DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. 96D–0058]

International Conference on Harmonisation; Draft Guideline on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin; Availability

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is publishing a draft guideline entitled ''Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin.'' The draft guideline was prepared under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The draft guideline describes viral safety testing and evaluation of biotechnology products derived from characterized cell lines of human or animal origin, and outlines data that should be submitted in marketing applications.

DATES: Written comments by August 8, 1996.

ADDRESSES: Submit written comments on the draft guideline to the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857. Copies of the draft guideline are available from the Division of Communications Management (HFD–210), Center for Drug Evaluation and Research, Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301–594–1012. An electronic version of this guideline is also available via Internet by connecting to the CDER file transfer protocol (FTP) server (CDVS2.CDER.FDA.GOV).

FOR FURTHER INFORMATION CONTACT:

Regarding the guideline: Ruth Wolff, Center for Biologics Evaluation and Research (HFM–30), Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852, 301–594–5660.

Regarding ICH: Janet J. Showalter, Office of Health Affairs (HFY–20), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301–827–0864.

SUPPLEMENTARY INFORMATION: In recent years, many important initiatives have been undertaken by regulatory authorities and industry associations to promote international harmonization of regulatory requirements. FDA has participated in many meetings designed to enhance harmonization and is committed to seeking scientifically based harmonized technical procedures for pharmaceutical development. One of the goals of harmonization is to identify and then reduce differences in technical requirements for drug development among regulatory agencies.

ICH was organized to provide an opportunity for tripartite harmonization initiatives to be developed with input from both regulatory and industry representatives. FDA also seeks input from consumer representatives and others. ICH is concerned with harmonization of technical requirements for the registration of pharmaceutical products among three regions: The European Union, Japan, and the United States. The six ICH sponsors are the European Commission, the European Federation of Pharmaceutical Industries Associations, the Japanese Ministry of Health and Welfare, the Japanese Pharmaceutical Manufacturers Association, the Centers for Drug Evaluation and Research and Biologics Evaluation and Research, FDA, and the Pharmaceutical Research and Manufacturers of America. The ICH Secretariat, which coordinates the preparation of documentation, is provided by the International Federation of Pharmaceutical Manufacturers Associations (IFPMA).

The ICH Steering Committee includes representatives from each of the ICH sponsors and the IFPMA, as well as observers from the World Health Organization, the Canadian Health Protection Branch, and the European Free Trade Area.

At a meeting held on November 29, 1995, the ICH Steering Committee agreed that a draft guideline entitled ''Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin'' should be made available for public comment. The draft guideline is the product of the Quality Expert Working Group of the ICH. Comments about this draft will be considered by FDA and the Quality Expert Working Group. Ultimately, FDA intends to adopt the ICH Steering Committee's final guideline.

The draft guideline describes approaches for evaluating the risk of viral contamination and for removing viruses from biotechnology products derived from human or animal cell lines. The draft guideline emphasizes the value of many strategies including: (1) Thorough characterization/screening of the cell substrate starting material in order to identify which, if any, viral contaminants are present; (2) assessment of risk by a determination of the human tropism of the contaminants; (3) incorporation of studies that assess virus inactivation and removal steps in the production process; (4) careful design of viral clearance studies to avoid pitfalls and provide interpretable results; and (5) use of different methods of virus inactivation or removal in the same production process in order to achieve maximum viral clearance.

In the past, guidelines have generally been issued under § 10.90(b) (21 CFR 10.90(b)), which provides for the use of guidelines to state procedures or standards of general applicability that are not legal requirements but are acceptable to FDA. The agency is now in the process of revising § 10.90(b). Although this guideline does not create or confer any rights for or on any person and does not operate to bind FDA, it does represent the agency's current thinking on viral safety evaluation of biotechnology products.

Interested persons may, on or before August 8, 1996, submit to the Dockets Management Branch (address above) written comments on the draft guideline. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The draft guideline and received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

The text of the draft guideline follows:

Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin

I. Introduction

This document is concerned with testing and evaluation of the viral safety of biotechnology products derived from characterized cell lines of human or animal origin (i.e., mammalian, avian, insect), and outlines data that should be submitted in the marketing application/registration package. For the purposes of this document, the term virus excludes nonconventional transmissible agents like those associated with Bovine Spongiform Encephalopathy (BSE) and scrapie. Applicants are encouraged to discuss issues associated with BSE with the regulatory authorities.

The scope of the document covers products derived from cell cultures initiated from characterized cell banks. It covers products derived from in vitro cell culture, such as interferons, monoclonal antibodies, and recombinant deoxyribonucleic acid (DNA)-derived products including recombinant subunit vaccines, and also includes products

derived from hybridoma cells grown in vivo as ascites. In this latter case, special considerations apply and additional information on testing cells propagated in vivo is contained in Appendix 1. Inactivated vaccines, all live vaccines containing self-replicating agents, and genetically engineered live vectors are excluded from the scope of this document.

The risk of viral contamination is a feature common to all biotechnology products derived from cell lines. Such contamination could have serious clinical consequences and can arise from the contamination of the source cell lines themselves (cell substrates) or from adventitious introduction of virus during production. To date, however, biotechnology products derived from cell lines have not been implicated in the transmission of viruses. Nevertheless, it is expected that the safety of these products with regard to viral contamination can be reasonably assured only by the application of a virus testing program and assessment of virus removal and inactivation achieved by the manufacturing process, as outlined below.

Three principal, complementary approaches have evolved to control the potential viral contamination of biotechnology products:

(1) Selecting and testing cell lines and other raw materials, including media components, for the absence of undesirable viruses which may be infectious and/or pathogenic for humans;

(2) Assessing the capacity of the production processes to clear infectious viruses; and

(3) Testing the product at appropriate stages of production for the absence of contaminating infectious viruses.

All testing suffers from the inherent limitation of quantitative virus assays in that the ability to detect low viral concentrations depends for statistical reasons on the size of the sample. Therefore, no single approach will necessarily establish the safety of a product. Confidence that infectious virus is absent from the final product will in many instances not be derived solely from direct testing for their presence, but also from a demonstration that the purification regimen is capable of removing and/or inactivating the viruses.

The type and extent of viral tests and viral clearance studies required at different stages of production will depend on various factors and should be considered on a case-by-case and step-by-step basis. The factors that should be taken into account include the extent of cell bank characterization and qualification, the nature of any viruses detected, culture medium constituents, culture methods, facility and equipment design, the results of viral tests after cell culture, the ability of the process to clear viruses, and the type of product and its intended clinical use.

The purpose of this document is to provide a general framework for virus testing, experiments for the assessment of viral clearance, and a recommended approach for the design of viral tests and viral clearance studies. Related information is described in the appendices and selected definitions are provided in the Glossary.

Manufacturers should adjust the recommendations presented here to their specific product and its production process. The approach used by manufacturers in their overall strategy for ensuring viral safety should be explained and justified. In addition to the detailed data which is provided, an overall summary of the viral safety assessment would be useful in facilitating the review by regulatory authorities. This summary should contain a brief description of all aspects of the viral safety studies and strategies used to prevent virus contamination as they pertain to this document.

II. Potential Sources of Virus Contamination

Viral contamination of biotechnology products may arise from the original source of the cell lines or from adventitious introduction of virus during production processes.

A. Viruses That Could Occur in the Master Cell Bank (MCB)

Cells may have latent or persistent virus infection (e.g., herpesvirus) or endogenous retrovirus which may be transmitted vertically from one cell generation to the next, since the viral genome persists within the cell. Such viruses may be constitutively expressed or may unexpectedly become expressed as an infectious virus.

Viruses can be introduced into the MCB by several routes such as: (1) Derivation of cell lines from infected animals; (2) use of virus to establish the cell line; (3) use of contaminated biological reagents such as animal serum components; and (4) contamination during cell handling.

B. Adventitious Viruses That Could Be Introduced During Production

Adventitious viruses can be introduced into the final product by several routes including, but not limited to, the following: (1) Use of contaminated biological reagents such as animal serum components; (2) use of a virus for the induction of expression of specific genes encoding a desired protein; (3) use of a contaminated reagent, such as a monoclonal antibody affinity column; and (4) use of a contaminated excipient during formulation.

III. Cell Line Qualification: Testing for Viruses

An important part of qualifying a cell line for use in the production of a biotechnology product is the appropriate testing for the presence of virus.

A. Suggested Virus Tests for MCB, Working Cell Bank (WCB), and Cells at the Limit of In Vitro Cell Age Used for Production

Table 1 shows an example of virus tests to be performed once only at various cell levels, including MCB, WCB, and cells at the limit of in vitro cell age used for production.

1. Master Cell Bank

Extensive screening for both endogenous and nonendogenous viral contamination should be performed on the MCB. For heterohybrid cell lines in which one or more partners are human or nonhuman primate in origin, tests should be performed in order to detect viruses of human or nonhuman primate origin because viral contamination arising from these cells may pose a particular hazard.

Testing for nonendogenous viruses should include in vitro and in vivo inoculation tests and any other specific tests, including species-specific tests such as the mouse antibody production (MAP) test, that are appropriate, based on the passage history of the cell line, to detect possible contaminating viruses.

2. Working Cell Bank

Each WCB as a starting cell substrate for drug production should be tested for adventitious virus either by direct testing or by analysis of cells at the limit of in vitro cell age, initiated from the WCB. When appropriate nonendogenous virus tests have been performed on the MCB and cells cultured up to or beyond the limit of in vitro cell age have been derived from the WCB and used for testing for the presence of adventitious viruses, similar tests need not be performed on the initial WCB. Antibody production tests are usually not necessary for the WCB. An alternative approach in which full tests are carried out on the WCB rather than on the MCB would also be acceptable.

3. Cells at the Limit of In Vitro Cell Age Used for Production

The limit of in vitro cell age used for production should be based on data derived from production cells expanded under pilot-plant scale or commercial-scale conditions to the proposed in vitro cell age or beyond. Generally, the production cells are obtained by expansion of the WCB; the MCB could also be used to prepare the production cells. Cells at the limit of in vitro cell age should be evaluated once for those endogenous viruses that may have been undetected in the MCB and WCB. The performance of suitable tests (e.g., in vitro and in vivo) at least once on cells at the limit of in vitro cell age used for production would provide further assurance that the production process is not prone to contamination by adventitious virus. If any adventitious viruses are detected at this level, the process should be carefully checked in order to determine the cause of the contamination and completely redesigned if necessary.

B. Recommended Viral Detection and Identification Assays

Numerous assays can be used for the detection of endogenous and adventitious viruses. Table 2 outlines examples for these assays. They should be regarded as assay protocols recommended for the present, but the list is not all-inclusive or definitive. Since the most appropriate techniques may change with scientific progress, proposals for alternative techniques, when accompanied by adequate supporting data, may be acceptable. Manufacturers are encouraged to discuss these alternatives with the regulatory authorities. Other tests may be necessary depending on the individual case. Assays should include appropriate controls to ensure adequate sensitivity and specificity. Wherever a relatively high possibility of the presence of a specific virus can be predicted from the species of origin of the cell

substrate, specific tests and/or approaches may be necessary. If the cell line used for production is of human or nonhuman primate origin, additional tests for human viruses, such as those causing immunodeficiency diseases and hepatitis, should be performed unless otherwise justified. The polymerase chain reaction (PCR) may be appropriate for detection of sequences of these human viruses as well as for other specific viruses. The following is a brief description of a general framework and philosophical background within which the manufacturer should justify what was done.

1. Tests for Retroviruses

For the MCB and for cells cultured up to or beyond the limit of in vitro cell age used for production, tests for retroviruses, including infectivity assays in sensitive cell cultures and electron microscopy (EM) studies, should be carried out. If infectivity is not detected and no retrovirus or retrovirus-like particles have been observed by EM, reverse transcriptase (RT) or other appropriate assays should be performed to detect retroviruses which may be noninfectious. Induction studies have not been found to be useful.

2. In Vitro Assays

In vitro tests are carried out by the inoculation of a test article (see Table 2) into various susceptible indicator cell cultures capable of detecting a wide range of human and relevant animal viruses. The choice of cells used in the test is governed by the species of origin of the cell bank to be tested, but should include a human and/or a nonhuman primate cell line susceptible to human viruses. The nature of the assay and the sample to be tested are governed by the type of virus which may possibly be present based on the origin or handling of the cells. Both cytopathic and hemadsorbing viruses should be sought.

3. In Vivo Assays

A test article (see Table 2) should be inoculated into animals, including suckling and adult mice, and in embryonated eggs to reveal viruses that cannot grow in cell cultures. Additional animal species may be used depending on the nature and source of the cell lines being tested. The health of the animals should be monitored and any abnormality should be investigated to establish the cause of the illness.

4. Antibody Production Tests

Species-specific viruses present in rodent cell lines may be detected by inoculating test article (see Table 2) into virus-free animals and examining the serum antibody level or enzyme activity after a specified period. Examples of such tests are the MAP test, rat antibody production (RAP) test, and hamster antibody production (HAP) test. The viruses currently screened for in the antibody production assays are discussed in Table 3.

C. Acceptability of Cell Lines

It is recognized that some cell lines used for the manufacture of product will contain endogenous retroviruses, other viruses, or viral sequences. In such circumstances, the action plan recommended for manufacture is described in section V. of this document. The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or for causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk.

IV. Testing for Viruses in Unprocessed Bulk

The unprocessed bulk constitutes one or multiple pooled harvests of cells and culture media. When cells are not readily accessible (e.g., hollow fiber or similar systems), the unprocessed bulk would constitute fluids harvested from the fermenter. A representative sample of the unprocessed bulk, removed from the production reactor prior to further processing, represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic).

In certain instances it may be more appropriate to test a mixture consisting of both intact and disrupted cells and their cell culture supernatants removed from the production reactor prior to further processing. Data from at least 3 lots of unprocessed bulk at pilot-plant scale or commercial scale should be submitted as part of the registration/marketing application package.

It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent, and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material sources, and results of viral clearance studies. In vitro screening tests, using one or several cell lines, are generally employed to test unprocessed bulk. If appropriate, a PCR test or other suitable methods may be used.

Generally, harvest material in which adventitious virus has been detected should not be used to manufacture the product. If any adventitious viruses are detected at this level, the process should be carefully checked to determine the cause of the contamination, and appropriate actions taken.

V. Rationale and Action Plan for Viral Clearance Studies and Virus Tests on Purified Bulk

It is important to design the most relevant and rational protocol for virus tests from the MCB level through the various stages of drug production to the final product, including evaluation and characterization of viral clearance from unprocessed bulk. The evaluation and characterization of viral clearance plays a critical role in this scheme. The goal should be to obtain the best reasonable assurance that the product is free of virus contamination.

In selecting viruses to use for a clearance study, it is useful to distinguish between the need to evaluate processes for their ability to clear viruses that are known to be present and the desire to estimate the robustness of the process by characterizing the clearance of nonspecific ''model'' viruses (described later). Definitions of ''relevant'', specific and nonspecific ''model'' viruses are given in the Glossary. Process evaluation requires knowledge of how much virus may be present in the process, such as the unprocessed bulk, and how much can be cleared in order to assess product safety. Knowledge of the time dependence for inactivation procedures is helpful in assuring the effectiveness of the inactivation process.

When a manufacturing process is characterized for robustness of clearance using nonspecific ''model'' viruses, less extensive virus removal/inactivation studies are appropriate. Indepth, time-dependent inactivation studies, demonstration of reproducibility of inactivation/removal, and evaluation of process parameters are not required. These studies should be performed on the manufacturing process in Cases A through E as described below.

Table 4 presents an example of an action plan in terms of process evaluation and characterization of viral clearance as well as virus tests on purified bulk, in response to the results of virus tests on cells and/or the unprocessed bulk. Various cases are considered. In all cases, characterization of clearance using nonspecific ''model'' viruses should be performed. The most common situations are Cases A and B. Production systems contaminated with a virus other than a rodent retrovirus are normally not used. Where there are convincing and well justified reasons for drug production using a cell line from Cases C, D, or E, these should be discussed with the regulatory authorities. With Cases C, D, and E, it is important to have validated effective steps to inactivate/remove the virus in question from the manufacturing process.

Case A: Where no virus, virus-like particle, or retrovirus-like particle has been demonstrated in the cells or the unprocessed bulk, virus removal and inactivation studies should be performed with nonspecific ''model'' viruses as previously stated.

Case B: Where only a rodent retrovirus (or a retrovirus-like particle which is believed to be nonpathogenic, such as rodent A- and R-type particles) is present, process evaluation using a specific ''model'' virus, such as a murine leukemia virus, should be performed. Purified bulk should be tested using suitable methods having high specificity and sensitivity for the detection of the virus in question. For marketing authorization, data from at least 3 lots of purified bulk at pilot-plant scale or commercial scale should be provided. Cell lines such as Chinese hamster ovary (CHO), C127, baby hamster kidney (BHK), and murine hybridoma cell lines have frequently been used as substrates for drug

production with no reported safety problems related to viral contamination of the products. For these cell lines in which the endogenous particles have been extensively characterized and clearance has been demonstrated, it is not usually necessary to assay for the presence of the noninfectious particles in purified bulk.

Case C: When the cells or unprocessed bulk are known to contain a virus, other than a rodent retrovirus, for which there is no evidence of capacity for infecting humans, (such as those identified by footnote 2 in Table 3, except rodent retroviruses (Case B)), virus removal and inactivation evaluation studies should use the identified virus. If it is not possible to use the identified virus, ''relevant'' or specific ''model'' viruses should be used to demonstrate acceptable clearance. Time-dependent inactivation for identified (or ''relevant'' or specific ''model'') viruses at the critical inactivation step(s) should be obtained as part of process evaluation for these viruses. Purified bulk should be tested using suitable methods having high specificity and sensitivity for the detection of the virus in question. For the purpose of marketing authorization, data from at least 3 lots of purified bulk manufactured at pilot-plant scale or commercial scale should be provided.

Case D: Where a known human pathogen, such as those indicated by footnote 1 in Table 3, is identified, the product may be acceptable only under exceptional circumstances. In this instance, it is recommended that the identified virus be used for virus removal and inactivation evaluation studies and specific methods with high specificity and sensitivity for the detection of the virus in question be employed. If it is not possible to use the identified virus, ''relevant'' and/or specific ''model'' viruses (described later) should be used. The process should be shown to achieve the removal and inactivation of the selected viruses during the purification and inactivation processes. Time-dependent inactivation data for the critical inactivation step(s) should be obtained as part of process evaluation. Purified bulk should be tested using suitable methods having high specificity and sensitivity for the detection of the virus in question. For the purpose of marketing authorization, data from at least 3 lots of purified bulk manufactured at pilot-plant scale or commercial scale should be provided.

Case E: When a virus which cannot be classified by currently available methodologies is detected in the cells or unprocessed bulk, the product is usually considered unacceptable since the virus may prove to be pathogenic. In the very rare case where there are convincing and well justified reasons for drug production using such a cell line, this should be discussed with the regulatory authorities before proceeding further.

VI. Evaluation and Characterization of Viral Clearance Procedures

Evaluation and characterization of the virus removal and/or inactivation procedures plays an important role in establishing the safety of biotechnology products. Many instances of contamination in the past have occurred with agents whose presence was not known or even suspected, and though this happened to biological products derived from various source materials other than fully characterized cell lines, assessment of viral clearance will provide a measure of confidence that any unknown, unsuspected, and harmful viruses may be removed.

The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition (''spiking'') of significant amounts of a virus to the crude material and/or to different fractions obtained during the various process stages and demonstrating its removal or inactivation during the subsequent stages. It is not necessary to evaluate or characterize every stage of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps.

The reduction of virus infectivity may be achieved by removal of virus particles or by inactivation of viral infectivity. For each production stage assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. For inactivation steps, the study should be planned in such a way that samples are taken at different times and an inactivation curve constructed. Studies should be carried out in a manner that is well documented and controlled (see section VI.B.5).

Viral clearance evaluation studies are performed to demonstrate the clearance of a virus known to be present in the MCB and/or to provide some level of assurance that adventitious viruses which could not be detected, or might gain access to the production process, would be cleared.

In contrast to viral clearance studies described above for viruses known to be present, studies to characterize the ability to remove and/or inactivate other viruses should be conducted. The purpose of the studies with viruses that are not known or expected to be present is to characterize the robustness of the procedure rather than to achieve a specific inactivation or removal goal. They are not performed to evaluate a specific safety risk. Therefore, a specific clearance value need not be achieved.

A. The Choice of Viruses for the Evaluation and Characterization of Viral Clearance

Viruses for clearance evaluation and process characterization studies should be chosen to resemble viruses which may contaminate the product and to represent a wide range of physico-chemical properties in order to test the ability of the system to eliminate viruses in general. The manufacturer should justify the choice of viruses in accordance with the aims of the evaluation and characterization study and the guidance provided in this guideline.

1. ''Relevant'' Viruses and ''Model'' Viruses

A major issue in performing a viral clearance study is to determine which viruses should be used. Such viruses fall into three categories: ''Relevant'' viruses, specific ''model'' viruses, and nonspecific ''model'' viruses.

''Relevant'' viruses are viruses used in process evaluation of viral clearance studies which are either the identified viruses, or of the same species as the viruses that are known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process. The purification and/or inactivation process should demonstrate the capability to remove and/or inactivate such viruses. When a ''relevant'' virus is not available or when it is not well adapted to process evaluation of viral clearance studies (e.g., it cannot be grown in vitro to sufficiently high titers), a specific ''model'' virus should be used as a substitute. An appropriate specific ''model'' virus may be a virus which is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties to the observed or suspected virus.

Cell lines derived from rodents usually contain endogenous retrovirus particles or retrovirus-like particles, which may be infectious (C-type particles) or noninfectious (cytoplasmic A- and R-type particles). The capacity of the manufacturing process to remove and/or inactivate rodent retroviruses from products obtained from such cells should be determined. This may be accomplished by using a murine leukemia virus, a specific ''model'' virus in the case of cells of murine origin. When human cell lines secreting monoclonal antibodies have been obtained by the immortalization of B lymphocytes by Epstein-Barr Virus (EBV), the ability of the manufacturing process to remove and/or inactivate a herpes virus should be determined. Pseudorabies virus may also be used as a specific ''model'' virus.

When the purpose is to characterize the capacity of the manufacturing process to remove and/or inactivate viruses in general, i.e., to characterize the robustness of the clearance process, viral clearance characterization studies should be performed with nonspecific ''model'' viruses with differing properties. Data obtained from studies with ''relevant'' and/or specific ''model'' viruses may also contribute to this assessment. It is not necessary to test all types of viruses. Preference should be given to viruses that display a significant resistance to physical and/or chemical treatments. The results obtained for such viruses provide useful information about the ability of the production process to remove and/or inactivate viruses in general. The choice and number of viruses used will be influenced by the quality and characterization of the cell lines and the production process.

Examples of useful ''model'' viruses representing a range of physico-chemical structures and examples of viruses which have been used in viral clearance studies are given in Appendix 2 and Table A–1.

2. Other Considerations

Additional points to be considered are as follows:

(a) Viruses which can be grown to high titer are desirable, although this may not always be possible.

(b) There should be an efficient and reliable assay for the detection of each virus

used, for every stage of manufacturing that is tested.

(c) Consideration should be given to the health hazard which certain viruses may pose to the personnel performing the clearance studies.

B. Design and Implications of Viral Clearance Evaluation and Characterization Studies

1. Facility and Staff

It is inappropriate to introduce any virus into a production facility because of good manufacturing practice (GMP) constraints. Therefore, viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process.

2. Scaled-down Production System

The validity of the scaling-down should be demonstrated. The level of purification of the scaled-down version should represent as closely as possible the production procedure. For chromatographic equipment, column bed-height, linear flow-rate, flow-rate-to-bed-volume ratio (i.e., contact time), buffer and gel types, pH, temperature, and concentration of protein, salt, and product should all be shown to be representative of commercial-scale manufacturing. For other procedures, similar considerations apply. Deviations which cannot be avoided should be discussed with regard to their influence on the results.

3. Analysis of Step-wise Elimination of Virus

When viral clearance studies are being performed, it is desirable to assess the contribution of more than one production step to virus elimination. Essential stages of the purification process should be individually assessed for their ability to remove and inactivate virus and careful consideration should be given to the exact definition of an individual stage. Sufficient virus should be present in the material of each stage to be tested so that an adequate assessment of the effectiveness of each step is obtained. Generally, virus should be added to in-process material of each stage to be tested. In some cases, simply adding high titer virus to unpurified bulk and testing its concentration between steps will be sufficient. When virucidal buffers are used in multiple steps within the manufacturing process, alternative strategies such as parallel spiking in less virucidal buffers may be carried out as part of the overall process assessment. The virus titer before and after each step being tested should be determined. Quantitative infectivity assays should have adequate sensitivity and reproducibility and should be performed with sufficient replicates to ensure adequate statistical validity of the result (see Appendix 3). Quantitative assays not associated with infectivity may be used if justified. Appropriate virus controls should be included in all infectivity assays to ensure the sensitivity of the method. Also, the statistics of sampling virus when at low concentrations should be considered (Appendix 4).

4. Determining Physical Removal Versus Inactivation

Reduction in virus infectivity may be achieved by the removal or inactivation of virus. For each production stage assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. If little clearance of infectivity is achieved by the production process, and the clearance of virus is considered to be a major factor in the safety of the product, specific or additional inactivation/removal steps should be introduced. It may be necessary to distinguish between removal and inactivation for a particular step, e.g., when there is a possibility that a buffer used in more than one clearance step may contribute to inactivation during each step, i.e., the contribution to inactivation by a buffer shared by several chromatographic steps and the removal achieved by each of these chromatographic steps should be distinguished. Assurance should be provided that any virus potentially retained by the production system would be adequately destroyed or removed prior to reuse of the system. For example, such evidence may be provided by demonstrating that the cleaning and regeneration procedures do inactivate or remove virus.

5. Inactivation Assessment

For assessment of viral inactivation, unprocessed crude material or intermediate material should be spiked with infectious virus and the reduction factor calculated. The determination of initial virus load for assessing inactivation potential may be derived from the titer of the spiking virus preparation. This may be of importance when virucidal buffers are used in multiple steps within the manufacturing process. Virus inactivation is not a simple, first order reaction and is usually more complex, with a fast ''phase 1'' and a slow ''phase 2.'' The study should, therefore, be planned in such a way that samples are taken at different times and an inactivation curve constructed. It is recommended that studies for inactivation include at least one time point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time. These types of data are particularly important where the virus is a ''relevant'' virus known to be a human pathogen and an effective inactivation process is being designed.

6. Function and Regeneration of Columns

Over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary. Some estimate of the stability of the viral clearance after several uses may provide support for repeated use of such columns. Assurance should be provided that any virus potentially retained by the production system would be adequately destroyed or removed prior to reuse of the system. For example, such evidence may be provided by demonstrating that the cleaning and regeneration procedures do inactivate or remove virus.

7. Specific Precautions

(a) Care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation, thus distorting the correlation with actual production.

(b) Consideration should be given to the minimum quantity of virus which can be reliably assayed.

(c) The study should include parallel control assays to assess the loss of infectivity of the virus due to such reasons as the dilution, concentration, filtration, or storage of samples before titration.

(d) The virus ''spike'' should be added to the product in a small volume so as not to dilute or change the characteristics of the product. Diluted, test-protein sample is no longer identical to the product obtained at commercial scale.

(e) Small differences in, for example, buffers, media, or reagents, can substantially affect viral clearance.

(f) Virus inactivation is time-dependent, therefore, the amount of time a spiked product remains in a particular buffer solution or on a particular chromatography column should reflect the conditions of the commercial-scale process.

(g) Buffers and product should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus may be necessary. If the product itself has antiviral activity, the clearance study may need to be performed without the product in a ''mock'' run, although omitting the product or substituting a similar protein that does not have antiviral activity could affect the behavior of the virus in some production steps. Sufficient controls to demonstrate the effect of procedures used solely to prepare the sample for assay (e.g., dialysis, storage) on the removal/inactivation of the spiking virus should be included.

(h) Many purification schemes use the same or similar buffers or columns repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the manufacturing stage at which it is used.

(i) Overall reduction factors may be underestimated where production conditions or buffers are too cytotoxic or virucidal and should be discussed on a case-by-case basis.

C. Interpretation of Viral Clearance Studies; Acceptability

The objective of assessing virus inactivation/removal is to evaluate and characterize process steps that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the manufacturing process. For virus contaminants, as in Cases B through E, it is important to show that not only is the virus eliminated or inactivated, but that there is excess capacity for viral clearance built into the purification process to assure an appropriate level of safety for the final product. The amount of virus eliminated or inactivated by the production

process should be compared to the amount of virus which may be present in unprocessed bulk. However, for inactivation studies in which nonspecific ''model'' viruses are used, or when specific ''model'' viruses are used as surrogates for virus particles such as the CHO intracytoplasmic retrovirus-like particles, it is sufficient to demonstrate reproducible clearance in at least two independent experiments. It is recommended that these studies for inactivation include at least one time-point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time.

To carry out this comparison, it is important to estimate the amount of virus in the unprocessed bulk. This estimate should be obtained using assays for infectivity or other methods such as transmission electron microscopy (TEM). The entire purification process should be able to eliminate substantially more virus than is estimated to be present in a single-dose-equivalent of unprocessed bulk. See Appendix 5 for calculation of virus reduction factors and Appendix 6 for calculation of estimated particles per dose.

A combination of factors must be considered when judging the data supporting the effectiveness of virus inactivation/removal procedures. These include:

(i) The appropriateness of the test viruses used;

(ii) The design of the clearance studies;

(iii) The log reduction achieved;

(iv) The time dependence of inactivation;

(v) The potential effects of variation in process parameters on virus inactivation/removal; and

(vi) The limits of assay sensitivities.

Effective clearance may be achieved by any of the following: Multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps. Since separation methods may be dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties, ''model'' viruses may be separated in a different manner than a target virus. Differences may originate from changes in surface properties such as glycosylation. However, despite these potential variables, effective removal can be obtained by a combination of complementary separation steps or combinations of inactivation and separation steps. Therefore, well-designed separation steps, such as chromatographic procedures, filtration steps, and extractions, can be effective virus removal steps provided that they are performed under appropriately controlled conditions.

An overall reduction factor is generally expressed as the sum of the individual factors. However, reduction in virus titer of the order of 1 $\log_{10}$ or less would be considered negligible and would be ignored unless assay variability were shown to be below that order of magnitude.

If little reduction of infectivity is achieved by the production process, and the removal of virus is considered to be a major factor in the safety of the product, a specific, additional inactivation/removal step or steps should be introduced. For all viruses, manufacturers should justify the acceptability of the reduction factors obtained. Results will be evaluated on the basis of the factors listed above.

D. Limitations of Viral Clearance Studies

Viral clearance studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved, but do not by themselves establish safety. However, a number of factors in the design and execution of viral clearance studies may lead to an incorrect estimate of the ability of the process to remove virus infectivity (see Appendices 2, 3, and 4). These factors include the following:

1. Virus preparations used in clearance studies for a production process are likely to be produced in tissue culture. The behavior of a tissue culture virus in a production step may be different from that of the native virus, for example, if native and cultured viruses differ in purity or degree of aggregation.

2. Inactivation of virus infectivity frequently follows a biphasic curve in which a rapid initial phase is followed by a slower phase. It is possible that virus escaping a first inactivation step may be more resistant to subsequent steps. For example, if the resistant fraction takes the form of virus aggregates, infectivity may be resistant to a range of different chemical treatments and to heating.

3. The ability of the overall process to remove infectivity is expressed as the sum of the logarithm of the reductions at each step. The summation of the reduction factors of multiple steps, particularly of steps with little reduction (e.g., below 1 $\log_{10}$), may overestimate the true potential for virus elimination. Furthermore, reduction values achieved by repetition of identical or near identical procedures should not be included unless justified.

4. The expression of reduction factors as logarithmic reductions in titer implies that, while residual virus infectivity may be greatly reduced, it will never be reduced to zero. For example, a reduction in the infectivity of a preparation containing 8 $\log_{10}$ infectious units per milliliter (mL) by a factor of 8 $\log_{10}$ leaves zero $\log_{10}$ per mL or one infectious unit per mL, taking into consideration the limit of detection of the assay.

5. Pilot-plant scale processing may differ from commercial-scale processing despite care taken to design the scaled-down process.

6. Addition of individual virus reduction factors resulting from similar inactivation mechanisms along the manufacturing process may overestimate overall viral clearance.

E. Statistics

The viral clearance studies should include the use of statistical analysis of the data to evaluate the results. The study results should be statistically valid to support the conclusions reached (see Appendices 3 and 4).

F. Re-Evaluation of Viral Clearance

Whenever significant changes in the production or purification process are made, the effect of that change on viral clearance should be considered and the system re-evaluated as needed. For example, changes in production processes may cause significant changes in the amount of virus produced by the cell line; changes in process steps may change the extent of viral clearance.

VII. Summary

This document suggests approaches for the evaluation of the risk of viral contamination and for the removal of virus from the product, thus contributing to the production of safe biotechnology products derived from animal or human cell lines, and emphasizes the value of many strategies, including:

A. Thorough characterization/screening of cell substrate starting material in order to identify which, if any, viral contaminants are present;

B. Assessment of risk by determination of the human tropism of the contaminants;

C. Incorporation of studies which assess virus inactivation and removal steps into the production process;

D. Careful design of the viral clearance studies to avoid pitfalls and provide interpretable results; and

E. Use of different methods of virus inactivation or removal in the same production process in order to achieve maximum viral clearance.

Glossary

Adventitious Virus. See virus.

Cell Substrate. Cells used to manufacture product.

Endogenous Virus. See virus.

Inactivation. Reduction of virus infectivity caused by chemical or physical modification.

In Vitro Cell Age. A measure of the period between thawing of the MCB vial(s) and harvest of the production vessel measured by elapsed chronological time in culture, population doubling level of the cells, or passage level of the cells when subcultivated by a defined procedure for dilution of the culture.

Master Cell Bank (MCB). An aliquot of a single pool of cells which generally has been prepared from the selected cell clone under defined conditions, dispensed into multiple containers, and stored under defined conditions. The MCB is used to derive all working cell banks. The testing performed on a new MCB (from a previous initial cell clone, MCB, or WCB) should be the same as for the MCB, unless justified.

Minimum Exposure Time. The shortest period for which a treatment step will be maintained.

Nonendogenous Virus. See virus.

Process Characterization of Viral Clearance. Viral clearance studies in which nonspecific ''model'' viruses are used to assess the robustness of the manufacturing process to remove and/or inactivate viruses.

Process Evaluation Studies of Viral Clearance. Viral clearance studies in which ''relevant'' and/or specific ''model'' viruses are used to determine the ability of the manufacturing process to remove and/or inactivate these viruses.

Production Cells. Cell substrate used to manufacture product.

Unprocessed Bulk. One or multiple pooled harvests of cells and culture media. When

cells are not readily accessible, the unprocessed bulk would constitute fluid harvested from the fermenter.

Virus. Intracellularly replicating infectious agents that are potentially pathogenic, possessing only a single type of nucleic acid (either ribonucleic acid (RNA) or DNA), are unable to grow and undergo binary fission, and multiply in the form of their genetic material.

Adventitious Virus. Unintentionally introduced contaminant viruses.

Endogenous Virus. Viral entity whose genome is part of the germ line of the species of origin of the cell line and is covalently integrated into the genome of animal from which the parental cell line was derived. For the purposes of this document, intentionally introduced, nonintegrated viruses such as EBV used to immortalize cell substrates or Bovine Papilloma Virus fit in this category.

Nonendogenous Virus. Viruses from external sources present in the Master Cell Bank.

Nonspecific Model Virus. A virus used for characterization of viral clearance of the process when the purpose is to characterize the capacity of the manufacturing process to remove and/or inactivate viruses in general, i.e., to characterize the robustness of the purification process.

Relevant Virus. Virus used in process evaluation studies which is either the identified virus, or of the same species as the virus that is known, or likely to contaminate the cell substrate or any other reagents or materials used in the production process.

Specific Model Virus. Virus which is closely related to the known or suspected virus (same genus or family), having similar physical and chemical properties to those of the observed or suspected virus.

Viral Clearance. Elimination of target virus by removal of viral particles or inactivation of viral infectivity.

Virus-like Particles. Structures visible by electron microscopy which morphologically appear to be related to known viruses.

Virus Removal. Physical separation of virus particles from the intended product.

Working Cell Bank (WCB). The WCB is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.

TABLE 1.—VIRUS TESTS TO BE PERFORMED ONCE AT VARIOUS CELL LEVELS

	MCB	WCB[1]	Cells at the limit[2]
Tests for Retroviruses and Other Endogenous Viruses			
Infectivity	+	–	+
Electron microscopy[3]	+[3]	–	+[3]
Reverse transcriptase[4]	+[4]	–	+[4]
Other virus-specific tests[5]	as appropriate[5]	–	as appropriate[5]
Tests for Nonendogenous or Adventitious Viruses			
In vitro Assays	+	–[6]	+
In vivo Assays	+	–[6]	+
Antibody production tests[7]	+[7]	–	–
Other virus-specific tests[8]	+[8]	–	–

[1] See text—section III.A.2.
[2] Cells at the limit; cells at the limit of in vitro cell age used for production (See text—section III.A.3.)
[3] May also detect other agents.
[4] Not necessary if positive by retrovirus infectivity test.
[5] As appropriate for cell lines which are known to have been infected by such agents.
[6] For the first WCB, this test should be performed on cells at the limit of in vitro cell age, generated from that WCB; for WCB's subsequent to the first WCB, a single in vitro and in vivo test can be done either directly on the WCB or on cells at the limit of in vitro cell age.
[7] e.g., MAP, RAP, HAP—usually applicable for rodent cell lines.
[8] e.g, tests for cell lines derived from human, nonhuman primate, or other cell lines as appropriate.

TABLE 2.—EXAMPLES OF THE USE AND LIMITATIONS OF ASSAYS WHICH MAY BE USED TO TEST FOR VIRUS

Test	Test article	Detection capability	Detection limitation
Anbibody production	Lysate of cells and their culture medium	Specific viral antigens	Agents not infectious for animal test system.
in vivo virus screen	Lysate of cells and their culture medium	Broad range of viruses pathogenic for humans	Agents failing to replicate or produce diseases in the test system.
in vitro virus screen for		Broad range of viruses pathogenic for humans	Agents failing to replicate or produce diseases in the test system.
1. Cell bank characterization	1. Lysate of cells and their culture medium (for cocultivation, intact cells should be in the test article)		
2. Production screen	2. Unprocessed bulk harvest or lysate of cells and their cell culture medium from the production reactor		
TEM on:		Virus and virus-like particles	Qualitative assay with assessment of identity.
1. Cell substrate	1. Viable cells		
2. Cell culture supernatant	2. Concentrated cell-free supernatant		

TABLE 2.—EXAMPLES OF THE USE AND LIMITATIONS OF ASSAYS WHICH MAY BE USED TO TEST FOR VIRUS—Continued

Test	Test article	Detection capability	Detection limitation
Reverse transcriptase (RT)	Cell-free culture supernatant	Retroviruses and expressed retroviral RT	Only detects enzymes with optimal activity under preferred conditions. Interpretation may be difficult due to presence of cellular enzymes; background with some concentrated samples.
Retrovirus (RV) infectivity	Cell-free culture supernatant	Infectious retroviruses	RV failing to replicate or form discrete foci or plaques in the chosen test system.
Cocultivation infectivity endpoint	Viable cells	Infectious retroviruses	RV failing to replicate. See above under RV infectivity.
TEM endpoint			See above under TEM.[1]
RT endpoint			See above under RT.
PCR (Polymerase chain reaction)	Cells, culture fluid and other materials	Specific virus sequences	Primer sequences must be present. Does not indicate whether virus is infectious.

[1] In addition, difficult to distinguish test article from indicator cells.

TABLE 3.—VIRUS DETECTED IN ANTIBODY PRODUCTION TESTS

MAP	HAP	RAP
Ectromelia Virus[2,3]	Lymphocytic ChoriomeningitisVirus (LCM)[1,3]	Hantaan Virus[1,3]
Hantaan Virus[1,3]	Pneumonia Virus of Mice (PVM)[2,3]	Kilham Rat Virus (KRV)[2,3]
K Virus[2]	Reovirus Type 3 (Reo3)[1,3]	Mouse Encephalomyelitis Virus (Theiler's, GDVII)[2]
Lactic Dehydrogenase Virus (LDH)[2]	Sendai Virus[1,3]	Pneumonia Virus of Mice (PVM)[2,3]
Lymmphocytic Choriomeningitis Virus (LCM)[1,3]	SV5	Rat Coronavirus (RCV)[2]
Minute Virus of Mice (MVM)[2,3]		Reovirus Type 3 (Reo3)[1,3]
Mouse Adenovirus (MAV)[2,3]		Sendai Virus[1,3]
Mouse Cytomegalovirus (MCMV)[2,3]		Sialoacryoadenitis Virus (SDAV)[2]
Mouse Encephalomyelitis Virus (Theiler's, GDVII)[2]		Toolan Virus (HI)[2,3]
Mouse Hepatitis Virus (MHV)[2]		
Mouse Rotavirus (EDIM)[2,3]		
Pneumonia Virus of Mice (PVM)[2,3]		
Polyoma Virus[2]		
Reovirus Type 3 (Reo3)[1,3]		
Sendai Virus[1,3]		
Thymic Virus[2]		

[1] Viruses for which there is evidence of capacity for infecting humans or primates.
[2] Viruses for which there is no evidence of capacity for infecting humans.
[3] Virus capable of replicating in vitro in cells of human or primate origin.

TABLE 4.—ACTION PLAN FOR PROCESS ASSESSMENT OF VIRAL CLEARANCE AND VIRUS TESTS ON PURIFIED BULK

	Case A	Case B	Case C[2]	Case D[2]	Case E[2]
Status					
Presence of virus[1]	–	–	+	+	(+)[3]
Virus-like particles[1]	–	–	–	–	(+)[3]
Retrovirus-like particles[1]	–	+	–	–	(+)[3]
Virus identified	not applicable	+	+	+	–
Virus pathogenic for humans	not applicable	–[4]	–[4]	+	unknown
Action					
Process characterization of viral clearance using nonspecific ''model'' viruses	yes[5]	yes[5]	yes[5]	yes[5]	yes[7]
Process evaluation of viral clearance using ''relevant'' or specific ''model'' viruses	no	yes[6]	yes[6]	yes[6]	yes[7]
Test for virus in purified bulk	not applicable	yes[8]	yes[8]	yes[8]	yes[8]

[1] Results of virus tests for the cell substrate and/or at the unprocessed bulk level. Cell cultures used for production which are contaminated with viruses will generally not be acceptable. Endogenous viruses (such as retroviruses) or viruses that are an integral part of the MCB may be acceptable if appropriate viral clearance evaluation procesures are followed.
[2] The use of source material which is contaminated with viruses, whether or not they are known to be infectious and/or pathogenic in humans, will only be permitted under very exceptional circumstances.

[3] Virus has been observed by either direct or indirect methods.
[4] Believed to be nonpathogenic.
[5] Characterization of clearance using nonspecific ''model'' viruses should be performed.
[6] Process evaluation for ''relevant'' viruses or specific ''model'' viruses should be performed.
[7] See text under Case E.
[8] The absence of detectible virus should be confirmed for purified bulk by means of suitable methods having high specificity and sensitivity for the detection of the virus in question. For the purpose of marketing authorization, data from at least 3 lots of purified bulk manufactured at pilot-plant scale or commercial scale should be provided. However, for cell lines such as CHO cells for which the endogenous particles have been extensively characterized and adequate clearance has been demonstrated, it is not usually necessary to assay for the presence of the noninfectious particles in purified bulk.

Appendix 1

Products Derived From Characterized Cell Banks Which Were Subsequently Grown In Vivo

For products manufactured from fluids harvested from animals inoculated with cells from characterized banks, additional information regarding the animals should be provided.

Whenever possible, animals used in the manufacture of biotechnological/biological products should be obtained from well defined, specific pathogen-free colonies. Quarantine procedures for newly arrived as well as diseased animals should be described, and assurance provided that all containment, cleaning, and decontamination methodologies employed within the facility are adequate to contain the spread of adventitious agents. This may be accomplished through the use of a sentinel program. A listing of agents for which testing is performed should also be included. Veterinary support services should be available on-site or within easy access. The degree to which the vivarium is segregated from other areas of the manufacturing facility should be described. Personnel practices should be adequate to ensure safety.

Procedures for the maintenance of the animals should be fully described. These would include diet, cleaning, and feeding schedules, provisions for periodic veterinary care if applicable, and details of special handling that the animals may require once inoculated. A description of the priming regimen(s) for the animals, the preparation of the inoculum, and the site and route of inoculation should also be included.

The primary harvest material from animals may be considered an equivalent stage of manufacture to unprocessed bulk harvest from a bioreactor. Therefore, all testing considerations previously outlined in section IV. of this document should apply. In addition, the manufacturer should assess the bioburden of the unprocessed bulk, determine whether the material is free of mycoplasma, and perform species-specific assay(s) as well as in vivo testing in adult and suckling mice.

Appendix 2

The Choice of Viruses for Viral Clearance Studies

A. Examples of Useful ''Model'' Viruses:

1. Nonspecific ''model'' viruses representing a range of physico-chemical structures:

- SV40 (Polyomavirus maccacae 1), human polio virus 1 (Sabin), animal parvovirus or some other small, nonenveloped viruses;
- a parainfluenza virus or influenza virus, Sindbis virus or some other medium-to-large, enveloped, RNA viruses;
- a herpes virus (e.g., HSV–1 or a pseudorabies virus), or some other medium-to-large, DNA viruses.

These viruses are examples only and their use is not mandatory.

2. For rodent cell substrates, murine retroviruses are commonly used as specific ''model'' viruses.

B. Examples of Viruses Which Have Been Used in Viral Clearance Studies.

Several viruses which have been used in viral clearance studies. are listed in Table A–1. However, since these are merely examples, the use of any of the viruses in the table is not mandatory and manufacturers are invited to consider other viruses, especially those which may be more appropriate for their individual production processes. Generally, the process should be assessed for its ability to clear at least three different viruses with differing characteristics.

TABLE A–1.—EXAMPLES OF VIRUSES WHICH HAVE BEEN USED IN VIRAL CLEARANCE STUDIES

Virus	Family	Genus	Natural host	Genome	Envelope	Size	Shape	Resistance[1]
Vesicular stomatitis virus	Rhabdo	Vesiculovirus	Equine Bovine	RNA	yes	70x175 nm	Bullet	Low
Parainfluenza virus	Paramyxo	Paramyxovirus	Various	RNA	yes	100–200+ nm	Pleo/Spherical	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80–110 nm	Spherical	Low
Sindbis virus	Toga	Alphavirus	Human	RNA	yes	60–70 nm	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50–70 nm	Pleo-Spherical	Low
Pseudorabies virus	Herpes		Swine	DNA	yes	120–200 nm	Spherical	Medium
Poliovirus Sabin Type 1	Picorna	Enterovirus	Human	RNA	no	25–30 nm	Icosahedral	Medium
Encephalomyocarditis virus (EMC)	Picorna	Cardiovirus	Mouse	RNA	no	25–30 nm	Icosahedral	Medium
Reovirus 3	Reo	Orthoreovirus	Various	RNA	no	60–80 nm	Spherical	Medium
SV40	Papova	Polyomavirus	Monkey	DNA	no	40–50 nm	Icosahedral	Very high
Parvoviruses (canine, porcine)	Parvo	Parvovirus	Canine Porcine	DNA	no	18–24 nm	Icosahedral	Very high

[1] Resistance to physico-chemical treatments based on studies of production processes. Resistance is relative to the specific treatment and it is used in the context of the understanding of the biology of the virus and the nature of the manufacturing process. Actual results will vary according to the treatment. These viruses are examples only and their use is not mandatory.

Appendix 3

Statistical Considerations for Assessing Virus Assays

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays should be performed to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

1. Variation may arise within an assay as a result of dilution errors, statistical effects, and differences within the assay system that are either unknown or difficult to control.

These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

2. Assay methods may be either quantal or quantitative. Quantal methods include infectivity assays in animals or in tissue-culture-infectious-dose (TCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

3. The 95 percent confidence limits for results of within-assay variation normally should be on the order of ±0.5 $\log_{10}$ of the mean. Within-assay variation can be assessed by standard textbook methods. Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately 0.5 $\log_{10}$ of the mean estimate established in the laboratory for the assay to be acceptable. Assays with lower precision may be acceptable with appropriate justification.

4. The 95 percent confidence limits for the reduction factor observed should be calculated wherever possible in studies of clearance of ''relevant'' and specific ''model'' viruses. If the 95 percent confidence limits for the viral assays of the starting material are +s, and for the viral assays of the material after the step are +a, the 95 percent confidence limits for the reduction factor are in the equation below.

$$\pm\sqrt{S^2 + a^2}$$

Appendix 4

Probability of Detection of Viruses at Low Concentrations

At low virus concentrations (e.g., in the range of 10 to 1000 infectious particles per liter), it is evident that a sample of a few milliliters may or may not contain infectious particles. The probability, *p*, that this sample does not contain infectious viruses is:

$p = ((V\text{-}v)/V)^n$

where V (liter) is the overall volume of the material to be tested, v (liter) is the volume of the sample and n is the absolute number of infectious particles statistically distributed in V.

If V >> v, this equation can be approximated by the Poisson distribution:

$p = e^{-cv}$

where c is the concentration of infectious particles per liter.

or, $c = \ln p / -v$

As an example, if a sample volume of 1 mL is tested, the probabilities *p* at virus concentrations ranging from 10 to 1000 infectious particles per liter are:

c	10	100	1,000
p	0.99	0.90	0.37

This indicates that for a concentration of 1,000 viruses per liter, in 37 percent of sampling, 1 mL will not contain a virus particle.

If only a portion of a sample is tested for virus and the test is negative, the amount of virus which would have to be present in the total sample in order to achieve a positive result should be calculated and this value taken into account when calculating a reduction factor. Confidence limits at 95 percent are desirable. However, in some instances, this may not be practical due to material limitations.

Appendix 5

Calculation of Reduction Factors in Studies to Determine Viral Clearance

The virus reduction factor of an individual purification or inactivation step is defined as the $\log_{10}$ of the ratio of the virus load in the pre-purification material and the virus load in the post-purification material which is ready for use in the next step of the process. If the following abbreviations are used:

Starting material: vol v′; titer $10^{a'}$;
virus load: $v'.10^{a'}$,
Final material: vol v″; titer $10^{a''}$;
virus load: $v''.10^{a''}$,

the individual reduction factors Ri are calculated according to

$10^{Ri} = v'.10^{a'} / v''.10^{a''}$

This formula takes into account both the titers and volumes of the materials before and after the purification step.

Because of the inherent imprecision of some virus titrations, an individual reduction factor used for the calculation of an overall reduction factor should be greater than 1. The overall reduction factor for a complete production process is the sum logarithm of the reduction factors of the individual steps. It represents the logarithm of the ratio of the virus load at the beginning of the first process clearance step and at the end of the last process clearance step.

Appendix 6

Calculation of Estimated Particles per Dose

This is applicable to those viruses for which an estimate of starting numbers can be made, such as endogenous retroviruses. Example:

I. Assumptions

Measured or estimated concentration of virus in cell culture harvest = 10^6/mL
Calculated viral clearance factor = $>10^{15}$
Volume of culture harvest needed to make a dose of product = 1 liter (10^3mL)

II. Calculation of Estimated Particles/Dose

$$\frac{\left(10^6 \text{ virus units / mL}\right)\times\left(10^3 \text{ mL / dose}\right)}{\text{Clearance factor} > 10^{15}} = \frac{10^9 \text{ particles / dose}}{\text{Clearance factor} > 10^{15}}$$

$= <10^{-6}$ particles/dose

Therefore, less than one particle per million doses would be expected.

Dated: May 1, 1996.

William K. Hubbard,

Associate Commissioner for Policy Coordination.

[FR Doc. 96–11641 Filed 5–9–96; 8:45 am]

BILLING CODE 4160–01–F