conjunction with devices, for example, for *ex vivo* purging of cells to remove immune or tumor cells, for *ex vivo* cell collection (e.g. hematopoietic stem cells), or for purification of other products intended for *in vivo* administration. Generally, these mAb should meet the same criteria for safety and freedom from adventitious agents as mAb intended for direct administration to patients. Likewise, reagents that are commonly used in conjunction with mAb for *ex vivo* manipulations of cellular products intended for *in vivo* administration (e.g. complement, DNAase) should meet the same safety standards as mAb intended for direct administration to patients. However, in such cases, some procedures for virus inactivation or removal may be performed on the downstream product rather than on the mAb or other reagent (see II.C.7). Complete information on products that will be used in conjunction with the mAb, such as rabbit complement or DNAase, should be submitted before clinical studies begin. This information can be submitted as a part of the original IND submission or in the form of a Master File.

As used in this document, "**cocktails**" are defined as two or more mAb administered at a fixed ratio. Relevant targets may include multiple antigens on infectious pathogens and multiple tumor-associated antigens. The rationale for combining the products should be clear and based on the clinical context or previous clinical experience with individual products. Lack of interference among the mAb in the combination should be shown and synergistic or additive effects should be characterized. Dose-ranging for each of the components is highly desirable. In some instances, dose-setting may be based on preclinical or clinical data that show the necessity or superiority of a particular dose and ratio of mAb in the combination.

As used in this document, "**panels**" are defined as sets of mAb directed against related antigens from which one or more members would be used for an individual patient based on target antigen characterization. Such panels could be submitted for approval in a single license application. Examples of panels might include anti-idiotype mAb for lymphoma and mAb directed against different bacterial or viral serotypes. Dose-ranging for each mAb would be necessary. During the phase 3 trials to establish efficacy of the entire panel, some clinical experience with each member of the panel should be obtained.

C. FILING INFORMATION

It is not necessary to have all of the information discussed in this document available in the initial IND submission. Rather, much of the information may be developed during clinical development, with guidance from CBER or other appropriate Centers by means of frequent dialogue. At pre-IND meetings, CBER staff may provide guidance in planning clinical development and establishing the format and content of initial IND submissions. Such meetings may be particularly useful when the product is a novel molecular entity or is produced by a novel process, and when drug development plans are unusually complex.

The manufacture of mAb that are produced and controlled by similar procedures in the same facility may in some cases be documented in a single Master File. This may be particularly helpful when data from generic or modular virus clearance studies are used for multiple antibodies that differ only in the variable (v) or complementarity-determining region (CDR) and when multiple antibodies are purified by identical procedures (see Section II.C.6).

See references 3 and 4 for information on filing biologics license applications. An Establishment License Application is no longer required for mAb intended for in vivo use (3).

II. PRODUCT MANUFACTURE AND TESTING

A. GENERAL PRINCIPLES AND DEFINITIONS

Traditionally, most mAb are produced by hybridoma cell lines through immortalization of antibody-producing cells by chemically-induced fusion with myeloma cells. In some cases, additional fusions with other lines have created "triomas" and "quadromas". We anticipate an increase in recombinant mAb (e.g. chimeric or humanized mAb, single-chain or dimeric Fvs, mAbs derived from phage display libraries etc.) and human mAb in the future. These may be produced in animal cell lines (e.g. CHO, SP2/0) transfected with recombinant DNA constructs, in human cells (e.g. immortalized lymphoid cells), in bacteria, yeast, insect cells etc. Novel methods of production for mAb or mAb-derived recombinant proteins may include insects, plants or transgenic animals,.
The principles reviewed in Sections II.B. 1 through 4 may be applied, in general to all hybridoma and heterohybridoma generated products, regardless of the species of origin. All steps in manufacturing of mAb to be used in trials intended to support licensure and of licensed mAb should comply with current Good Manufacturing Practices (cGMPs), as appropriate for the stage of product development.

While manufacturing details and safety issues may be different for different expression systems, some general principles can be applied. The establishment of a reliable and continuous source from which the antibody can be consistently produced is highly recommended (e. g. master cell banks for cell cultures, seed banks for transgenic plants, founder strains for transgenic animals). If transient expression systems are used, master vector seed stocks should be generated, and the genetic stability of the expression constructs used should be tested. Appropriate in-process testing which takes into consideration the specific safety concerns of the expression system used should be instituted. Sponsors are encouraged to consult the most recent available versions of the Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, the Points to Consider in the Production and Testing of New Drugs and Biologicals produced by Recombinant DNA Technology or the Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived From Transgenic Animals (1, 2, 5), the 1996 CBER/CDER Guidance Document on the Submission of Chemistry, Manufacturing and Controls Information for a Therapeutic Recombinant DNA-derived Product or a Monoclonal Antibody Product for In Vivo Use (4), as well as relevant International Conference on Harmonization (ICH) documents (e.g. 6, 7), if applicable to their expression systems. Sponsors considering novel expression systems not specifically covered by guidance documents are encouraged to consult with CBER.

B. MANUFACTURE AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES

1. Cell lines

The following information should be provided in the IND or biologics license application:

a. Source, name, and characterization of the parent cell line, including any immunoglobulin heavy or light chains that it synthesizes and/or secretes, the fusion partner in the case of hybridomas, or the host cell line in the case of transfected cells producing recombinant mAb.

b. Species, animal strain, characterization, and tissue origin of the immune cell.

c. Description of immortalization procedures, if any, used in generating the cell line.

d. Identification and characterization of the immunogen. A complete biochemical characterization may not be possible or necessary in all cases. However, we recommend that as much information as possible be gathered on the nature and characteristics of the material used as an immunogen. Such data can be useful in choosing appropriate potency assays, as well as in evaluating potential for cross-reactivity and possible clinical usefulness. For example, a determinant which is not expressed on the surface of target cells bind necrotic cells better than intact cells.

e. Description of the immunization scheme. In the case of human mAb, any *in vitro* or *in vivo* immunization procedures should be described, as well as any relevant aspects of the subject's medical history.

f. Description of the screening procedure used. In the case of human mAb, steps performed in order to enrich antigen-specific human B cell populations should be described.

g. Description of the cell cloning procedures. If changes in cell culture process (e.g. cells adapted from serum-containing to serum-free medium) are shown not to affect product quality, it is not necessary to reclone the cells or rebank the MCB or WCB. In this context, product quality includes not just the identity, purity, potency and pharmacological characteristics of the purified product, but also its safety profile. For example, possible changes in types and/or titers of viruses detectable in the unpurified bulk material and the ability of the purification process to remove or inactivate them should be addressed).

h. For transfected animal or plant cell substrates, as well as for microbial cell substrates (bacteria, yeast), a detailed description of the vector(s) and final construct(s) generation, including whether or not extraneous amino acid sequences are introduced into the product as a result of subcloning, and description of transfection/transformation, screening and selection procedures (see refs. 1-6). Determination of cDNA sequence(s) of the predominant transcript(s) is acceptable as an indication of clonality of transfected cell lines.

i. For cell culture systems using autonomously replicating vectors (e.g. baculovirus or other transient expression systems) a detailed description of the vector system, construct generation, selection, vector banking procedures, and infection/transfection procedures should be provided (1-6).

j. For all cell substrates, description of the seed lot system for establishing and maintaining the master cell bank (MCB) and the working cell bank (WCB).

2. Production in cell culture

The following information should be provided in the IND or biologics license application:

a. A description of the culture procedures if production is entirely *in vitro* or if cells are passaged *in vitro* prior to mouse inoculation.

b. A description of the culture media used, including certification and testing. Serum additives used in hybridoma propagation should be free of contaminants and adventitious agents.

c. The steps taken to prevent or control contamination by viruses, bacteria, fungi, mycoplasma and transmissible spongiform encephalopaties (TSE) agents. These include, among other things, a description of the equipment, transfers, room classification, employee gowning procedures etc.

d. The acceptance criteria for cells or tissue culture supernatants intended for further manufacture.

3. Production in animals or plants

The following information should be provided in the IND or biologics license application:

a. A description of the cell line used as the inoculum (if any) should be provided (see 2.a. above).

b. Animal care should be in accordance with the NIH Guide for Care and Use of Laboratory Animals. For ascites production, the use of specific pathogen free (SPF) mice is recommended. To ensure manufacture of consistent, high quality ascites for production of mAb, an animal health monitoring program should be in place that encompasses quarantine procedures, sentinel animals, and an in-house health surveillance program (including screening for mycoplasma). Frequency of serological testing of sentinel mice should be established and is usually based on the incidence of virus contamination. Screening programs for known infectious agents should be updated to reflect advances in the knowledge of infectious diseases. Sponsors should be responsible for the adequacy of screening programs.

c. All protocols for ascites production should also incorporate information on: *i*) species, sex and age of animals used; *ii*) animal supplier; *iii*) volume of pristane; *iv*) volume and concentration of cell inoculum; *v*) timing of priming, inoculation, and ascites harvesting; *vi*) frequency and procedure for ascites harvesting; *vii*) definition of a batch; *viii*) animal bedding, food and water; *ix*) number of animals housed together; *x*) environmental conditions under which each procedure takes place and *xi*) number of times cells are passaged from one animal to another, if applicable.

d. For production in transgenic animals, the vectors, constructs and procedures used for gene transfer should be described. The genetic background and characterization of founder animals, the generation and selection of production herds and animal maintenance procedures should be described as well (see ref. 5 for details). Health monitoring programs for animal herds or colonies should be in place, including screening for zoonoses known to exist in captive animals of the relevant species in North America. Programs for screening and detection of known infectious agents should be tailored for the animal species and periodically updated to reflect advances in the knowledge of infectious diseases. Sponsors should be responsible for the adequacy of screening programs (see paragraph b above). When initially establishing transgenic animal strains, the following considerations should be kept in mind: *i*) non-transgenic animals to be used for breeding or gene transfer procedures should be obtained from closed herds or colonies that are serologically well characterized and as free as possible of pathogens of concern for the animal species or for humans; *ii*) the use of imported animals or first generation offspring from imported animals is discouraged and *iii*) animals from species in which TSE have been documented should be obtained from closed herds with documented absence of dementing illnesses and controlled food sources.

e. For production using autonomously replicating vectors (e.g. baculovirus) in live insect larvae, larvae maintenance procedures should be described in detail, including procedures used to control and monitor bioburden.

f. For production in plants (e.g. transgenic plants or autonomously replicating vectors using plants as bioreactors), early consultation with CBER staff is recommended.

4. Purification

Purification schemes for mAb should be described in the IND or biologics license application. We

recommend that mAb purification schemes incorporate the following characteristics:

a. Production techniques that will prevent the introduction of and eliminate contaminants, including animal proteins and materials, DNA, endotoxin, other pyrogens, culture media constituents, components that may leach from columns, and viruses.

b. Incorporation of one or more steps known to remove or inactivate retroviruses in excess of the endogenous particle load, whenever applicable (see Reference 8 and discussion of virus clearance studies in Section II.C.). As a general guidance, we recommend that each purification protocol include at least two orthogonal (i.e. based on different mechanisms) robust virus removal steps (see below). Including these steps would not obviate the need for virus clearance studies, except in the case of products intended for use in feasibility trials in serious or life-threatening conditions (see Section II.D.2.)

> *i.* **Robust virus removal/inactivation steps** are defined as those that have been shown to work well under a variety of conditions (e.g. pH or ionic strength of column buffers) with a variety of mAb. Robust steps include low pH, heat, solvent/detergent treatments, and filtration (see Table III). Sponsors have the option of providing adequate evidence indicating that a step different from these is robust, or is reliably effective for removal/inactivation under the conditions employed. An estimate of the efficiency of robust steps in removing virus may be demonstrated by: (a) cross-referencing master files or reliable scientific literature published in peer-reviewed journals or (b) generic or modular clearance studies (see Section II.C.6. for definition).

c. Demonstration of the ability of the purification scheme to remove adventitious agents and other contaminants, by means of a clearance study. For some contaminants, e.g. DNA, pristane or protein A, such a clearance study, if appropriately carried out, may be an acceptable alternative to routine testing for the contaminant. In the case of virus clearance studies, we recommend the use of several model viruses encompassing large and small particles, DNA and RNA genomes, as well as chemically sensitive and resistant lipid enveloped and non-enveloped strains. Human blood products should be avoided in production of other biologicals. When human blood products that may be contaminated with hepatitis C virus (HCV) or other infectious agents must be used in production (e.g. as media additives), such schemes should include viruses that are acceptable models for HCV, such as bovine viral diarrhea virus (BVDV) or Sindbis virus. Retrovirus clearance studies should be performed prior to phase 1 trials, except for products intended for use in the setting of serious or life-threatening conditions in feasibility trials (see Section II.D.). Clearance studies for other viruses and/or other contaminants should be carried out prior to production for phase 2/3 trials and may need to be repeated if the final manufacturing process has changed. ICH guidelines are currently being drafted to address in further detail the viral safety evaluation of biotechnology products derived from cell lines of human or animal origin.

d. Limits should be prospectively set on the number of times a purification component (e.g. a chromatography column) can be reused. Such limits should be based upon actual data obtained by monitoring the component s performance over time.

e. As a product is developed, retention samples from each production should be saved under appropriate conditions so that side-to-side comparisons may be made to determine product comparability (see Section II.E.).

f. A description of the purification room(s) design features, HVAC and other support systems, equipment, transfers and personnel should be provided. Emphasis should be placed on operational features that minimize the risk of contamination from the environment or cross-contamination from other products.

5. Characterization of purified unmodified mAb

Before a mAb is studied in humans, a precise and thorough characterization of antibody structural integrity, specificity, and potency should be conducted and described in the IND. The mAb should be as free as possible of non-Ig contaminants. A properly qualified in-house reference standard with known characteristics, specificity, and potency, and that is stored under appropriate conditions and periodically tested to ensure its integrity, should be used for lot-to-lot comparisons. Reference standards should be updated as a product evolves but should be finalized by the start of phase 3 trials. Appropriate standard operating procedures (SOPs) should be developed for qualification of a new reference standard.

a. Structural Integrity

A combination of SDS-PAGE, IEF, HPLC, mass spectrometry, or other appropriate physicochemical methods should be used to show that the purified antibody is not fragmented, aggregated, or otherwise modified (e.g. loss of carbohydrate side chains). Side-by-side comparisons of production lots to the in-house reference standard should be performed.

b. Specificity

Assays should provide evidence that the binding of the mAb to the target antigen is specific. Once the specificity of the antibody is characterized, it should be screened for cross-reactivity with human tissues (see Section III.A.). The following are some suggestions on the design of specificity studies:

i. Direct binding assays should include both positive and negative antibody and antigen controls. At least one isotype-matched, irrelevant (negative) control antibody should be tested. Negative antigen controls should include a chemically similar, antigenically unrelated compound, if available (e.g. similar chemical nature, size, charge, and charge density).

ii. Whenever possible, the protein, glycoprotein, glycolipid, or other molecule bearing the reactive epitope, should be biochemically defined, and the antigenic epitope, itself, determined. If the antigenic determinant is a carbohydrate, the sugar composition, linkage, and anomeric configuration should be established.

iii. If possible, fine specificity studies using antigenic preparations of defined structure (e.g. oligosaccharides or peptides) should be conducted to characterize antibody specificity by means of inhibition or other techniques. For complex biological mixtures, the lots of test antigen and/or inhibitors used for direct binding tests should be standardized. Inhibition of antibody binding by soluble antigen or other antibodies should be measured quantitatively.

iv. Once the specificity of an antibody has been determined, it is important to quantitate antibody binding activity by affinity, avidity, immunoreactivity, or combinations of these assays, as appropriate. A number of published methods are suitable for measurement of antibody binding activity (9, 10).

c. Potency Assays and Potency Specifications

Potency assays are used to characterize the product, to monitor lot-to-lot consistency, and to assure stability of the product. Potency may be measured by a binding assay, a serologic assay, activity in an

animal model, and/or a functional assay performed *in vitro* or *in vivo*. It is desirable that the assay(s) bear the closest possible relationship to the putative physiologic/pharmacologic activity of the product and be sufficiently sensitive to detect differences of potential clinical importance in the function of the product. In particular, when the performance of the antibody depends not only upon antigen binding but also on other critical functions, it is desirable that the potency assay(s) measure all such functions. Documentation of the potency assay's performance, including sensitivity, intra- and inter-assay variation and robustness, should be provided.

i. Antibody binding activity may be quantitated by ELISA, RIA, radioimmune precipitation, cytotoxicity, flow cytometry, or any other standard, appropriate method. Activity should be expressed as specific antigen-binding units per mg or • g of antibody. Product should be compared to an in-house reference standard. Appropriate measurements of antibody affinity, if established, may be a useful adjunct to other assays. Parallel line bioassay or a similar, valid statistical procedure should be used in calculating potency.

ii. The potency of a mAb may also be tested by measurement of *in vivo* function in animal models, although such assays are often cumbersome and difficult to standardize and should not be the sole measure of potency.

iii. The permissible range of values in potency assays that reflects adequate biological activity of a product should be based on experience with a particular antibody. Ideally, potency assays should be correlated with *in vivo* activity in order to develop control tests which will ensure an effective product. This implies that multiple production lots should be used during the clinical development program and potency assay results should be correlated with clinical performance. When clinical performance is measured by *in vitro* tests used as surrogates of efficacy, such tests should be validated in a phase 3 clinical trial of appropriate design.

6. Anti-idiotype vaccines

The following issues should be addressed for anti-idiotype vaccines:

a. In the case of an anti-idiotype vaccine (Ab2 vaccine), the Ab2 immunogen should be characterized as to the Ab2 type, e.g. classical type (Ab2•) or antigen mimic (Ab2•) (11).

b. Ab2• vaccines should be shown to be reactive with the appropriate population of human Ab1 (antibody to nominal antigen) if such antibodies are available.

c. The Ab2 preparation should be studied for the appropriateness of response (to target antigen) in xenogeneic as well as syngeneic animals (12).

7. Monoclonal antibodies conjugated with toxins, drugs, radionuclides or other agents (immunoconjugates)

Immunoconjugates are typically produced by chemical processes using specific reagents to link the unconjugated antibody with a non-antibody agent. Alternatively, immunoconjugates can be obtained as chimeric recombinant proteins containing non-immunoglobulin and immunoglobulin sequences in the same polypeptide chain. In addition to previously discussed recommendations for unconjugated (naked) mAb, manufacturers of immunoconjugates should address the following:

a. Construction of the Immunoconjugate.

A full description of the reagents and the process used to construct an immunoconjugate should be submitted, including:

i. A description of components such as toxins, drugs, enzymes, and cytokines that are linked to the mAb, including: the source, structure, production, purity (including demonstration of freedom from adventitious agents), and characterization of all components (if components are purchased, a certificate of analysis should be supplied).

ii. A description of chemical components, such as linkers and chelating agents, that will be used in preparing the immunoconjugate. These should include documentation of the sources of reagents and method of preparation and determinations of residual impurities from synthesis or purification. Charts of the synthetic reaction pathways and any relevant published or in-house data concerning the toxicity of chemicals used in the production of an immunoconjugate should be provided.

iii. The average ratio of coupled material to antibody and the number of conjugated moieties per antibody should be determined as the first step in establishing lot release criteria for the final product and developing the relationship between immunoglobulin substitution number, potency, and stability.

iv. Products prepared using recombinant DNA technology (e.g., derived from transfected cell lines or microbial cell substrates, chimeric, reshaped, complementarity determining region [CDR] grafted, single chain Fv antibodies, and recombinant immunoconjugates) should follow recommendations discussed in references 1-7, as appropriate. The stability of recombinant immunoconjugates should be studied carefully, as such chimeric proteins may have altered conformational stability, solubility or tendency to aggregate compared to their component polypeptides in their native structures. Loss of specific immunoreactivity due to denaturation or formation of aggregates (e.g. diabodies formed by recombinant Fvs) may lead to altered pharmacokinetics and/or binding to non-target tissues.

b. Purity of the Immunoconjugate

i. Special care should be taken to ensure that the antibody preparations are as free as possible of extraneous immunoglobulin and non-immunoglobulin contaminants as such contaminants could react with nuclides, toxins or drugs during the construction of the immunoconjugate.

ii. The amount of free antibody and free components in the final product should be determined with limits set for each. Reactive intermediates should be inactivated or removed.

c. Immunoreactivity, Potency and Stability of the Immunoconjugate

Coupling of toxin or drug to an antibody may alter the activity of either component.

i. Immunoreactivity before and after coupling should be assessed using appropriate methodology (9, 10).

ii. Activity of the non-immunoglobulin component of immunoconjugates, should be assessed by a potency assay whenever appropriate (e.g., toxins, cytokines or enzymes, but not radio-

immunoconjugates intended for use in imaging)

iii. Limits on the percent change in immunoreactivity resulting from construction of the immunoconjugate should be established as part of product specifications.

iv. The immunoconjugate should be tested for stability *in vitro* by incubation in pooled human serum at 37° C under sterile conditions. Plasma may be used instead of serum, provided that the anticoagulant used does not affect the stability of the immunoconjugate (e.g., chelating agents may react with some radioisotopes, heparin may interact with basic proteins, etc.). Aliquots should be analyzed at timed intervals for the concentration of intact immunoconjugate and degradation products. The conditions under which product stability is evaluated and the positive and negative controls used should be fully described. Stability in human serum or plasma is not relevant for topically administered immunoconjugates which are demonstrated not to be absorbed into the bloodstream. It should be established whether or not such immunoconjugates or their components are detectable in plasma after topical administration, and whether or not they elicit an immune response.

d. Specific Issues Related to mAb Coupled to Radionuclides

The preparation of the radioimmunoconjugate should be performed in a standardized, well-controlled, and validated manner. Methods should be developed to estimate the percent radioactivity in each of the three species of concern: free isotope, conjugated mAb, and labeled, non-mAb substances.

i. It is recommended that the initial IND submission for a radiolabeled mAb contain analytical results from two to three radiolabeling runs that demonstrate the preparation of an immunoreactive, sterile, and pyrogen-free product. These radiolabeling runs should be performed by the same personnel who will radiolabel the mAb for the study, using the reagents that will be used for the study.

ii. Radiopharmaceutical grade isotopes should be used when preparing immunoconjugates. The sterility and pyrogen-free nature of each isotope should be documented by submission of a certificate of analysis and letters of cross-reference for manufacturing information.

iii. The concentrations of covalently-bound and free isotope in the final product as well as residual levels of labeling reagents and their decomposition products should be determined during the trial labeling runs.

iv. Quality control tests that will be performed before and/or after each patient administration should be described.

v. When appropriate, colloid formation by the radio-immunoconjugate should be determined and limits set for it.

C. QUALITY CONTROL AND PRODUCT TESTING

1. Cell line qualification

Qualification of the cell line for production of a mAb to be used as a biologic therapeutic should include screening the master cell bank (MCB) and the working cell bank (WCB), at least on a one-time basis, for endogenous and adventitious agents utilizing the tests outlined in Table I and described in the Points to

Consider in the Characterization of Cell Lines Used to Produce Biologicals (1). Because the WCB is derived from the MCB and propagated for only a few additional passages in tissue culture, abbreviated testing for detecting newly introduced contaminants is acceptable. Any virus contaminant should be quantified and, wherever possible, identified in order to establish the extent of virus clearance that the purification process should achieve (see also Section II.C.5.). The tropism of virus contaminants for human cells should be determined by appropriate infectivity assays. In the case of tissue culture or fermenter production, end-of-production cells (EPC) should be tested at least once to evaluate whether new contaminants are introduced or induced by the growth conditions. EPC should also be re-examined when there are changes in culture medium or in the scale of production. Cells at the limit of *in vitro* age used for production can be tested in lieu of EPC to allow for possible extensions in the length of time cells are kept in culture as manufacturing schemes are developed. The term EPC will be used throughout this text for ease of consultation. For cell lines which are known not to be susceptible to infection with mammalian viruses (e.g. plant cells, some insect cells), bacterial and fungal sterility testing, and in some cases testing for Mycoplasma or other mollicutes such as Spiroplasma, will be the most important concerns. Consultation with CBER is advised before using these cell substrates.

Table I
Cell Line Qualification

Tests	MCB	WCB	EPC
Sterility	+	+	+
Mycoplasma	+	+	+
Virus			
Adventitious	+	-	+
Species-specific*	+	-	-
Retrovirus	+	-	+
Authenticity	+	+	+

* Tests for rodent, primate, or human viruses (other than retroviruses), as appropriate
Retrovirus testing is not required for murine hybridomas. All other cell substrates should be tested as described in Section II.C.1.d.

a. Cell lines should be free from bacterial and fungal contamination as demonstrated by sterility testing. Recommended testing procedures for mycoplasma (cultivable and non-cultivable) are described in Ref. 1.

b. Screening for adventitious viruses (other than retroviruses) should include routine *in vivo* and *in vitro* tests (1).

c. Screening for species-specific viruses (other than retroviruses):

i. The mouse antibody production (MAP) test for murine cell lines (see Appendix II), the HAP test for hamster lines, and the RAP test for rat lines should be used. *In vivo* testing for lymphocytic choriomeningitis (LCM) virus, including non-lethal strains, is recommended. Testing of hamster cell lines should include minute virus of mice (see III.B.1.c.).

ii. Material that is contaminated with LCM, reovirus, Sendai virus, or Hantaan virus should not be used for mAb production.

iii. Cell lines from non-human primates should be screened for the following: herpes viruses (simiae and SA-8), cytomegalovirus (sCMV), encephalomyocarditis virus, simian hemorrhagic fever virus (SHF), varicella virus of simians (sVZV), adenovirus, SV-40, monkeypox, rubeola, and Ebola virus. Any other zoonotic agents suggested by the cell line derivation history should be screened for.

iv. Human cell lines should be screened for Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B (HBV) and HCV, human herpes virus 6 (HHV-6), and any other viruses that are suggested by the medical history of the donor and type of tissue used to establish the original line. Cells from patients who are known to have developed Creutzfeld-Jakob disease (CJD) or other TSE, or from persons with two or more genetically related family members with CJD, should not be used.

v. Heterohybridomas using cells from 2 different species should be tested as appropriate for both species of origin.

vi. For cell lines of other species please consult with CBER.

d. Retrovirus testing of cell lines: Retrovirus contamination of cells from different species varies. The following should be considered when designing studies to detect retrovirus:

i. Murine cells used to produce monoclonal antibodies should be considered inherently capable of producing infectious murine retrovirus. The amount of retrovirus in the unprocessed bulk should be quantitated on a series of bulk harvests and shown to be consistent from lot to lot (1). Endogenous virus particle burden should be determined at the end of a typical fermentation, prior to purification, preferably by thin-section EM on material pelleted by ultracentrifugation. Particle burden determination is preferable to infectivity assays at this stage of production because it does not depend upon the susceptibility to infection of the cell lines used for virus amplification and it provides a "worst case scenario" of the level of viral contamination. Thin-section EM also allows morphological observation of viruses. Other, novel methods of equal or superior sensitivity and general applicability may be acceptable, if appropriately validated. Sufficient retrovirus removal by the purification scheme should be demonstrated (see also Section II.C.4.).

ii. Rat myeloma cell lines and hybridomas may not express retrovirus (13).The absence of detectable retrovirus, however, should be demonstrated by co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay, including examination of EPC and several production lots. If retrovirus is not detected by infectivity assays or electron microscopy, further clearance studies may not be needed. It is suggested that purification schemes for mAb produced by rat cell lines include one or more robust retrovirus inactivation or removal step.

iii. CHO cell lines express defective retrovirus particles (14). Whether hamster cell lines express infectious retroviruses has not been shown. Sponsors should demonstrate the lack of infectious hamster retroviruses by means of the most sensitive infectivity assays available. These include co-cultivation of the test article with a cell line(s) susceptible to a wide range of retroviruses combined with a sensitive detection assay. As a product moves into pivotal clinical trials, it may be necessary to make additional attempts to detect potential infectious virus by utilizing a wider range of indicator cells, including human cell lines (15, 16). Because of uncertainty about the validity of infectivity assays for hamster retroviruses, sufficient retrovirus particle removal by the purification scheme should be demonstrated (see also Section II.C.4-5). It is suggested that purification schemes for mAb produced by hamster cell lines include one or more robust retrovirus inactivation or removal step.

iv. Hybridomas or transfected clones produced from cells of non-human primate or human origin should be examined for the presence of retrovirus. Generic assays, such as transmission electron microscopy (TEM) or reverse transcriptase (RT) can be used to assess the presence of retrovirus. Other assays may be used, as long as they are appropriately validated. In addition, all primate cell lines should be screened for simian immunodeficiency virus (SIV), simian T lymphotropic virus (STLV), Foamy virus, human T lymphotropic virus (HTLV), and human immunodeficiency virus (HIV). Cell lines from non-human primates should be additionally tested for presence of simian retroviruses (SRV).

e. Each cell clone generated by stable transfection of widely used parental lines (e.g. CHO) should be considered as a new cell line from the standpoint of viral safety. Such clones may have significantly

different genetic characteristics compared to the parental line as a result of the transfection procedure itself, the clonal selection process and positional effects due to random integration of vector DNA into the cellular genome. Each clone should be screened for retroviruses and for adventitious viruses as described above and in Table I. Screening for species-specific viruses can be done once, on the MCB of the parental line.

f. Authenticity testing should confirm the cell line's species of origin, identity, and lack of cell-line cross-contamination.

2. Lot-to-lot quality control monitoring of unprocessed bulk lots and purified bulk lots, and final product specifications

Quality control monitoring should be performed on each lot of product, as defined in 21 CFR 600.3(x). Table II summarizes lot-to-lot product safety testing.

Table II
Lot-to-lot product safety tests

Tests	Unprocessed Bulk	Purified Product	Final Product
Sterility	+*	+	+
Mycoplasma	+	-	-
Virus			
Adventitious	+	-	-
Species-specific	+	-	-
Retrovirus	+	-	-
Polynucleotide	-	+	-
Endotoxin	-	-	+

*Bioburden testing with acceptable limits is sufficient at this stage.

In vitro testing with three indicator cell lines should be performed routinely for non-ascites material. *In vivo* testing is generally done once for non-ascites material but should be repeated when production methods change.

MAP, RAP or HAP testing for ascites only..

Quantitation of retrovirus (preferably by TEM) in the unprocessed bulk is important for murine hybridomas. For other hybridomas, generic assays for detection and quantitation of retrovirus, such as TEM coupled with appropriate co-cultivation assays are important if MCB or EPC are positive.

a. Unprocessed Bulk Lots

i. There should be set limits for bacterial bioburden in unprocessed bulk material. If bioburden testing of pooled ascites harvests shows the presence of viable contaminants, they should be quantified, and allowable limits for bacterial contamination should be set based on manufacturing experience. The identity of the bacterial species should be determined on a periodic basis and whenever the allowable limits for contamination have been exceeded. Filtration of ascites

harvests through a • 0.45 • m filter prior to storage is recommended (see also II.C.2.a.*ii.*).

ii. Tests for cultivable and non-cultivable mycoplasma should generally be performed on unprocessed bulk hybridoma supernatants, prior to any clarification by filtration (1). The filtration of unprocessed bulk ascites through a 0.45 • m filter followed by storage at • -60° C prior to testing for mycoplasma is acceptable if samples of unfiltered material are retained for testing. If mycoplasma contamination of animals or unpurified bulk ascites or hybridoma supernatants is detected, these should not be used or processed further.

iii. *In vitro* virus testing with three indicator cell lines (e.g. Vero, MRC5, 3T3) should be performed routinely. *In vivo* testing is generally done once (as part of cell line qualification, Section II.C.1) but should be repeated when production methods change (1). Bioreactors containing hamster cells can become contaminated with minute virus of mice that may escape detection in routine *in vitro* assays. MAP testing or PCR testing for this virus appear to be more sensitive. In all cases, the frequency of monitoring should be specified in SOPs and justified based on actual experience when using continuous production in contrast to batch production. When contamination with a particular virus is encountered in a facility, consideration should be given to modifying the routine testing program in order to detect that virus.

iv. Species-specific virus testing should be performed (see Table II and II.C.1.c.).

v. Murine retrovirus contamination should be quantitated routinely for bulk ascites harvests. This may not be necessary if the sponsor can provide data showing that little variation exists in the concentration of mAb and retroviral load of their bulk ascites over several consecutive manufacturing runs, and the purification scheme used can remove substantially more than the highest load observed. If ascites production uses different groups of mice, periodic serologic monitoring for species-specific viruses should be performed on each group prior to their use for producing ascites. For tissue culture harvests, retrovirus contamination should be quantitated on three clinical grade production lots in order to establish the level of virus contamination for the specific cell line and manufacturing process (1) (see II.C.4). This quantitation of retrovirus should be done preferably by generic assays such as TEM or alternatively by sensitive infectivity assays (see also Section II.C.1.d.). Quantitation should be repeated when changes in tissue culture media, duration or scale of culture are made.

vi. For hybridomas of non-murine origin or other cell substrates, see section II.C.1.d. for appropriate assays to determine whether retrovirus is present. In those cases where MCB or EPC are positive for retrovirus, each lot of unpurified bulk should be examined for detection and quantitation of retrovirus by generic assays, such as TEM, coupled with appropriate co-cultivation assays.

b. Purified Bulk Lots (Drug Substance)

In addition to lot-to-lot safety testing summarized in Table II, routine testing on purified bulk lots of unmodified and modified mAb product should include the following determinations (for discussion of immunoconjugates see Section II.B.7.):

i. If a cell bank containing a known infectious agent is used, CBER staff should be consulted before proceeding with development, and clearance studies should be conducted to demonstrate the removal/inactivation of this agent by the mAb purification scheme. Testing for murine

retrovirus during clearance studies should employ infectivity assays which detect ecotropic recombinant murine retrovirus (ERV) and the polytropic or mink-cell focus-forming murine retrovirus (MCF). The infectivity assay should be comprised of an amplification period on a cell line sensitive to infection by these murine retroviruses (for example, Mus dunni cells, 17) coupled with an appropriate indicator assay (for example, PG4 S+L- assay for MCF virus, immunofluorescence assay with appropriate antibodies for detection of ERV). Assays which do not rely on infectivity, such as PCR-based assays, may be substituted, provided they have been validated for sensitivity and specificity, and that their results are correlated with those of infectivity assays. The first consecutive 3-5 lots of purified bulk should be tested to confirm that the contaminant was removed by the purification scheme. For cell lines containing viruses or virus-like particles, the absence of detectable 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 or commercial scale should be provided. However, for cell lines for which the endogenous particles have been extensively characterized, such as CHO cells, and adequate clearance has been demonstrated, it is not usually necessary to assay for the presence of non-infectious particles in purified bulk. Should a human infectious agent be identified in the cell bank, every lot of purified product should be tested and consultation with CBER staff is recommended before extensive product development.

ii. Chemical purity including the residual amounts of extraneous animal proteins, e.g., albumin, immunoglobulin or other contaminants in the final product. An SDS-PAGE analysis, under both reducing and non-reducing conditions, of increasing amounts of purified material should be provided. Generally, silver staining methods are more sensitive but less quantitative than Coomassie blue for SDS-PAGE.

iii. Molecular integrity, including the presence of aggregated, denatured or fragmented product.

iv. Immunoglobulin class or subclass, if used as a test of identity.

v. IEF pattern of the antibody (or its heavy and light chains) in each bulk lot with comparison to the in-house reference standard.

vi. Sterility.

vii. Lot-to-lot testing for DNA content, prior to any excipient addition, is recommended as a way to monitor purification efficiency and reproducibility. DNA content in the final product should be as low as possible, as determined by a highly sensitive method. Low cell viability at harvest may contribute to high DNA levels in unprocessed bulk. It is suggested that, whenever possible, the final product contain no more than 100 pg cellular DNA per dose (18). It is suggested that a method with a sensitivity of 10 pg be used to determine DNA levels (2). An appropriately conducted clearance study for DNA removal may be an acceptable substitute for lot-to-lot testing.

viii. Tests for detection and quantitation of potential contaminants or additives (e.g., antibiotics, other media components, host cell proteins, chromatography reagents, preservatives, or components that may be leached from affinity chromatography columns such as protein A). Whenever possible, contaminants introduced by the recovery and purification process should be below detectable levels using a highly sensitive analytical method. However, for many of these potential contaminants, depending upon their potential for toxicity or immunogenicity, an

appropriately conducted clearance study may be an acceptable substitute for lot-to-lot testing. For products intended for marketing, at least 3 exhibit lots should be tested to confirm the removal of the contaminant(s) for which clearance studies have been conducted. Such clearance studies for product contaminants may have to be repeated when manufacturing schemes are changed. We recommend that antibiotics, particularly penicillin or other beta lactams, not be used. However, if they are used, their removal must be demonstrated by an adequate clearance study. The acceptability of trace contaminants that cannot be removed by standard methods should be discussed with CBER prior to the submission of an IND. Pristane, if used in the propagation of ascites fluid, should be shown to be undetectable by a sensitive test.

ix. A brief description of the formulation process and areas to be used for it should be provided. This description should incorporate information on the processing area, support systems, personnel and product transfers in sufficient detail to highlight the design or operational features utilized to minimize contamination or cross-contamination.

c. Final-Filled Product (Drug Product)

The following tests should be performed on the contents of final containers from each filling of product as defined in 21 CFR 600.3(y). In certain situations (e.g. user-radiolabeling), special approaches to final container testing may need to be developed on a case-by-case basis after discussion with CBER:

i. Protein quantity.

ii. Potency (21 CFR 610.10).

iii. Purity (21 CFR 610.13). Electrophoretic migration of the product in both the native and reduced states on polyacrylamide gels with comparison to the in-house reference standard can be used as a test for purity.

iv. Sterility (21 CFR 610.12).

v. A test for endotoxin. The Limulus Amebocyte Lysate (LAL) assay may be an acceptable equivalent method as allowed in 21 CFR 610.9 when validated by the rabbit pyrogen test as described in 21 CFR 610.13. A U.S. licensed test system should be used to perform the LAL assay. Comparative testing should be repeated when LAL lots made by a different manufacturer are used. Conditions necessary for comparative testing of the rabbit pyrogen and LAL assay procedures should be discussed with CBER on a case-by-case basis. See also "Guidelines on Validation of the LAL Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices", December , 1987 (19).

vi. An appropriate identity test (21 CFR 610.14).

vii. Moisture (21 CFR 610.13) testing, when appropriate.

viii. Preservative (21 CFR 610.15) testing, when appropriate.

ix. Excipients, when appropriate

x. pH, when appropriate.

xi. The areas used for final fill of drug products should follow the recommendations provided in the Guidelines on Sterile Drug Products Produced by Aseptic Processing (20). For information on validation of equipment used in aseptic processing, refer to the Guidelines for Submitting Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (21).

3. Stability of product

Product stability should meet the demands imposed by the clinical protocol. Accelerated stability testing data may be supportive but do not substitute for real-time data for product approval and labeling.

a. A stability testing program should be developed that includes tests for physico-chemical integrity (e.g., fragmentation or aggregation), potency, sterility, and, as appropriate, moisture, pH and preservative stability, at regular intervals throughout the dating period. See also "Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics" (22) and the relevant ICH document (7). For products that are in clinical trials, significant changes that occur during storage should be reported to CBER. For license applications, and preferably prior to each stage of development, stability tests that support the proposed dating period should be performed on the final-filled product, using the container and closure configuration intended for distribution. For biologics license applications, storage of production intermediates (e.g. unpurified bulk, purified bulk) also should be supported by stability testing.

b. Stability tests for assuring biological activity (e.g. quantitative *in vitro* potency assays) should include a manufacturer's in-house reference standard. Whenever possible, a single lot of test antigen (e.g. purified antigen, cells or tissue) should be used throughout the study. A quantitative potency assay(s) should be used to permit a meaningful comparison of activities.

c. Accelerated stability studies, i.e. stability testing after storage at temperatures exceeding routine storage temperatures, may help to identify and establish which tests are stability-indicating. Specific parameters that indicate stability should be monitored by trend analysis on every lot on the stability testing program.

4. General considerations on quantitation and removal of a retrovirus contaminant

The amount of any retrovirus contaminant in the unprocessed bulk product should be estimated. If a retrovirus is detected, any possible or suspected tropism for human cells should be explored (e.g. by co-cultivation assays). This should be followed by demonstration of removal of the contaminant, using the contaminant itself or a representative analogue of the known contaminant (e.g. a model retrovirus) in a model purification system (1).

Unprocessed bulk supernatant concentrates or ascites should be assayed prior to any manipulation other than clarification by low speed centrifugation, unless it can be shown that virus testing would be made more sensitive by initial partial processing. We recommend that retroviruses contaminating ascites or supernatants produced by rodent cell lines be quantitated by TEM of concentrated samples of supernatant. If TEM results are negative, it should be assumed that the titer of retrovirus is equivalent to the lowest limit of detection ($1x10^6$/ml). Ascites and supernatants produced by non-rodent or hybrid cell substrates should be assayed as described (see Section II.C.2.b.*vi.*). Demonstration that retroviruses are removed or inactivated by the purification scheme should occur prior to phase 1 studies, except for mAb intended for use in feasibility trials in serious or life-threatening conditions (see Section II.D.). References 1, 2 and 8,

and paragraphs 5 and 6 below, also discuss the design of studies to demonstrate retrovirus removal. The goal of such studies is to demonstrate that the purification process is able to remove substantially more virus than is estimated to be present in a single dose equivalent of starting material.

5. General considerations on the design and interpretation of virus clearance studies

These considerations are provided here solely for guidance purposes in the design of virus clearance (i.e., removal/inactivation) studies.

a. General Experimental Design

The objective of a virus clearance study is to provide a quantitative estimate of the level of virus reduction provided by the removal/inactivation procedures. Thus, the study should be designed, conducted and analyzed in a manner that will provide accurate information to reliably assess the ability of these procedures to remove/inactivate viruses. The study, including virus infectivity assays, should be designed according to good scientific practice to yield data with accuracy and precision that are amenable to statistical analysis. Each clearance study should include an appropriate control experiment performed in parallel to the experimental condition to assess virus inactivation caused by experimental manipulation (dilution, concentration, filtration, storage etc.). Any observed difference should be used to adjust the virus reduction/inactivation values for each removal/inactivation procedure. It is preferable that the study be conducted under current Good Laboratory Practices (21 CFR, Part 58).

b. Statistics

Virus clearance studies should be supported by appropriate statistical analysis demonstrating that the study is valid and reliable. Virus infectivity assays used to quantitate the virus titer should be sensitive, reproducible and conducted with sufficient replicates to demonstrate statistical accuracy. Sufficient sample volumes should be tested to ensure that there is a high probability of detecting virus in the sample if present. The experimental variability present in virus titration should be determined in virus infectivity assays and reported using confidence intervals. Within assay variability should be calculated using conventional means (standard deviation, standard error of the mean, etc.). Between assay variation can be monitored by the inclusion of an appropriate virus reference preparation run in parallel with unknown samples. The 95% confidence intervals for within assay variation should be established and reported for each virus infectivity assay, and should be in the order of • 0.5 $\log_{10}$ of the mean. The quantitative estimate of virus reduction for each procedure should be reported as the reduction factor. This is defined as the $\log_{10}$ of the ratio of the virus load in the starting material to the virus load in the post reduction material ($\log_{10}$ (volume x virus titer of starting material / volume x virus titer of post-reduction material)). In general, this virus reduction factor should be based upon the amount of virus detected in the spiked starting material. The 95% confidence interval for each reduction factor is calculated from the 95% confidence interval of the virus infectivity assays at the beginning (+s) and end (+a) of each procedure using the formula • $\sqrt{a^2+b^2}$. Such confidence intervals should be calculated whenever possible in studies of clearance using relevant or specific model viruses (with respect to viruses actually detected in the cell line as opposed to non-specific model viruses). The reduction factors for each procedure are summed to calculate an overall reduction factor for the entire process. Additivity of reduction factors assumes that different steps are independent, i.e. have different mechanisms of action so that virus forms (mutant, aggregates etc.) escaping one step do not have a higher likelihood of also escaping the other. Reduction factors from two steps with the same mechanism of action (e.g. two incubations at low pH) are not necessarily additive. Due to the high intrinsic variability of infectivity assays, reduction factors of one log or less are considered negligible and are not included in the overall reduction factor.

6. Generic or modular virus clearance studies

a. A generic clearance study is one in which virus removal and inactivation is demonstrated for several steps in the purification process of a model antibody. These data may then be extrapolated to other antibodies following the same purification and virus removal/inactivation scheme as the model antibody.

b. A modular clearance study is one that demonstrates virus removal or inactivation of individual steps during the purification process (column chromatography, filtration, pasteurization, solvent/detergent, low pH, etc). Each module in the purification scheme may be studied independently of the other modules. Different model mAb may be used to demonstrate viral clearance in different modules, if necessary. If the purification process of a product mAb differs at any of the virus removal or inactivation modules from the model mAb, this module must be studied independently from the model. The other, identical modules in the procedure may be extrapolated to the product mAb.

c. Applicability: For monoclonal antibodies manufactured at one site, generic or modular clearance data may be extrapolated to other monoclonal antibodies of the same species and H and L chain class and, generally, subclass, derived from the same source (e.g. ascites or tissue culture) and cell substrate. Generic and/or modular clearance will apply only when the mAb have similar biochemical properties and are purified by identical methods. Particular attention should be paid to column elution buffer conditions, including pH and ionic strength, the sequence of columns, protein concentration, dwell times, flow rate, pressure, temperature, and potential problems associated with scale-up, at all steps of virus inactivation and removal. In some cases, sponsors may demonstrate virus removal/inactivation for a particular module at two different values of a given parameter (e.g. ionic strength, dwell time, temperature) and use any values of that parameter falling within this range. In order to apply generic or modular clearance algorithms to a specific product, it is necessary to determine the virus load in the unpurified bulk for each specific product using at least 3 lots (see Section II.C.2.). This information is then used to determine whether the total reduction/inactivation factor provided by the purification procedure ensures that substantially more virus is removed or inactivated than the estimated unpurified bulk titer. For example, generic clearance might apply to a series of murine mAb of different specificities but of the same H and L chain isotypes that are purified in an identical manner, or to a series of humanized mAb of the same H and L chain isotype but with different CDRs, that are purified by identical methods. The concepts of generic and modular virus clearance studies do not apply to products of entirely human origin or to products that have the potential to be contaminated by human pathogens. Consultation with CBER is advised before applying generic or modular virus clearance studies.

d. Virus Clearance Master Files: A sponsor purifying mAb for a variety of applications or a manufacturer producing mAb for a variety of sponsors may submit a Master File containing the data demonstrating virus removal or inactivation for different purification schemes. This Master File may then be cross-referenced in INDs/IDEs or license applications using mAb purified by these schemes if applicability criteria are met.

7. Product testing requirements for mAb used as ancillary products

Testing requirements for mAb used as ancillary products (see I.B. for definition) vary depending on the clinical indication and the stage of product development. From a safety standpoint, they should be characterized in the same way as products intended for *in vivo* administration. However, production steps which follow purification of the mAb can be used as part of the virus removal/inactivation scheme. These include, for example, conjugation of mAb to solid phases for affinity purification, sanitation of affinity columns etc. The concepts of generic and modular clearance studies apply to ancillary products as well. Purity of the ancillary product may not be as critical as for products intended for *in vivo* administrations, provided that the performance of the ancillary product in the production of the final product is acceptable

and reproducible, limits are set for impurities and the nature of impurities is known. Leaching of mAb or impurities from the mAb preparations into the final product should be taken into consideration in testing the final product, as appropriate. Removal of mAb or impurities from the mAb preparation may be demonstrated by means of a clearance study. Labeling for the final product may need to carry precautionary statements about potentially toxic or immunogenic residual impurities.

D. PRODUCT SAFETY TESTING FOR FEASIBILITY CLINICAL TRIALS IN SERIOUS OR IMMEDIATELY LIFE-THREATENING CONDITIONS

1. General considerations

The extent of product testing necessary before a particular clinical trial is initiated depends on the source and nature of the product, the stage of product development and the clinical indication. Abbreviated testing needs described in this Section (II.D.) apply to feasibility clinical trials in serious or immediately life-threatening conditions for which no effective alternative treatment exists. Abbreviated testing does not apply to human products made in human cell substrates, but may apply to recombinant products made from transfected human genes, depending on the cell substrate. Consultation with CBER is strongly advised for sponsors considering the application of abbreviated testing in this setting to products that have the potential to be contaminated by human pathogens. For the purpose of this document, the following definitions should be considered:

> *i*) **Feasibility clinical trials.** These are pilot studies whose objectives include, among others, early characterization of safety and initial proof of concept in a specific patient population. These trials are limited in scope, and are generally conducted in a single center, with a small number of patients (e.g. 5-20). These trials cannot be used by themselves to support licensure of a product. Studies conducted in normal volunteers are not included in this definition.
>
> *ii)* **An immediately life-threatening condition** is "a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment" (21 CFR 312.34).

See 21 CFR 312.34 and the Federal Register vol. 52, No. 99 (May 1977) for a discussion of serious or life-threatening conditions. Application of abbreviated testing requirements to serious conditions which are not immediately life-threatening as defined in 21 CFR 312.34 will depend upon an assessment of the potential risks and benefits to the patient(s). Factors that should be considered in such a risk-benefit analysis include, among others: *i*) the nature and manufacture of the product; *ii*) the nature and severity or stage of the disease; *iii*) the anticipated effect(s) of product administration (e.g., diagnosis, palliation or cure); *iv*) the availability of comparable or satisfactory alternate treatments *v*) characteristics of the patient population (e.g. age, response to previous therapy); *vi*) the number of patients involved and *vii*) the design of the clinical trial (e.g. patient follow-up, safety monitoring etc.). Pre-IND consultation with CBER staff is strongly recommended for sponsors planning to use the abbreviated testing described below for serious but not immediately life-threatening diseases. The guiding principle for these trials is that sufficient information should be provided before testing in human subjects to assure that patients and their contacts will not be put at unacceptable risk. Informed consent issues for these trials are discussed in Section IV.A.1.e. The limited testing described below should not be used to support development beyond the stage of feasibility trials. Therefore, sponsors are encouraged to plan for additional testing and characterization as described in Section II.C. when they intend to pursue advanced clinical development and seek licensure. In designing the purification process, it is advisable to include at least two orthogonal robust virus inactivation/removal steps. This would further reduce the testing necessary to begin initial clinical trials.