identical.

MUTAGENESIS - The induction of genetic mutation by physical or chemical means to obtain a characteristic desired by researchers.

MUTATION - A change in the genetic material, either of a single base pair (point mutation) or in the number or structure of the chromosomes.

MYELOMA - Tumor cell line derived from a lymphocyte.

NICK TRANSLATION - In vitro method used to introduce radioactively labelled nucleotides into DNA.

NICK - A break in the sugar- phosphate backbone of a DNA or RNA strand.

OLIGONUCLEOTIDES - Short segments of DNA or RNA, i.e.; a chain of a few nucleotides.

OPERATOR GENE - A gene which switches on adjacent structural gene(s).

OPERON - Complete unit of bacterial gene expression consisting of a regulator gene(s), control elements (promoter and operator) and adjacent structural gene(s).

PATHOGEN - A disease- producing agent, usually restricted to a living agent, such as a bacterium or virus.

PEPTIDE BOND - Chemical bond between the carboxyl (- COOH) group of one amino acid and the amino (- NH2) group of another.

PLAQUE - Clear area in a plated bacterial culture due to lysis by a phage.

PLASMID - An extrachromosomal, self- replicating, circular segment of DNA; plasmids (and some viruses) are used as "vectors" for cloning DNA in bacterial "host" cells.

POLYCLONAL - Derived from different types of cells.

PROKARYOTE - An organism (e.g. bacterium, virus, blue- green algae) whose DNA is not enclosed within a nuclear membrane.

PROTEIN - A polypeptide consisting of amino acids. In their biologically active states, proteins function as catalysts in metabolism and, to some extent, as structural elements of cells and tissues.

PYROGENICITY - The tendency for some bacterial cells or parts of cells to cause inflammatory reactions in the body, which may detract from their usefulness as pharmaceutical products.

RECOMBINANT DNA - DNA that contains genes from different sources that have been combined by methods of genetic engineering as opposed to traditional breeding experiments.

RESTRICTION MAP - Linear arrangement of various restriction enzyme sites.

RESTRICTION SITE - Base sequence recognized by an enzyme.

RETROVIRUS - RNA virus which replicates via conversion into a DNA duplex.

REVERSE TRANSCRIPTASE - An enzyme that catalyzes the synthesis of DNA from RNA.

RNA (RIBONUCLEIC ACID) - Basic biochemical component of the chromosome that is found mainly in the nucleolus and ribosomes. Messenger RNA transfers genetic information from the nucleus to the ribosomes in the cytoplasm and also acts as a template for the synthesis of polypeptides. Transfer RNA transfers activated amino acids from the cytoplasm to messenger RNA.

RNA POLYMERASE - An enzyme that catalyzes the synthesis of RNA in transcription.

SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPORESIS (SDS- PAGE) (See Test Methods)

STRAIN - A group of organisms of the same species having distinctive characteristics, but not usually considered a separate breed or variety.

T- HELPER CELLS - T- lymphocytes with the specific capacity to help other cells, such as B- lymphocytes, to make antibodies. T- helper cells are also required for the induction of other T- lymphocyte activities. Synonym is T inducer cell, T4 cell, or CD 4 lymphocyte.

T- SUPPRESSOR CELLS - T- lymphocytes with specific capacity to inhibit T- helper cell function.

TRANSCRIPTION - The first stage in the expression of a gene by means of genetic information being transmitted from the DNA in the chromosomes to messenger RNA.

TRANSLATION -The second stage in the expression of a gene by means of genetic information being transmitted from the mRNA to the synthesis of protein.

VECTOR - A plasmid, phage or cosmid into which foreign DNA may be inserted for cloning.

WESTERN BLOT - (See Test Methods).

WORKING CELL BANK (WCB) - A quantity of cells derived from one or more ampules of the Master Cell Bank and used to initiate the production batch.

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