

# Guidance for Industry

## Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans

### DRAFT GUIDANCE

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## **GUIDANCE FOR INDUSTRY**

### **Source Animal, Product, Preclinical and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans**

*This guidance document represents FDA's current thinking on the production, testing, and evaluation of products intended for xenotransplantation. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.*

#### **I. INTRODUCTION**

##### **A. Purpose of the Document**

This document is intended to provide guidance on the production, testing and evaluation of products intended for use in xenotransplantation. The guidance includes scientific questions that should be addressed by sponsors during protocol development and during the preparation of submissions to Food and Drug Administration (FDA or Agency), e.g., Investigational New Drug Application (IND) and Biologics License Application (BLA).

For the purpose of this document, xenotransplantation refers to any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues or organs. For the purpose of this document, xenotransplantation products include live cells, tissues or organs used in xenotransplantation. (See Definitions in section I.C.)

This document presents issues that should be considered in addressing the safety of viable materials obtained from animal sources and intended for clinical use in humans. The potential threat to both human and animal welfare from zoonotic (i.e., relating to diseases that arise from the transfer of infectious agents by normal contacts between animals and humans) or other infectious organisms warrants careful characterization of animal sources of cells, tissues, and organs. This document addresses issues such as the characterization of source animals, source animal husbandry practices, characterization of xenotransplantation products, considerations for the xenotransplantation product manufacturing facility, appropriate preclinical models for xenotransplantation protocols, and monitoring of recipients of xenotransplantation products. This document recommends specific practices intended to prevent the introduction and spread of infectious agents of animal origin into the human population. It is expected that new methods proposed by sponsors to address specific issues will be scientifically rigorous and that sufficient data will be presented to justify their use.

## **B. Background**

Recent advances in technology and pharmacology, which have been important for achieving success in allotransplantation, have led to the proposal that xenotransplantation, initially attempted nearly a century ago (reference 35), may provide a solution to the shortage of human allografts (reference 36). Proposed xenotransplantation protocols include implantation in humans of live organs, tissues or cells from a nonhuman animal source, and procedures in which human cells or fluids that are intended for administration to human recipients, have had ex vivo contact with live nonhuman cells, tissues or organs. Examples of xenotransplantation procedures include:

- transplantation of xenogeneic hearts, kidneys, or pancreatic tissue to treat organ failure,
- implantation of neural cells to ameliorate neurological degenerative diseases,
- administration to patients of human cells previously cultured ex vivo with live nonhuman animal antigen-presenting or feeder cells, and
- extracorporeal perfusion of a patient's blood or blood component perfused through an intact animal organ or isolated cells contained in a device to treat liver failure.

The use of these different xenotransplantation products has the potential for transmission of infectious disease from nonhuman animals to humans.

Potential public health risks posed by the use of xenotransplantation products include the following:

- (1) transmission of organisms that are pathogenic for humans but may not be pathogenic or even detectable in the source animal host,
- (2) transmission of organisms that may not normally be pathogenic in humans but can become so in the immunosuppressed or immunocompromised individual, and
- (3) recombination or reassortment of organisms, particularly viruses, with nonpathogenic or endogenous human infectious agents, to form new pathogenic entities.

Furthermore, it is difficult to predict the infectious agents that may cause disease in a recipient of a xenotransplantation product solely on the basis of analysis of naturally occurring zoonoses because there are major differences between normal contact of humans with animals and contact of a recipient with a xenotransplantation product. For example, the physical barrier or distance is eliminated in the recipient due to transplantation and vascularization of xenotransplantation products, or even implantation of nonvascularized cells or tissues, or ex vivo manipulations that cause intimate proximity or contact of xenotransplantation product materials with recipient cells, tissues, or fluids. The potential for viral adaptation in immunocompromised or iatrogenically immunosuppressed hosts and the potential for undetected spread of previously latent viral infections are of particular concern.

For these reasons, during product development it is important to consider the safety, not only of recipients and their contacts, but also of the public. Public discussion of these issues is

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important and will continue to take place through the FDA Biological Response Modifiers Advisory Committee-Subcommittee on Xenotransplantation, the Department of Health and Human Services (DHHS) Secretary’s Advisory Committee on Xenotransplantation, and other public fora.

Many issues relevant to the use of live materials obtained from nonhuman animal sources are addressed in previous FDA Guidance documents (see Guidance Documents in References) published by the Center for Biologics Evaluation and Research (CBER). In addition, the International Conference on Harmonization (ICH) has published a number of documents that are relevant to the use of xenotransplantation products in humans (see ICH Guideline in References). These documents should also be consulted.

The United States Public Health Service (PHS) has previously made recommendations regarding the infectious disease risks posed by use of xenotransplantation in humans. In 1996, the PHS published a “Draft PHS Guideline on Infectious Disease Issues in Xenotransplantation” (Federal Register notice, September 23, 1996 (61 FR 49920)). Based on comments received and advances in fields relating to xenotransplantation, the PHS updated and revised the draft guideline (reference 1) (hereafter referred to as “revised PHS Guideline”). This FDA guidance document reiterates many of the concepts in the revised PHS Guideline, but in addition includes specific advice regarding all aspects of xenotransplantation product development and production, and xenotransplantation clinical trials.

It is anticipated that FDA's approach to regulation of xenotransplantation products will evolve as the scientific knowledge in the area of xenotransplantation continues to accumulate. Thus, this document, as with other guidance documents will change as knowledge and experience pertinent to xenotransplantation accumulates. In addition, FDA realizes that it may not be appropriate to apply every aspect of the guidance to every xenotransplantation product. For example, some of the recommendations for animal husbandry may not be needed for xenotransplantation products obtained from well-characterized, long-established cell culture lines.

### **C. Definitions and Abbreviations**

Act: The Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321 et seq.).

AAALAC: Association for Assessment and Accreditation of Laboratory Animal Care, International. This organization inspects and accredits biomedical animal facilities.

Agents of concern: For the purpose of this document, agents of concern are infectious agents that may pose a risk to the recipient and/or public, i.e., agents that can, potentially could, or have an inadequately defined ability to infect, cause disease in, and/or transmit among humans.

BLA: Biologics License Application.

CDC: Centers for Disease Control and Prevention.

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cGMP: Current good manufacturing practice. For drugs, including biological drugs, cGMP regulations can be found at 21 CFR Parts 210 and 211. For biological products, see 21 CFR Part 600 Subpart B and Part 610. For blood and blood components, additional regulations can be found at Part 606 (21 CFR Part 606). For devices, quality system regulations can be found at Part 820 (21 CFR Part 820).

Closed herd or colony: Herd or colony governed by Standard Operating Procedures (SOPs) that specify criteria restricting admission of new animals to assure that all introduced animals are at the same or higher health standard compared to the residents of the herd or colony.

CPE: Cytopathic effects. An effect on nucleated cells in vitro caused by some viruses that are observable microscopically.

DPF: Designated pathogen free. This term is used to describe animals, animal herds, or animal facilities that have been rigorously documented to be free of specified infectious agents, and that are maintained using well-defined routines of testing for designated pathogens, and utilizing rigorous SOPs and practices of herd husbandry and veterinary care to assure the absence of the designated pathogens.

EM: Electron microscopy. A method used to visualize very small objects, such as subcellular particles, or organisms such as viruses.

FDA or Agency: Food and Drug Administration.

FSIS: Food Safety Inspection Service, Department of Agriculture.

Gnotobiotic: The science of rearing laboratory animals, the microflora and microfauna of which are specifically known in their entirety.

IACUC: Institutional Animal Care and Use Committee. A local institutional committee established to oversee the institution's animal program, facilities, and procedures. An IACUC carries out semiannual program reviews and facility inspections and reviews all animal use protocols and any animal welfare concerns. (See PHS Policy on Humane Care and Use of Laboratory Animals, September 1986; reprinted March 1996.)

IBC: Institutional Biosafety Committee. A local institutional committee established to review and oversee basic and clinical research conducted at that institution. The IBC assesses the safety of the research and identifies any potential risk to public health or the environment. (See section IV-B-2 of the NIH Guideline for Research Involving Recombinant DNA Molecules, reference 17.)

IDE: Investigational device exemption application. These are applications containing requests to use an unapproved device in clinical tests using human subjects. The statutory requirement is at section 520(g) of the Act (21 U.S.C. 360(g)), and the implementing regulations can be

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found at 21 CFR Part 812.

**IND:** Investigational new drug application. These applications are required for persons who intend to conduct clinical investigations involving unapproved drug products, including those subject to section 505 of the Act (21 U.S.C. 355) or to the licensure provisions of section 351 of the PHS Act (42 U.S.C. 262). The IND regulations are found at 21 CFR Part 312.

**IRB:** Institutional Review Board. A board, committee or other group designated by an institution established to review and approve biomedical and behavioral research involving human subjects in order to protect the rights and welfare of human subjects (See 21 CFR Part 56, Institutional Review Boards and 45 CFR Part 46, Protection of Human Subjects.)

**Lot:** Defined in 21 CFR 210.3(b)(10) as a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits, and in 21 CFR 600.3(x) as that quantity of uniform material identified by the manufacturer as having been thoroughly mixed in a single vessel. Each lot of final product is subjected to appropriate tests to ascertain adherence to specifications prior to release of the product for clinical use. Licensed biological products may be subject to lot release as described in 21 CFR 610.2(a). Often in the case of xenotransplantation products, an entire lot is used for treating a single recipient.

**Master File:** Master Files are submitted to the FDA and contain information regarding a product, such as product manufacture or general procedures. Procedures and information contained in the Master File can be cross-referenced in INDs and IDEs on written permission from the Master File sponsor, but confidentiality of the information within the Master File is maintained. (See 21 CFR 314.420.)

**PBMC:** Peripheral blood mononuclear cells.

**PCR:** Polymerase chain reaction. An enzymatic technique, using a thermophilic enzyme to catalyze synthesis of short DNA sequences, that allows detection of nucleic acids by amplification of specific DNA sequences.

**PHS Act:** The Public Health Service Act (42 U.S.C. 201 et seq.).

**PMA:** Premarket approval application. This is a marketing application for certain devices under section 515 of the Act. The regulations for PMAs can be found at 21 CFR Part 814.

**PTC:** Points to Consider. These documents, published by CBER, represented an earlier version of what CBER now calls “Guidance for Industry.”

**Recipient:** An individual who receives or who undergoes ex vivo exposure to a xenotransplantation product (as defined in xenotransplantation).

**RT:** Reverse Transcriptase. An enzyme found particularly in retroviruses, that catalyzes the synthesis of DNA from RNA.

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SAF: Source animal facility.

SOP: Standard operating procedure.

Source animal: An animal from which cells, tissues, and/or organs for use in xenotransplantation are obtained.

TSE: Transmissible spongiform encephalopathy. TSEs are fatal, subacute degenerative diseases of humans and animals with characteristic neuropathology (spongiform change and deposition of an abnormal form of a prion protein present in all mammalian brains). TSEs are experimentally transmissible by inoculation or ingestion of diseased tissue. The abnormal prion protein is hypothesized to be the agent of transmission. Alternatively, other unidentified co-factors or an as-yet-unknown viral agent may be necessary for transmission.

USDA: United States Department of Agriculture.

Xenotransplantation: For the purpose of this document, any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues or organs.

Xenotransplantation product(s): For the purpose of this document, xenotransplantation products include live cells, tissues or organs used in xenotransplantation (defined above).

Zoonosis: A disease of animals that may be transmitted to humans under natural conditions (e.g., brucellosis, rabies).

## **II. REGULATORY RESPONSIBILITY**

Xenotransplantation products, including live organs, tissues, or cells from a nonhuman source, or xenotransplantation product materials used in encapsulated form or in which nonhuman live organs, tissues or cells have ex vivo contact with human body fluids, cells, tissues or organs that are subsequently given to a human recipient, require premarket approval by FDA. If xenotransplantation products are to be used in clinical investigation, they require an appropriate investigational application to FDA (21 CFR 312.20). Most xenotransplantation products will be regulated as biological products by CBER. CBER regulates biological products, including cellular therapies, under authority of section 351 of the PHS Act (42 U.S.C. 262), and the Act (21 U.S.C. 321 et seq.). Regulations for drugs, biological products and devices are found in Title 21 of the Code of Federal Regulations (e.g., 21 CFR Part 312 for regulations governing Investigational New Drugs (IND), and 21 CFR Part 601 for regulations governing licensing of biological products).

Some products may be combination products consisting of a biologic and a device, such as

xenogeneic cells contained in a device used for extracorporeal hemoperfusion. Others may be combinations of a biologic and a drug, such as if a novel immunosuppressive agent were to be used only in the context of transplantation of a specific xenotransplantation product. The regulation of combination products is determined by the primary mode of action of the product. Refer to 21 CFR Part 3 for issues regarding regulation and assignment for pre-market review of combination products.

### **III. SOURCE ANIMAL CHARACTERIZATION**

#### **A. General Considerations**

The cross-species infectious potential of specific animal pathogens should be a major consideration in the selection of the source animal species. Anatomic and physiologic considerations are also of importance. For example, whether an organ is of appropriate size, will function adequately across species barriers and will become integrated in the various physiologic, inflammatory and neuroendocrine processes are considerations, as are certain immunologic concerns including the suitability of current regimens in prevention of rejection of the nonhuman live cells, tissues or organs. Species that are endangered or protected should be excluded from use. Sponsors should consult all relevant PHS and FDA guidance documents on this subject prior to submitting an application, and specifically should consult the document, “Guidance for Industry: Public Health Issues Posed by the Use of Nonhuman Primate Xenografts in Humans” (reference 2) before submitting an application to FDA that involves the use of nonhuman primates as sources of a xenotransplantation product. (The term “xenograft” in the above referenced document is synonymous with the term “xenotransplantation product” in current use by DHHS and in this guidance.)

Due to potential infectious disease risks associated with the use of xenotransplantation products, appropriate source animal qualifications should be developed and should include herd management and programs for prevention and screening for infectious agents. Although testing of the final xenotransplantation product for infectious agents is crucial, appropriate control of animal sources and husbandry provides important additional assurance for the safety of such products by controlling infections by both known and potentially even unknown agents. Therefore, the specific information supplied by the sponsor regarding animal husbandry including housing, feeding, veterinary care, drug and biologic treatment of source animal herds and individual source animals, will be crucial for FDA evaluation of the potential for safe use of cells, tissues, or organs from such source animals.

The SAF, production process, and records are subject to FDA inspection under section 704 of the Act (21 U.S.C. 374) and section 351(c) of the PHS Act (42 U.S.C. 262(c)).

#### **B. Animal Welfare Concerns**

Another area of consideration for SAFs and manufacturers of xenotransplantation products is the welfare of the animal sources. Procedures for animal husbandry, tissue harvesting, and

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termination of animals should be approved by an appropriate Institutional Animal Care and Use Committee, in accordance with the Animal Welfare Act (7 U.S.C. Sec. 2131 et seq.). In cases where funds are received from the PHS, procedures must also comply with the PHS Policy on Humane Care and Use of Laboratory Animals according to section 495 of the PHS Act (42 U.S.C. Sec. 289d). It is recommended that the SAF be accredited by the AAALAC. Standards for accredited facilities are provided in the National Research Council's Guide for the Care and Use of Laboratory Animals (reference 4).

### **C. Animal Origin**

#### 1. Animal History

The sponsor should document the geographic origin, species, strain, and genealogy of the source animal(s) and herd(s). The documentation of source animal history should describe factors that may pose risks to recipients, such as possible exposure of the predecessor animals to sources of transmissible spongiform encephalopathies (TSEs) or other adventitious or infectious agents of concern (see Definitions, section I.C.). Source animals should be bred and raised in captivity and be derived from closed herds. Artificial insemination, embryo transfer, cloning, or hysterotomy plus foster feeding may be used to establish animal herds with fewer endemic pathogens. In particular, the revised PHS Guideline suggests that breeding programs use cesarean derived animals whenever possible (reference 1). The animal history should document the use of these procedures.

#### 2. Animal and Herd Qualification

Source animals should only be derived from closed herds with documented health screening programs. Individuals with expertise in infectious diseases of the species involved should develop a list of viruses, bacteria (including the rickettsiae), mycoplasma, fungi, TSEs, and parasites for which the herd is screened and supply this information to FDA. All infectious agents known to infect the source species should be considered. The rationale for omitting agents that are found in the source animal species from the herd screening program should be justified in the FDA submission requesting investigational use (e.g., IND application). For example, the geographic location of the herd may allow exclusion of certain infectious agents. Source animals from TSE-susceptible species should be obtained only from closed herds that are documented to be free from TSE diseases or TSE-associated agents (see also section III.C.3.c.). Animals obtained from geographic areas in which TSEs are known to exist in the source species should not be used as source animals. The frequency of the screening, the method of assay, and the method of identifying which and what proportion of animals are sampled should be described and justified. As data are accumulated that demonstrate product safety, the screening program may be modified in consultation with the FDA.

The same considerations should be applied to semen donors, whether or not they are

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members of the herd including, for example, screening for infectious agents that may be transmitted by semen.

3. Source Animals from Outside the U.S.

a. Animals from outside the U.S. or their first generation offspring should not be used as sources for the production of xenotransplantation products unless they are of a species or strain not available in the United States, or have specific qualities that provide a unique and scientifically justified clinical advantage, such as transgenic animals.

b. If the use of source animals from outside the U.S. is necessary and justifiable, the manufacturers should apply the same considerations for these animals as for source animals bred in the U.S. (e.g., see section III.D. for Animal Health and Husbandry). A quarantine period of sufficient length to demonstrate the absence of infectious agents of concern should be used, and extensive screening of the animals should be performed. Thorough documentation should be submitted to demonstrate that such source animals have been derived from closed herds, have been housed under appropriate conditions and subjected to recommended health maintenance procedures and screenings, and have not been fed rendered or recycled mammalian materials for at least two generations. Agents that are endemic in the country of origin should be included in the screening. Methods and conditions of transport of imported animals should be described. Descriptions should include means of transport and husbandry during transport including isolation, caging, handling, animal treatment, and presence of other animals of the same or different species. If animals from countries outside the U.S. are needed, they should be used as founders for a domestic herd that will be well-characterized for an extended period of time prior to use, using procedures sufficient to validate the herd's acceptability as source animals.

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c. Source animals should not be imported from any country or geographic region where TSEs are known to be present in the source species. The USDA has identified countries from which the importation of ruminants and some ruminant products are restricted or prohibited (9 CFR Parts 94 and 96).

d. The USDA and, when appropriate, CDC should be consulted for their requirements regarding importation of animals or animal tissues.

4. Range and Wild Animals

Animals that are raised under free-ranging conditions should not be used as source animals. Such animals have a higher likelihood of harboring infectious agents due to potential exposure of the source animal to other animals, birds, insects, or other uncontrolled environmental factors.

Wild-caught animals should not be used as source animals.

5. Animal Sources Obtained from Slaughterhouses or Abattoirs

Animals from slaughterhouses or abattoirs are unsafe for use as source animals. Appropriate documentation and histories of animals from slaughterhouses may not be available because the animals are often obtained from geographically divergent farms or markets, and exposure to other animals or potential sources of infectious agents during transit or after arrival at the slaughterhouse is unknown. Therefore, such animals should not be used as source animals.

**D. Animal Health and Husbandry**

Production of animals as sources of live cells, tissues, or organs for use in xenotransplantation products involves an adequately designed facility and a program for the operation of the facility to minimize the animals' exposure to infectious agents.

Source animals should be obtained exclusively from SAFs. Detailed plans for maintaining source animals should be included in the FDA submission as part of the application for investigational use (e.g., IND) or as a Master File. These plans should include standard operating procedures detailing the containment and housing of animals, feeding and obtaining feed, water and bedding, performance and monitoring of the health screenings, removal from production and disposal of the animals and their byproducts, and identifying individual animals and recording their movements to, through, and out of the facility. These procedures should take into consideration the source animal species and xenotransplantation product(s) as appropriate.

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### 1. Facilities

Animals should be housed in facilities built and operated in accordance with recommendations described in the National Research Council's Guide for the Care and Use of Laboratory Animals (reference 4) and be accredited by the AAALAC. SAFs should not be located in geographic proximity to manufacturing or agricultural activities that could compromise the facility biosecurity by providing or enabling a source of infections.

SAFs are subject to the regulations in 21 CFR Part 600 Subpart B on establishment standards, including the requirements regarding animals and personnel in §§ 600.10 and 600.11. SAFs also are subject to the regulations in 21 CFR Part 600 Subpart C, regarding inspections. These facilities are subject to inspection by designated representatives of the clinical protocol sponsor and public health agencies.

A detailed description of the facilities and procedures for housing source animals should be included with the FDA submission (e.g., IND or Master File). The information provided should include plans for the shelters, the feeding areas, the washing areas, the fencing, air handling systems (particularly in quarantine areas), and other physical attributes of the animal environment. Facility descriptions should also include information on physical barriers and operational measures intended to eliminate or minimize exposure to insects, birds, or other animals that may transmit disease to the source animals. Records should be kept which report any biological or physical compromise of the animal environment as well as measures taken in response to this problem. These descriptions should also cover the procedures and schedules followed for cleaning and other routine maintenance of the animal enclosure. Procedures for elimination of animal wastes should be included. Include in the description how qualified source animals will be housed (for example as a batch or individuals) and the methods used to decontaminate the housing after the source animals are used.

The SAF staff should include veterinarians with expertise in the infectious diseases and agents prevalent in the particular animal species being raised in the facility. If an infectious disease veterinarian is not on staff, documentation should be provided that an individual with the appropriate expertise is available for consultation. Staff should also include adequate numbers of caretaker personnel with appropriate training in the care and health of the species being housed (e.g., §§ 600.10 and 600.11).

### 2. Maintenance of Source Animals

#### a. General

Source animals should be maintained in accordance with standard operating procedures appropriate to the species, xenotransplantation product, and the intended clinical application. SOPs should provide for admission of new

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animals to the SAF and source animal pool, for quarantine, and for removal, isolation, or elimination of diseased animals, and this information should be provided to FDA. Animals that have been removed from the source animal pool due to illness or infection should not be reintroduced.

Procedures should be developed to identify incidents that negatively affect the health of the herd or colony. This information is relevant to the safety review of every xenotransplantation product application. Such information, as well as the procedures to collect the information, should be reported to FDA.

### b. Health Screening

i. It is recommended that source animals be maintained in barrier facilities that are considered free of designated pathogens. For the purpose of this document such facilities are termed Designated Pathogen Free (DPF), and animals derived from them are termed DPF animals. Initial screening and routine monitoring are important to validate that such facilities maintain DPF status. Protocols for monitoring the herd for disease and infectious agents should exist, and a copy or a summary of the SOPs should be included in the FDA submission requesting investigational use (e.g., IND). The frequency of testing may be modified as the reliability of the production system is established using data from earlier screens. Appropriate experts such as infectious disease consultants, virologists, microbiologists, accredited microbiological laboratories, and veterinarians should be consulted to generate a list of agents for which all source animals should be screened, and a list of appropriate diagnostic tests. In addition to screening for specific infectious agents, more general assays for detection of classes of agents should be used. For example, feces from source animal herds should be examined on a regular basis for evidence of parasitic infections. If infectious agents including normal flora that could potentially be infectious in an immunosuppressed recipient have been identified in source animals, the use of such animals should be avoided. However, the use of such animals may be warranted under certain circumstances. The sponsor should consult with CBER if the use of such animals is contemplated (see, for example, section V.C.4.d.). Techniques for introducing new animals such as artificial insemination, cesarean section, cloning or novel gnotobiotic techniques should be fully described.

ii. Subclinical infections of source animals may not be apparent at the time of harvest of the nonhuman live cells, tissues or organs, and may be identified only retrospectively. Sampling of individual animals from the herd of origin for screening, and the use of

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sentinel animals, should help minimize this problem and may help identify infections in recipients post transplantation. A sentinel animal program that includes periodic necropsy and extensive histology and pathology evaluations should be considered. The screening procedures should be appropriate for the animal species, the xenotransplantation product, and the clinical application. Specific screening procedures should include appropriate physical examination and laboratory tests, and should underscore zoonoses known to exist in the species or geographical regions in which the source animals originate and are maintained.

iii. Individual source animals should be quarantined and screened prior to harvest of cells, tissues or organs, as discussed in section III.D.4.b below and in the revised PHS Guideline (reference 1).

### c. Health Care

The herd health surveillance system should include comprehensive documentation of all veterinary care received by source animals. These include documentation of all illnesses, medical care, procedures, drugs administered, vaccinations, routine physical exams and any other treatments received by each animal. Use of antimicrobial agents should be carefully documented due to potential risk to allergic recipients receiving unprocessed nonhuman live cells, tissues or organs. Residual drug levels should be validated to be insignificant in cells, tissues or organs taken from source animals that previously have received medications. Exclusive use of killed vaccines generally is warranted both in the source animal and in the herd with which it is associated. Live vaccinations should be used only when alternative immunogens for vaccinations are not available, and only if scientific evidence exists to support that the live cells, tissues or organs from the vaccine-treated animal will not pose a risk of infection for the human recipient. Procedures to deal with illnesses or other incidents that affect the health of the herd should be in place and provided to FDA. Animals requiring treatment with blood, blood products, or tissues obtained from animals outside the closed herd should not be used as source animals and should be removed from the herd. Aseptic techniques and sterile equipment should be used for all parenteral interventions including vaccinations, treatment with drugs or biologics, phlebotomy, and biopsies. If animals within the closed herd have been treated with a biological product (e.g., vaccine, monoclonal antibody) such treatment should be documented in the application to FDA requesting investigational use (e.g., IND), and copies of package inserts or labeling should be provided. Treatment of animals with drugs for any reason should be documented and maintained in the SAF's records. Procedures for disposal of dead animals should be developed (see section III.G.).

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d. Feed

The storage and delivery of feed, water, and other consumables should be described. Records should include manufacturer, batch numbers, and other pertinent information, and record keeping should be documented in an SOP. The vendor and contents of feed given to a source animal for at least two generations prior to use as a source for live cells, tissues or organs used in xenotransplantation should be recorded in the individual source animal's records. Feeds containing rendered or recycled mammalian material, or significant drug contamination or pesticide or herbicide residues should not be used for source animals. Natural, non-sterile, foods such as hay should be avoided to minimize potential risks of exposure to pests or infectious agents. Water should be of sufficient quality to prevent unnecessary exposure of animals to infectious or adventitious agents, drugs, pesticides, herbicides, and fertilizers. Pasteurized milk products may be included in feeds. Newborn animals should be fed colostrum or milk from dams only if the dams have been specifically qualified by the same procedures used for herd qualification.

e. Caretakers

SOPs for animal caretakers should be provided in the FDA submission requesting investigational use (e.g., IND), and should include entry and exit procedures, clothing requirements and all interactions with the animals, e.g., feeding, watering, exercising, delivery of immunizations and medications, etc., (e.g., § 600.11). There should be a documented training program for personnel as described in the cGMP regulations (§ 211.25).

The health of humans in contact with animals should be monitored on a routine basis (reference 5). The program for screening and monitoring of caretaker and other staff should be predetermined and customized to maximize screening information, and should be described in an SOP. Health monitoring of humans who come into contact with the animals should include physical exams with periodic sampling and storage of serum or plasma for individuals having frequent and close contact with source animals, to less rigorous monitoring for those with occasional contact. Baseline samples should be obtained from all caretakers. Health monitoring of caretakers should be described in SOPs.

3. Animal and Personnel Traffic Through the Source Animal Facility

SOPs for entry and exit of animals should be developed and should include transportation of animals to and from the facility. All animals entering the facility should be subjected to a defined quarantine period allowing for completion of any screening procedures. The minimum quarantine period for animals used in manufacture is 7 days (§ 600.11(f)(2)). However, longer quarantine periods that

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extend beyond the incubation period for infectious agents in the source animal species should be used for animals entering a SAF. A tracking system should be devised that allows unique identification of each individual animal in the facility. Entry and exit of animals and human staff should be minimized to avoid exposures to transmissible infectious agents. The use of an ‘all in/all out’ or batch approach for moving qualified source animals is encouraged as a method of minimizing the potential for infectious agent transmission.

Personnel traffic patterns should be described in the FDA submission requesting investigational use (e.g., IND), and should minimize transmission of infectious agents. Caretakers should not work in more than one animal facility or with more than one species of animal. Caretakers should not work with more than one isolated group of animals or more than one herd within any given day unless validated SOPs for caretaker decontamination and disinfection are used.

#### 4. Individual Source Animal Qualification

##### a. Testing for infectious agents

All individual source animals should be screened for presence of the same infectious agents used for herd qualification. In addition, further laboratory tests for infectious agents as described in section V. for testing of the xenotransplantation product (e.g., viral cocultivation assays,) should be performed on appropriate samples of source animal blood or tissue. When fetal or neonatal animals will be used as source animals, testing of the mothers should be conducted and may supplant testing of the fetus or neonate if technical and temporal difficulties render such testing unfeasible.

When feasible, a biopsy of the live animal cells, tissue or organ or other relevant tissue should be examined by histopathology and tested for infectious agents by appropriate assays. Remaining biopsy tissue should be archived as described in section III.E.3.

All tests should be performed at a time as close as possible to the date of harvest of the live cells, tissues or organs, but which allows the results to be obtained prior to their use. If more than 3 months have elapsed since the initial testing or biopsy of the source animal, tests should be repeated prior to harvest.

The nature, timing, and results of surveillance of the herd from which the individual animal is procured should be considered in designing appropriate screening of individual animals.

##### b. Quarantine

Individual source animals generally should be quarantined for a minimum of 3

weeks prior to harvest of their live cells, tissues or organs. It may be appropriate to modify individual quarantine periods depending on the characterization and surveillance of the source animal herd, the design of the facility, and the clinical indication. If the quarantine is shortened, justification should be provided. During the quarantine, in addition to tests for infectious agents, source animals should undergo physical examination by a veterinarian including complete blood count, peripheral blood smear, and fecal exam for parasites.

**E. Harvest of Nonhuman Live Cells, Tissues or Organs for Use in Producing Xenotransplantation Products**

1. Harvest and Documentation

The procedures and physical facilities used for harvesting of live cells, tissues or organs from source animals should be described in detail in the application to FDA requesting investigational use (e.g., IND). Validated procedures for avoiding the introduction of infectious agents during harvesting should be in place. Validation of the procurement and screening procedures should include documented performance of the processes, with documented results supporting successful harvest of live cells, tissues or organs from source animals that meet lot release criteria including identity, potency (or activity) and safety (e.g., microbiological sterility). Source animal anesthesia should not be harmful to the human recipient. A summary of the health records regarding the source animal (e.g., health status and microbiological screening reports, results of lot release tests, and anesthetic used, if relevant) should accompany the xenotransplantation product and should be incorporated into the recipient's records.

SOPs should permit rapid, accurate, and facile tracking of tissue from an individual source animal to the recipient.

2. Transportation

Transportation of source animals may expose them to risks not encountered in closed herds and should be avoided if possible. It is therefore recommended that, when feasible, and particularly in cases where source animal tissues or cells are going to be processed further prior to use, live cells, tissues or organs should be procured at the animal facility prior to shipping. In some cases, particularly when the xenotransplantation product is a whole organ intended for immediate transplantation, it may be necessary to ship live animals. In those cases where transportation is necessary, barriers equivalent to, or better than, those in place at the SAF should be maintained during transit to ensure that source animal contamination does not occur en route. Transportation should occur in dedicated vehicles in which source animals are not exposed to any other animals, and the method should be documented in the submission to FDA. If there is any question regarding the effectiveness of the transportation and containment procedures, animals should be quarantined and re-screened in a fashion

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comparable to that used for entry of new animals into a closed herd.

The method of transporting the live animal cells, tissues or organ from harvest site to the clinical xenotransplantation site should be detailed in the application to FDA requesting investigational use (e.g., IND). Procedures for avoiding shipping errors, avoiding contamination, and documenting transfer of animal materials to the correct patient should be developed and implemented.

### 3. Source Animal Sample Archive

#### a. Timing of Sample Acquisition

If the source animal is sacrificed at the time its live cells, tissues or organs are harvested, a full necropsy should be conducted including gross, histopathological, and microbiological evaluation and archival samples including portions of the product should be obtained for storage as described in section III.E.3.b.

If the source animal is not sacrificed at the time its cells, tissues or organs are harvested, portions of the harvested material and plasma and leukocytes from the source animal should be archived, and the health of the source animal should be monitored for life.

When source animals die or are euthanized, a full necropsy should be performed and archival samples should be obtained for storage as described in section III.E.3.b.

#### b. Samples to be Archived and Storage Conditions

Archived samples of source animal tissues and body fluids should be stored at -70°C or lower temperatures, as appropriate for preserving the sample, or maintained at room temperature for fixed samples. Section 3.7.1 of the revised PHS Guideline, (reference 1) recommends that at least ten 0.5 cc aliquots of citrated- or EDTA-anticoagulated plasma and at least five aliquots of viable leukocytes ( $1 \times 10^7$ /aliquot, for subsequent isolation of nucleic acids and proteins or for use as a source of viable cells for co-culture or other tissue culture assays) should be cryopreserved. The conditions of cryopreservation and storage for viable samples should be selected to maintain cell viability for the period of storage (see III.E.3.c.). Appropriate tissue samples for formalin fixation and paraffin-embedding and for cryopreservation should be collected from source animals at the time the live cells, tissues or organs are procured. Tissue samples representative of major organ systems of source animals (e.g., spleen, liver, bone marrow, central nervous system, lung,) should be collected and cryopreserved at necropsy. As appropriate to the xenotransplantation product, other body fluids, such as cerebrospinal fluid, should be archived at

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the time of procurement of the product and/or necropsy. If sentinel animals are used, tissue samples and body fluids obtained at necropsy should also be archived.

### c. Archive Rationale, Duration, and Responsibility

The revised PHS Guideline (reference 1) recommends that a sufficient quantity of materials be harvested and cryopreserved for three different uses:

- (i) dedicated sample(s) for use by the PHS (see reference 1),
- (ii) for use if needed for recipient diagnosis and care, and
- (iii) for use by the sponsor, as appropriate.

Detailed plans for obtaining and storing the archive samples should be included in the application to FDA requesting investigational use (e.g., IND application). The revised PHS Guideline (reference 1) recommends that samples should be stored for 50 years from the time of sample acquisition. Responsibility for the archives and access to the specimens should be clearly designated.

### 4. Herd Records

Records should be kept pertaining to the source animals and facilities. These records are subject to inspection and should be maintained for 50 years beyond the date of procurement of the nonhuman live cells, tissues or organs for use in xenotransplantation.

### 5. Disposition of Records on Closing of a Source Animal Facility

If a SAF ceases operation, all records and archived samples should be transferred to the respective sponsors or the sponsors should be notified of a new archive site. The sponsor should make provisions for all records to be maintained for the requested period in the event that the establishment ceases operation. If a sponsor ceases to exist, FDA should be consulted regarding the disposition of records and archive samples.

## **F. Source Animal History for Xenogeneic Cell Lines**

Cell lines from animals may be established and used in the production of xenotransplantation products. The history of the cell line should be included in the application to FDA requesting investigational use. Especially for long-term cultures, it need not always include all the detailed information about the source animal and source animal facility mentioned above. However, it should include at a minimum the species and tissue of derivation. Information such as age and sex of the source animal, laboratory of derivation, date of derivation, and the immediate provider of the cell line should be included whenever possible. For short-term cultures (e.g., derived less than one year previously), it should also include a description of the husbandry and

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health status of the particular source animal and herd or colony. The history of the cell line should also include the above information on feeder cells or animals used for passage in vivo, if such techniques were used to develop the cell line. The final product should be characterized and tested as described in section V. The “Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals” (reference 25) may also be consulted for pertinent recommendations regarding the production, identification and characterization of cell lines used in manufacture.

### **G. Disposal of Animals and Use of Byproducts**

There is a need for advance planning for the ultimate disposition of source animals, including those animals in which the insertion of genetic information failed ("no-takes"), and sentinel animals bred for use in producing xenotransplantation products, especially animals of species ordinarily used to produce food. Food or feed derived from such animals may be adulterated under the Act. Generally, such animals should not be used as sources of human food via milk or meat as ingredients of feed for other animals. Such animals should not be used as pets or breeding animals because of the potential for pets or breeding animals of species commonly used as food to enter the food chain. Source animals should be disposed of in a manner consistent with the disposal of infectious medical waste in compliance with federal, state, and local requirements.

There may be infrequent situations where animals from xenotransplantation facilities can be considered safe for human food use or as feed ingredients when disposed of through rendering. Persons wishing to offer animals into the human food or animal feed supply or who have food safety questions should first consult with FDA's Center for Veterinary Medicine. CBER will refer food safety issues from sponsors to CVM, or sponsors may contact CVM directly through the Division of Compliance, HFV-235, FDA, Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855, 301-827-0181.

## **IV. CHARACTERIZATION OF XENOTRANSPLANTATION PRODUCTS**

### **A. General Considerations**

In general, the final xenotransplantation product should be tested for safety, identity, purity, and potency. 21 CFR Part 610 describes types of assays that are required for licensed biologics. Similar tests should also be used during investigational stages of product development. Assays for safety testing including infectious agents tests, and tests for endotoxin, are discussed in more detail in section V. of this document. Assays for testing identity and potency will depend on the product itself. Assays for purity should include tests for endotoxin or pyrogen, and for certain xenotransplantation products should include measurements of cell populations in the xenotransplantation product. For further guidance in this section, see references 6, 31, 32, and 33.

Additional recommendations and comments regarding microbiologic tests are found in section V. of this document

### **B. Considerations for Classes of Xenotransplantation Products**

#### **1. Xenotransplantation Products Used Immediately after Procurement from the Source Animal**

When xenotransplantation products are transplanted directly after removal from a source animal, it may not be possible to perform all tests on the final product and have the results available prior to use. However, the sponsor should use a biopsy of the organ or a relevant surrogate sample (e.g., adjacent tissues or contra-lateral organs) for assay of the xenotransplantation product. Safety analyses should include fungal and bacterial sterility, mycoplasma and virus testing. Tests for endotoxin or pyrogen should also be performed. Although it is realized that results of these tests will not be available prior to transplantation, assay or culture periods should still be completed and the results recorded. Histology, performed on a retention sample or biopsy of the xenotransplantation product, may be used to document identity of the product.

#### **2. Stored or Processed Xenotransplantation Products**

For live xenogeneic cells or tissues that are stored, processed, or expanded *ex vivo*, in addition to safety testing, additional product characterization to measure identity, purity, and potency should be performed. As much as possible, results of these assays should be available prior to xenotransplantation, and used for lot release. These same product characterization steps should also be applied to xenotransplantation products comprised of human cells that have had *ex vivo* contact, for example by co-culture, with cells or tissues of nonhuman origin.

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### a. Safety

Tests for bacterial and fungal sterility, mycoplasma, and viral agents, generally considered safety tests, are discussed in detail in section V. of this document.

### b. Identity

A means to assess identity of the active component of the xenotransplantation product should be developed. This may include identification of relevant cell or tissue types using immunological, immunohistological or biochemical cell markers. In some cases, histological evaluation may be used. Depending on the manufacturing process it may also be necessary to verify the species or strain identity of the final product, such as when the SAF handles more than one strain or species of animal.

### c. Purity

If the final product is a heterogeneous xenotransplantation product, i.e., a tissue possessing several types of cells, or a cellular implant containing extraneous tissues or cells which may be incompletely removed during tissue dissection, cell processing or ex vivo culture, it is especially important that the purity of the cell population be determined. The sponsor should develop a quantitative method to assess the presence of the putatively active cell type as well as contaminating cell types in the final product. This may be achieved, for example, using morphologic, histologic, molecular genetic, biochemical and/or immunocytochemical techniques to identify contaminating cells and/or their products. For xenotransplantation products comprised of human cells that have had ex vivo contact with cells or tissues of nonhuman origin, quantitative assays to assess the presence of nonhuman cells in the final product should be performed. Purity assays should be validated and are important for production of a consistent product. Results of such tests should be used as a lot release specification if possible. In those cases where the final product is a purified population of cells of a single or few types, such as an established cell line, the product should still be tested for purity, and tests for identity of the cells should be developed.

Endotoxin levels should be measured on the final product and results should be available for use as a lot release. Tests for endotoxin are also discussed in the context of tests for infectious agents (section V.C.3.).

d. Potency

Potency assays that measure and reflect the intended biological activity of the final product should be performed. For example, potency assays may measure biologically active molecules secreted/produced by the xenotransplantation product, such as cytokines, hormones or neurotransmitters. If necessary, development of appropriate potency assays may proceed along with product development. In addition, cell viability should be assayed and used for lot release.

**V. MICROBIOLOGICAL TESTING OF XENOTRANSPLANTATION PRODUCTS**

**A. General Considerations**

1. Framework

This section of the guidance document is intended to provide a general framework for the microbiological testing of xenotransplantation products. Some specific examples of tests and organisms are suggested. However, sponsors are encouraged to consider all available up-to-date information regarding potential pathogens and testing strategies to evaluate their own systems, perform experiments to identify potentially infectious agents, and to propose and validate appropriate tests in consultation with CBER. During the initial stages of investigations, it may not be necessary for all assays, with the exception of standard sterility tests, to be completely validated. However, the specificity, sensitivity and reproducibility should be established for all procedures used to detect infectious agents to the extent possible.

2. General Biological Products Standards

For general standards on testing of biologics for infectious agents refer to 21 CFR Part 610 (see references 6, 7, 25, 32, and 33).

Additional guidance on these issues as they relate to xenotransplantation can also be found in section 3.3 of the revised PHS Guideline (reference 1).

3. Inactivation or Removal of Infectious Agents

Whenever possible, without compromising the integrity and effectiveness of the xenotransplantation product, validated procedures for inactivation or removal of adventitious agents, infectious agents, or other microbiological contaminants should be incorporated into the manufacture of the product. The Agency realizes that the use of such methods may be difficult but encourages sponsors to develop methods to accomplish inactivation/removal of potentially infectious agents in xenotransplantation

products.

4. Archiving

Samples of all final xenotransplantation products (i.e., cells or tissues, or biopsies of organs), whether fresh or from culture *ex vivo*, should be cryopreserved and archived for future testing, as may be needed. In some cases, for example if the xenotransplantation product is a whole intact organ, it may be acceptable to archive a relevant surrogate sample (e.g., adjacent tissues or contra-lateral organ). If the final product consists of human cells, tissues or organs that have been in contact *ex vivo* with live nonhuman cells, tissues or organs, samples of both the final product and the nonhuman animal cells tissues or organs should be archived. As in the case of the animal source samples (see section III.E.3.c.), sufficient quantities and numbers of replicates of the xenotransplantation product should be harvested and cryopreserved for three different uses:

- (a) dedicated sample(s) for use only by PHS (see reference 1),
- (b) for use if needed for recipient diagnosis and care, and
- (c) for use by the sponsor as appropriate.

Detailed plans for obtaining and storing archive samples should be included in the application to FDA requesting investigational use (e.g., IND). Samples should be stored for 50 years from the time of manufacture of the xenotransplantation product. Responsibility for the archives and access to the specimens should be clearly described.

5. Sponsors should make provisions for all samples and attendant records to be maintained for the requested period of time in the event that an establishment ceases operation.

**B. Considerations for Classes of Xenotransplantation Products**

1. Xenotransplantation Products Used Immediately after Procurement from the Source Animal

In procedures in which the xenotransplantation product is transplanted immediately after removal from the source animal, such as xenotransplantation of whole organs, results of testing of the xenotransplantation product may not be available prior to its clinical use. In such cases, testing of the source animal itself may be all the testing that is possible prior to the procedure. Testing of samples taken from such xenotransplantation products or appropriate relevant biological surrogates, e.g., adjacent tissues or contra-lateral organs, is also warranted even though the results will not be available prior to use of the xenotransplantation product because results may contribute to patient management and to development of a scientific data base. (See also section IV.B.1.)

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### 2. Stored or Processed Xenotransplantation Products

For xenogeneic cells or tissues that are stored, processed, or expanded ex vivo, testing for infectious agents should be accomplished or, at a minimum, initiated prior to xenotransplantation. If cells or tissues are maintained in culture, cell culture procedures and reagents should be validated for maintenance of microbial sterility, including both xenogeneic infectious agents and other cell culture adventitious agents. Testing should be performed periodically during the culture period. It may not be necessary to perform all tests at every time point, but a scientific rationale should be supplied to support the selection of tests performed at each given time. As an example, samples may be tested:

- (a) at the initiation of culture ex vivo,
- (b) before cryopreservation if performed as a step in manufacture,
- (c) as late as possible during culture such that final results (or useful preliminary results) will be available prior to the release and use of the product,
- (d) two to three days before clinical use (e.g., for microbiological cultures used in lot release), and
- (e) at the time of final product harvest, though results may not be available before clinical use.

### 3. Xenotransplantation Product/Device Combination Products

In certain biologic/device combination products, the xenogeneic component is separated from human fluids or tissues by physical barriers that might prevent or reduce transmission of certain classes of infectious agents. If such claims are to be made, or if the existence of the physical barrier is to be used in lieu of certain other precautions to lower the risk of transmission, the sponsor should provide the results of validation studies that demonstrate the inhibition of transmission of specific infectious agents and the maintenance of device/barrier integrity. For specific guidance on the design of these types of studies, see reference 33. For example, if such claims are to be made in the patient informed consent document, the results of these studies should be provided in the application to FDA requesting investigational use; if such claims are to be made during marketing, the data should be provided in the pre-market application. The design of these studies should take into consideration the following parameters:

- (a) Conditions of normal physiologic use of the xenotransplantation product/device combination product, and conditions under which the combination is subjected to physical and biological stress.
- (b) Use of microorganisms that are representative of infectious agents potentially present in the xenotransplantation product. Note that the size and plasticity of selected agents should be considered.
- (c) Use of agents that would demonstrate the physical properties of the barrier (i.e., permeability to viruses or other particles with differing properties such

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as size, charge, hydrophobicity, shape, etc.).

Without supporting data obtained from such studies, xenotransplantation products contained within a barrier should not be assumed to present lesser risk of infection to humans than xenotransplantation products implanted directly into a recipient.

**C. Assay Design for the Detection of Infectious Agents**

1. General

The choice of tests will vary depending on the animal source, including the species, strain and geographic origin, the histological type of tissue, the processing of tissue prior to use, and the proposed use or clinical indication. Special consideration should be given to organisms known to infect the source animal and those known to cause zoonoses. The list of infectious agents to be tested for should be based on that used for individual source animal qualification. Discussions with CBER are encouraged. Data should be included in the FDA application for investigational use to document the specificity, sensitivity, and reproducibility of novel assays used to detect infectious agent(s).

2. Tests for Bacteria, Fungi, and Mycoplasma

Standards concerning the types of methods used for detection of bacteria, fungi, and mycoplasma in licensed biologics can be found in 21 CFR Part 610. Alternative methods may be used during product development but use of such methods should be supported by data on the sensitivity, specificity, and reproducibility of the method. For xenotransplantation products, such data should be obtained using infectious agents appropriate to the source animal species, geographic origin of the source animal, and the cells, tissues or organ(s) to be used. These data should be submitted to FDA.

In addition to testing the final product for viable organisms, Gram stains should be performed on appropriate samples of all final xenotransplantation products. The results of these stains should be available prior to use of the product in humans, and a negative Gram stain should be set as a lot release criterion.

3. Endotoxin Test

During the product development phase, a bacterial endotoxin test may be performed in lieu of the rabbit pyrogen test as described for licensed products (§ 610.13(b)). The type of endotoxin assay, and its specificity and sensitivity should be described in the application for investigational use submitted to FDA (e.g., IND). If the manufacturer intends to use an endotoxin assay in lieu of the rabbit pyrogen test after licensure, equivalency with the pyrogen test for the specific xenotransplantation product will need to be demonstrated at the time of license application (§ 610.9).

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Since it is possible to perform an endotoxin assay within a few hours, an appropriate assay should be selected and performed. Results should be available prior to use for any xenotransplantation product that has been cultured, stored, or processed for more than the few hours required to perform the assay. These results should be used as a lot release specification. Consult the “Guideline on Validation of the Limulus Amoebocyte Lysate Test as and End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices,” 1987 (reference 7), for additional guidance on endotoxin assays.

### 4. Viruses

#### a. Culture Assays

Xenogeneic cells used for xenotransplantation (fresh or cultured) should be tested by co-culture with a panel of appropriate indicator cells to amplify potential viral contaminants. The panel of cells used in this analysis should include a cell line representative of the source animal species, a cell line representative of the animal tissue(s) type used in the manufacture of the xenotransplantation product, and a human cell line. For additional guidance see the “Points To Consider in the Characterization of Cell Lines Used to Produce Biologicals” (reference 25). When possible, manipulated and/or unmanipulated source animal cells should also be co-cultivated with recipient cells, such as peripheral blood cells. Co-cultivation cultures should be observed routinely for CPE, focus formation, RT activity, and changes in cell growth or other unexpected changes. Visualization of co-cultures by EM is recommended to identify morphologic changes or to recognize certain viruses. Efforts should be made to identify any viruses detected using immunoassay, PCR, or other assays using virus-specific probes. At the end of the culture period, cultures should be tested for hemagglutination and hemadsorption with erythrocytes of three different species (reference 25). Additional efforts may be necessary to characterize viruses that are detected that may be novel or for which specific probes may not yet be available.

Lot release specifications should be set based on available data. They should be used for release of xenotransplantation products for which results can be available prior to administration of the product to humans, such as for products that can be cryopreserved. For cells that are manipulated *ex vivo*, if time allows, viral tests should be performed during the period of culture or manipulation, so that the results are available prior to delivery of the product to the recipient. If it is not possible to obtain the results prior to use, samples of each product lot should still be tested. In these instances, assay procedures should be qualified and data obtained on a number of representative final product lots prior to beginning human trials.

#### b. Activation of Latent Viruses

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Special consideration should be given to testing for viruses known to occur in the latent state. Transmission of viruses with long clinical latencies is of concern due to the possibility of transmission of these viruses from the recipient to the recipient's contacts in the absence of symptoms or signs of disease. Immunosuppression and transplantation, either alone or in combination, may activate latent viruses (reference 42). Manipulation or culture of cells *ex vivo* may also activate latent viruses (see references 43 and 44).

Determination of which experiments might be appropriate to detect latent viruses in animal cells, tissues or organs would depend upon the tissue type and the virus in question. Examples of experiments that have been used to detect viral activation and may be useful in the xenotransplantation product setting, include the following:

- the expression of endogenous retroviruses is induced by culturing *in vitro*, or by treatment with iododeoxyuridine or demethylating agents such as 5-aza-cytidine (reference 37); and
- cultivation *in vitro* of ganglia latently infected with Herpes simplex virus results in the production of infectious virus (reference 38).

In certain cases positive result may not necessarily preclude use of such tissue (see section V.C.4.d. for information regarding xenotransplantation products containing porcine endogenous retrovirus (PERV)), but the identification and characterization of the resulting virus may provide useful information and materials for monitoring the recipient of the xenotransplantation product for the presence of the activated virus (see section VIII.F.3.).

If either the processing or clinical use of the xenotransplantation product will involve conditions with the potential to activate latent viruses (e.g., PERV), attempts should be made to evaluate that potential prior to use.

### c. In Vivo Assays for the Detection of Viruses

Xenotransplantation products should be tested by assay *in vivo* for detection of certain viruses that may not be found by culture methods *in vitro*. For example, many serotypes of Coxsackie A virus are only detected upon inoculation of newborn mice (reference 38). It is therefore recommended that if there are no reliable *in vitro* assays, that appropriate *in vivo* assays should be applied (see reference 25).

### d. Assays Suitable for the Detection of Porcine Endogenous Retroviruses (PERV)

All live cells, tissues or organs derived from pigs contain sequences for porcine

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endogenous retroviruses in their genome (reference 39). It has been demonstrated that in some instances, and in some primary porcine cells, that these sequences are expressed, resulting in production of infectious retrovirus (references 40, 41, 43, and 44). In light of data demonstrating that PERV can infect human cell lines in vitro (references 43, 44, and 45), FDA recommends that all porcine-derived xenotransplantation products be evaluated using appropriate assays for the production of infectious retrovirus.

Xenotransplantation products (e.g., a fresh sample of the xenotransplantation product or relevant surrogate tissue e.g., adjacent tissues or contra-lateral organs or the cultured xenogeneic cells) should be tested by co-culture with appropriate indicator cells to amplify any infectious retrovirus(es). Indicator cells that have been demonstrated to be permissive for PERV replication, include the human embryonic kidney cell line 293 (American Type Culture Center (ATCC CRL-1573)), mink lung fibroblasts (ATCC CCL-64), certain feline cell lines (such as PG-4, ATCC CRL-2032), and a swine testis cell line ST (ATCC CRL-1746). One or more of these cell lines should be chosen for initial analysis of the porcine xenotransplantation product or appropriate relevant proxy tissue. After co-culture for a period of at least 30 days or 10 cell passages, the cells should be analyzed for the transfer of PERV from the porcine cells to the indicator cell by either an optimized RT assay (reference 46) or use of PERV-specific primers to amplify, by PCR, reverse-transcribed viral RNA or cellular RNA (references 43, 44, and 45). Evidence for virus production will not necessarily result in the xenotransplantation product being considered unsuitable for clinical use. Rather, additional characterization of the virus should be pursued in consultation with CBER in order to ensure appropriate reagents are available for recipient follow-up (section VIII.F.). Additional characterization may include analysis for the cell substrate most sensitive to infection by the particular strain of PERV present in the xenotransplantation product and sequence analysis of the infectious virus produced by the xenotransplantation product. These steps will provide important information and development of diagnostic tools to optimize the protocols for follow-up of recipients for evidence of infection (section VIII.F.).

## **VI. MANUFACTURING AND PROCESS-RELATED GMP CONSIDERATIONS FOR HARVEST AND PROCESSING OF XENOTRANSPLANTATION PRODUCTS**

### **A. General Considerations**

Facilities used for the harvest and/or processing of xenotransplantation products should be designed to minimize the potential for contamination of the harvested and/or processed xenogeneic cells, tissue or organs and cross-contamination between lots of these cells, tissues or organs.

For sponsors of investigational trials, the validation activities described in this section should be phased in during the investigational phase, as the clinical studies progress toward submission of an application for premarket approval (e.g., BLA). The exception to this is sterility assurance validation, which should be completed before initiating clinical trials. Manufacturing process controls should be in adherence to cGMP regulations (21 CFR Parts 210 and 211). The IND regulations (21 CFR 312.23(a)(7)) allow that some controls may be introduced as appropriate for the phase of development.

### **B. Contamination/Cross-Contamination Precautions**

Precautions should be taken to prevent contamination/cross-contamination during harvest and manipulation of xenogeneic cells or tissues. Consideration should be given to:

- personnel, animal, material and waste flows into and out of the facility;
- proposed air cleanliness classifications;
- cleaning/sanitizing agents used and demonstration of their efficacy in relation to facility isolates, viruses and other potential adventitious agents; and,
- environmental monitoring and gowning procedures.

#### **1. Flows**

Personnel, animal, material, product and waste flows into and out of the facility should be designed to exclude mixing of “clean” and “dirty” activities. Ideally, flows should be one way so that personnel, animals, materials and product enter and exit separately. Using this design, waste would only exit through designated airlocks, pass-throughs and/or autoclaves. Alternatively, segregation of activities may be accomplished procedurally and/or temporally. In this case, special care should be taken to avoid contamination or cross-contamination. For example, more stringent cleaning and sanitization schedules should be in place.

Of special concern is the transfer of animals to the harvesting area (i.e., operating room). The animals should be prepared in such a way as to exclude potential surface contaminants, which may be carried from the animal facility.

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### 2. Cleaning and Sanitizing Agents

Agents used for cleaning and sanitizing work surfaces and equipment, as well as other surfaces in the harvesting and processing areas (e.g., floors, walls), should be demonstrated to be effective against facility isolates, viruses and other potential adventitious agents. Cleaning schedules should be established which maintain acceptable control in relation to the activities performed in the specified area. It is expected that validation studies demonstrating the efficacy of the agents used will be performed as the trial progresses towards submission of an application for premarket approval, e.g., BLA.

### 3. Environmental Monitoring

A program for monitoring the environment in the harvesting and processing areas should be established based on the criticality of the manufacturing process involved.

Nonviable particulate monitoring should be performed to verify air cleanliness classifications in the harvesting and processing areas (see section VI.C.1. for recommended air cleanliness classifications). This verification should include laminar flow areas in the harvesting area and biological safety cabinets in the processing area (reference 8). After initial verification, nonviable particulate monitoring should be performed at established intervals to demonstrate maintenance of the assigned air cleanliness classification.

Viable particulates, (i.e., microbes), may be monitored using a variety of techniques. The use of settling plates during harvesting and processing activities, while not quantitative, provides some assurance that the quality of the environment has not been compromised. Quantitative methods should be established as clinical trials progress towards submission of an application for premarket approval, e.g., BLA. Surfaces, including those of personnel performing production activities (e.g., gloved hands), should be monitored using contact plates or swabs to demonstrate the continued efficacy of the cleaning regimen, and maintenance of asepsis for personnel. It is recommended that personnel engaged directly in harvesting and processing activities be monitored at the conclusion of each critical activity (e.g., surgery, aseptic surgery). Additionally, random sampling of operators performing cell expansion activities may be undertaken.

### 4. Changeover Procedures

Changeover procedures designed to prevent contamination between harvests of xenotransplantation products should be in place, followed and documented. These procedures should include clearance of all materials and waste from the operating room or cell processing cabinet, and cleaning/sanitization of surfaces. In addition, segregation procedures, if multiple lots of xenogeneic cells or tissues are processed at

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the same time, should be addressed. Adequate labeling of processing vessels (e.g., tissue culture flasks) and dedication of equipment or portions of equipment (e.g., shelves within incubators) are examples of such segregation procedures. Centrifuges used for processing are of particular concern in terms of cross-contamination. It is recommended that only one lot of xenogeneic cells or tissues be centrifuged at a time. Integrity of centrifuge tubes should be demonstrated or closed systems employed, when possible. Centrifuges should be adequately cleaned between each lot operation.

### **C. Validation and Qualification**

As noted previously, validation and qualification efforts should be ongoing as clinical trials progress. Minimally, assurance that systems and equipment are functioning as needed, is expected. Validation protocols and data summaries should be submitted to FDA for review as part of the ongoing investigational file.

#### 1. Air Handling Systems

Heating, Ventilation and Air Conditioning (HVAC) systems should be designed to provide adequate air quality for harvesting and processing of xenotransplantation products. Laminar flow units may be employed above the operating table to provide high quality air during harvesting operations. Biological Safety Cabinets may be employed to maintain aseptic conditions during processing. It is expected that this equipment be capable of producing Class 100 conditions for the most critical of processes, although it is understood that maintenance of these conditions may be difficult during harvesting. Minimally, the environment surrounding the Class 100 laminar flow units and/or biological safety cabinets should be Class 100,000. Proceeding towards licensure, areas surrounding critical Class 100 processes should meet Class 10,000 conditions.

Validation of these systems and units should include verification of air changes and pressure differentials, and that the desired cleanliness level is achieved (see section VI.B.3.). Testing of the High Efficiency Particulate Air (HEPA) filters contained in the system should address integrity and efficiency.

Routine environmental monitoring (see section VI.B.3.), pressure differential checks and recertification of HEPA filters should demonstrate maintenance of the desired conditions.

#### 2. Water

It is expected that water used to formulate necessary reagents, or for critical cleaning purposes (i.e., equipment and surfaces in the harvesting and processing areas), will meet the United States Pharmacopoeia (USP) XXIV monograph for Water for Injection (WFI) (reference 9). If WFI is purchased, lot specific testing should be performed and hold times validated for open containers. If WFI is generated at the

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facility, the system should be properly validated and routinely monitored to ensure continued quality.

### 3. Equipment

Equipment used for harvesting and/or processing of xenogeneic cells and tissues should be adequately calibrated and qualified. Temperature controlled equipment, such as refrigerators/freezers and incubators, should then be routinely monitored to assure proper conditions. Carbon dioxide supplied to incubators used for cell expansion should be 0.2 micron filtered to minimize the potential for contamination. If water baths are used, maintenance procedures for water quality should be employed. This may include the addition of agents to control contamination.

### 4. Aseptic Processing

Generally, manipulation or expansion of xenogeneic cells or tissues is an entirely aseptic process, i.e., there is no final sterilizing filtration of the product. In order to validate this process, media fills (substitution of media for product) should be performed to demonstrate that sterility may be maintained consistently. Assurance of sterility of the final product is necessary from the very beginning of the clinical studies (reference 10). Personnel performing these functions should be adequately trained and monitored to assure consistent performance during normal production.

All product contact equipment should be sterile and free of pyrogens when aseptically processing cells or tissues. Disposable labware (e.g., flasks) may be employed, where possible. The sterility and depyrogenicity of the containers and closures used for the final product are of particular importance. For equipment and components that must be sterilized, there should be evidence that the autoclave cycle(s) is validated to provide an acceptable level of sterility assurance. Minimally, basic load configurations should be established and followed, and biological indicators placed within each load to verify lethality. As studies progress, it is expected that formal validation of all sterilizing/depyrogenating processes will be performed.

### 5. Process Validation

Ultimately, prior to licensure all processes used to manufacture the product should be validated. The Agency has previously defined process validation (reference 11). It is expected that process validation, when performed, will be prospective and at full scale, with the exception of studies performed to demonstrate viral clearance (removal/inactivation). Laboratory studies may also help to establish appropriate operating and process parameters and may be used in support of the formal study. It is expected that information on the validation protocol(s) and summaries of data resulting from its execution will be included in the license application.

## **VII. PRECLINICAL CONSIDERATIONS FOR XENOTRANSPLANTATION**

### **A. General Considerations**

This section is intended to serve as a general framework for the preclinical testing of xenotransplantation products prior to use in clinical trials. The general principles as set forth in the document generated by the International Conference on Harmonization (ICH) on the safety of biotechnology-derived pharmaceuticals can also be applied to these products (reference 34). In general, studies to support the safety characterization of therapeutic agents should focus on the intended alteration to the human pathophysiologic state (i.e., activity), as well as unintended effects (i.e., toxicity) to the host system. Such studies serve to assess the potential for clinical risks and constitute an important component of a FDA application. Preclinical studies are particularly valuable for gaining insight into safety issues which cannot be evaluated in human recipients for ethical or practical reasons. Consequently, sponsors should design strong preclinical safety programs, and also consult the ICH guidance documents related to acute and chronic drug safety characterization (<http://www.ifpma.org/ich5s.html> under “ICH Safety” [S1-S5] or “Joint Safety/Efficacy” [M] headings).

Specific considerations in the design of preclinical studies that are intended to support the safety of xenotransplantation products should include:

- (1) the animal source for the xenotransplantation product,
- (2) the tissue’s anatomic and physiologic similarity to its human homologue,
- (3) the determination of function of the xenotransplantation product,
- (4) the animal model system,
- (5) the integrity of the device components (if a device is used),
- (6) the dose levels (based on tissue mass, as well as pharmacologic/metabolic activity or release kinetics of bioactive molecules),
- (7) the route of administration (site of implantation/injection, extracorporeal or ex vivo use),
- (8) the study duration (as related to potential human exposure),
- (9) reactions between source animal and host immune systems,
- (10) interspecies extrapolation (i.e., cross-species activity of secreted proteins/hormones at receptors), and
- (11) device biocompatibility.

Because a primary intent of the preclinical animal and in vitro studies is to identify potential clinical risk factors, these evaluations should focus on maximizing the similarity between animal and human testing strategies in test substance, route of administration, and dosing regimen. Animal models of human xenotransplantation should utilize a xenotransplantation system evaluating the cell, tissue or organ type being examined for use in humans, and should utilize clinically relevant immunosuppressive therapy. Rigorous preclinical program design is needed to ensure comparability of preclinical to clinical study design and is important for selecting appropriate clinical indication, inclusion/exclusion criteria, recipient monitoring scheme, dose, concomitant therapies, as well as for advising potential recipients of risks (informed consent).

## **B. Issues Related to Infectious Agents**

Since the transfer of infectious agents that are pathogenic, latent, or even non-pathogenic in their natural animal host may cause serious disease in an immunosuppressed patient, the microbiologic burden carried by the xenotransplantation product as well as the immune status of the recipient should be considered in preclinical study designs. Additionally, designs of preclinical studies should incorporate:

- (1) careful veterinary monitoring of animals, taking note of any early signs of infection, and
- (2) procedures needed to assign a cause of mortality (using appropriate serologic or immunohistochemical identification of pathogens).

In order to prevent the spread of demonstrable or potential infectious agents, animals should be cared for with appropriate precautions, including isolation if necessary. Deaths from infections in animal models may occur due to immunosuppressive regimens that may be intentionally more extreme than expected for use in humans in order to avoid rejection of the xenogeneic live cells, tissues or organs, and to obtain proof-of-concept data. Therefore, data identifying cause of death (e.g., xenogeneic infectious agents or activation of latent host infection) could assist in interpreting human risk, may be helpful in refining animal experimental models, and may identify pathogenic infectious agents in the source animal. Animal models of xenotransplantation, while exploring these issues, are limited by uncertainties in extrapolation of cross-species infectivity information; e.g., data indicating no infections in animal, even primate species, are not adequate to assure that humans will not be susceptible to infections transmitted by the xenotransplantation product.

Additional insight into refinements of animal immunosuppressant regimens may come from evaluation of host resistance. Host immunocompetence may be evaluated by measuring resistance to infection by various pathogens including those that may be contained within the xenotransplantation product.

## **C. Xenotransplantation Product-Host Interactions**

### **1. Immunologic Rejection**

Survival of the xenogeneic cells, tissues or organs should be assessed in animal models, with attention given to

- (a) identifying infiltration of immune or inflammatory cells into the xenotransplantation product or alteration of such cells in other relevant compartments such as the blood and cerebrospinal fluid,
- (b) fibrotic encapsulation of the xenotransplantation product, e.g., resulting in impaired function or xenotransplantation product loss,
- (c) xenotransplantation product necrosis,

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- (d) any evidence of Graft Versus Host Disease (GVHD),
- (e) in vivo function and durability of encapsulation or barriers intended to diminish rejection or inflammatory responses,
- (f) any special concerns regarding the site and nature of the xenotransplantation product, and
- (g) if relevant to the particular xenotransplantation product, the possibility that rejection of that product might predispose the recipient to rejection of subsequent xenotransplantation products or allotransplants.

### 2. Immunosuppression

Preclinical animal studies in which xenotransplantation is used in an immunosuppressed host may raise questions regarding the relevance of the model to clinical pharmacology, toxicology, or immunology. Consideration of how both the host and source species handle the immunosuppressive drugs may be necessary (for instance, where a nephrotoxic drug is metabolized by hepatic enzymes, but intra-species differences in metabolism exist). Immunosuppressive drugs often have very restricted therapeutic indices, so that pharmacokinetics and metabolism may markedly affect the activity and/or toxicity of the agents in the host or xenotransplantation products. Attempts should be made to delineate toxicities due to immunosuppressive drugs from toxicities due to the xenotransplantation product.

Relative activity of immunosuppression on the source species of the live xenogeneic cells, tissues or organs should be considered and studied where appropriate, since immunosuppressive treatment that selectively suppresses immunity in the host species may be permissive to GVHD. This might occur in cases where immunologically active cells are contained, either intentionally or inadvertently, within the xenotransplantation product.

### 3. Tumorigenicity in the Immunosuppressed Host

In addition, the tumorigenic potential of the xenotransplantation product, perhaps due to altered cell growth regulation or to immunosuppression of the host, is an important concern (refer to section VII.E.).

### 4 Cross-Species Compatibility of Bioactive Molecules

For xenotransplantation products where it is intended that the product synthesize and provide bioactive molecules, such as cytokines or hormones, data from preclinical experiments should be provided that support that the molecules produced will be active in humans. Experiments to address this issue should evaluate concentration-response issues, and should be performed in vitro, and/or in appropriate preclinical models in vivo.

Even when the xenotransplantation product is composed of a single cell type, the

product may secrete unintended molecules that could alter normal host physiology. Moreover, host substances might affect product function. Therefore, preclinical models should evaluate the overall health of the recipient as well as markers of activity such as production of intended and unintended bioactive substance by the xenotransplantation product. (See section VII.D.2. for further discussion on this topic.)

#### 5. Migration of Xenogeneic Cells

Cells from xenotransplantation products may migrate within the host, thus presenting clinical concerns regarding adverse reactions deriving from displaced, bioactive cells or unexpected anatomical impediments. This may be especially true for incompletely differentiated cells (see section VII.D.3.) and may be evaluated in animals using histopathology, possibly coupled with enhancing techniques such as fluorescent dye loading and/or species-specific antibodies, or more sensitive techniques such as PCR.

### **D. Considerations for the Use of Heterogeneous Xenotransplantation Products**

The following principles should be applied to the development of appropriate preclinical testing of heterogeneous xenotransplantation products in order to assess potential adverse effects. A xenotransplantation product may be considered heterogeneous if it is, for example, a tissue or solid organ, possessing many varieties of cells, or a cellular implant, containing extraneous tissues or cells which may be incompletely removed during tissue dissection or present in short term cultures *ex vivo*.

#### 1. Characterization of Constituent Cell Types in a Heterogeneous Xenotransplantation Product

The procedures used in preclinical studies for the collection, isolation, and, if used, for the activation or expansion of the xenotransplantation product, should mimic the procedures intended for use in clinical trials, and cell types in the product being tested should be characterized in an analogous fashion to the proposed clinical xenotransplantation product. See section IV.B.2.c. regarding recommendations for the evaluation of purity of heterogeneous xenotransplantation products.

#### 2. Secretion of Biologically Active Molecules by Xenotransplantation Products

It is also important to consider cell function in the characterization of xenotransplantation products. The establishment of the cross-species activity and therapeutic levels of the desired biological agent(s) (secreted/produced by the xenotransplantation product) is critical for the eventual evaluation of activity or efficacy. However, uncharacterized cells or tissues present in the xenotransplantation product may also produce biologically active molecules with unintended activities. Experiments should be performed to identify released, bioactive substances (e.g., neurotransmitters, hormones, cytokines) whether by intended or extraneous cell types in the xenotransplantation product. For example, samples of tissues being prepared for

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transplantation may be maintained or cultured *in vitro*, and the supernatants tested for activities or relevant bioactive substances. The ICH guidance document on safety preclinical evaluations in biotechnology (reference 34) should be consulted for additional guidance on these studies.

In addition to assessment *in vitro*, heterogeneous xenotransplantation products should be evaluated in appropriate animals. Because xenotransplantation products may secrete substances that alter normal host physiology, and because host substances may affect the function of the xenotransplantation product, preclinical transplant models should evaluate the overall health of the recipient (i.e., clinical signs, gross pathology, and histopathology) as well as markers of activity of the xenotransplantation product. Combination toxicity and activity studies can be used to evaluate both potential therapeutic and constitutive functions of the xenotransplantation product. In some instances, the ability to biopsy xenotransplantation products periodically is a potentially valuable tool for evaluating the histopathologic status of the product and host immune response, especially when evaluated in conjunction with clinical chemistries. Control experiments might also be performed to test the *in vivo* effects of live xenogeneic cells, tissues or organs taken from anatomic sites other than those used for therapeutic procurement of the xenotransplantation product, but lacking the therapeutic cell or tissue type and its anticipated pharmacologic activity.

### 3. Differentiation in Heterogeneous Xenotransplantation Products

Xenotransplantation products derived from fetal animal sources, dedifferentiated cells or tissues, or cells expanded *ex vivo* may comprise a heterogeneous population with regard to cell maturity. The degree of heterogeneity may depend on the cell or tissue type from which the xenotransplantation product is obtained, the period of fetal development during which the tissue is procured, and/or the time in culture. For such products, preclinical studies should compare the viable cell types initially transplanted with those that exist subsequently in the xenotransplantation product. This comparison may warrant preclinical studies with sequential sacrifice groups, or biopsies. Techniques such as immunohistochemical staining, trypan blue exclusion, bioassays, or PCR assays may be useful in identifying heterogeneous cell differentiation. An effort should be made to develop models to evaluate the effects of differentiation on the function of the xenotransplantation product, using, for example, measurements of release or secretion of biologically active molecules including those that may not be intended for efficacy of the xenotransplantation product but that it may produce. Viable products may change over time as they respond to, adapt to, and functionally integrate with the host environment. Therefore, monitoring cell viability, morphology, and functional endpoints (e.g., endocrine, behavioral, or immunological) over time may be used to guide development of clinical monitoring regimens.

## **E. In Vitro and In Vivo Tumorigenicity Models for Xenotransplantation Products Intended for Transplantation**

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Tumorigenicity is an important part of preclinical testing for certain xenotransplantation products, such as those manipulated *ex vivo*. For further guidance applicable to this topic, see references 25, 6, and 34.

Xenotransplantation products may be tumorigenic in a new species because of various factors, such as transgenic manipulations, endogenous viruses, *ex vivo* culture, and immunosuppression of the host. Therefore, for xenotransplantation products intended for implantation, consideration should be given to evaluation of tumorigenicity *in vivo* and *in vitro*.

1. Multiple models exist for testing tumorigenesis *in vivo*. The role of immune challenge, immunosuppressive drugs, and infectious disease exposure comprise an important set of safety concerns that may be addressed preclinically. Preclinical experiments should include careful evaluation of controls, background tumor growth rates, tumor incidence and type, location, and time of appearance of tumors over an extended period. These should make use of histopathologic evaluation as a primary endpoint.
2. Colony formation in soft agar (clonogenic assays) and growth in organ culture may be useful *in vitro* assays of the tumorigenic potential, particularly for cell lines. These tests may provide information on stability or abnormal characteristics of cell lines, and may substitute for testing in animals if the sponsor demonstrates that the tests have equivalent sensitivity.

For xenotransplantation products consisting of cells that have been expanded *ex vivo*, a change in cellular growth pattern, morphology, or growth factor dependence may suggest transformation and a need for more rigorous investigation.

### **F. Combinations of Xenotransplantation Products with Devices**

A number of products for therapeutic use are combinations of xenotransplantation products and device components, either for use as implants or extracorporeally. All of the preceding recommendations in section VII. apply to such products. These products also warrant further preclinical characterization for bioreactivity and biocompatibility of the device components. Preclinical testing often will include characterization of the device intended for human use, rather than a homologous product that has been made in scale with a small laboratory species. This in turn may dictate that the device is studied in an animal species with blood volume and size, and possibly, anatomic structures, close to that of humans.

Device elements may be reviewed jointly by staff in CBER and the Center for Devices and Radiological Health (CDRH). Failure of device components (e.g., membranes and filters) that serve to isolate animal tissue from the recipient is an important aspect of safety assessment and is addressed by review staff in CDRH. Additional device toxicity issues, also considered by staff at CDRH, are covered in biocompatibility guidance, published by the International Standards Organization (reference 21).

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Implanted devices may be intended for permanent or extended residence in the human body. Since these are considered chronic therapies, chronic risks (such as chronic inflammation, carcinogenicity, consequences of re-implantation, and local/systemic toxicities) of implanted xenotransplantation product/device combinations may require evaluation prior to product premarket approval, and to some extent, prior to initiation of investigational studies in humans. Studies prior to initiation of clinical investigational studies would usually be expected to last a minimum of 3 months. Toxicological program design will depend, in part, on clinical considerations of the patient population for which use of the xenotransplantation product/device combination is intended.

Membranes with pores may partially isolate xenogeneic tissue housed in devices from attack by host immune cells by membranes with pores, but proteins and pathogens from the xenogeneic tissue may still be released into the host along with desired pharmacologically active molecules. Such devices may reduce but may not eliminate a risk of xenogeneic infections. They also may act as a stimulus leading to local inflammation and fibrin deposition. Adhesions and granulomas may form in host tissue, and deposits on implants may interfere with activity and implanted cell viability. Additionally, encapsulated xenotransplantation products intended to be permeable to bioactive substances (such as encapsulated islets) should be evaluated for preimplantation activity, and should be retrieved and assessed for activity, capsule integrity, and tissue viability after various periods of time in the animals.

Extended animal studies (e.g., 12 to 24 months) should be conducted using the clinical route of administration (e.g., implant site) and clinical grade materials. Studies should be designed to include groups that elucidate reactions to the biomaterials alone, as well as groups exposed to clinical and supraclinical doses of the complete product. Toxicology studies for implanted biomaterials which have previously been utilized in non-cellular devices may be relevant to safety determinations of the xenotransplant/device combination products, but cannot completely satisfy the need for toxicity evaluation of the new product in its complete clinical form. Sponsors should be aware that later changes in formulation of the xenotransplantation product may necessitate the conduct of a new toxicology studies.

For devices used for extracorporeal hemoperfusion, studies should evaluate the hemodynamic effects of establishing and discontinuing the extracorporeal circuit, products released from the tissues housed in the device (e.g., proteins that could cause anaphylactic responses or stimulate unintended autoimmunity), deposition of blood cells (such as platelets) on device tubing or other components, irregularities in clotting or complement activation, and removal of drugs from the recipient's circulation through filtration or device-localized cellular metabolism. Assessment of the biologic activity of the combination product is often a component of preclinical safety evaluations. For instance, studies should evaluate the duration and predictability of cellular (e.g., cell cartridge) activity, so that the biologic component of the device may be replaced at appropriate intervals to maintain life-supporting pharmacologic or metabolic activity.

In summary, animal studies of xenotransplantation product/device combinations, as with other preclinical experiments, should be designed taking into consideration all aspects of the clinical

trial and the need to study both desired and undesired activities of the xenotransplantation product, as well as toxicities evaluated at the local and systemic levels.

## **VIII. CLINICAL ISSUES IN XENOTRANSPLANTATION**

### **A. General Considerations**

This section provides general principles rather than specific guidance. Because the available basic knowledge and clinical experience with xenotransplantation is limited, current issues may be resolved as new knowledge is acquired and new concerns may emerge.

### **B. Clinical Protocol Review**

Sponsors are responsible for ensuring reviews, as appropriate, by local review bodies, including Institutional Review Boards (IRBs), Institutional Animal Care and Use Committees (IACUCs), and Institutional Biosafety Committees (IBCs). (See reference 1)

In addition to the human subjects issues traditionally addressed by local IRBs, institutional review of xenotransplantation clinical trial protocols should also address:

- (1) the potential risks of infection for the recipient and contact populations (including health care providers, family members, friends, and the community at large);
- (2) source animal husbandry (e.g., screening program, animal quarantine); and
- (3) issues related to human and veterinary infectious diseases (including virology, laboratory diagnostics, epidemiology, and risk assessment).

### **C. Xenotransplantation Site**

The revised PHS Guideline has recommended that all clinical xenotransplantation procedures be performed in transplantation centers with appropriate experience and expertise for comparable allotransplantation procedures and with the capability to culture and to identify viral agents using in vitro and in vivo methods either on-site or through active and documented collaborations (reference 1).

### **D. Criteria for Patient Selection**

Because of the potentially serious public health risks of possible zoonotic infections, xenotransplantation should be limited to patients with serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available. Candidates should be limited to those patients who have potential for a clinically significant improvement with increased quality of life following the procedure. The patient's ability to comply with public health measures as stated in the protocol, including long-term monitoring, should also be considered.

## **E. Risk/Benefit Assessment**

It is understood that the lack of other therapeutic options and the severity of disease may raise the benefit-to-risk ratio for some individuals. However, consideration and evaluation of risks and benefits of xenotransplantation should address both recipient and public health concerns. The sponsor should consider the following in providing a benefit-to-risk analysis.

Infectious disease is among the potential risks both to the recipient and to the public posed by the use of xenotransplantation products. Transmission of microbial agents from xenotransplantation products could lead to systemic disease (for example, infection or neoplasia) or failure of the xenotransplantation product in the recipient. Immunological risks include rejection of the live xenogeneic cells, tissues or organs, and, in some cases, GVHD. In addition, transmission of infectious agents could result in outbreaks of zoonotic disease, silent transmission of latent viruses, or emergence of new strains of pathogens. Experience has shown that widespread horizontal or vertical transmission of new pathogens is possible before the pathogens are recognized (e.g., Human Immunodeficiency Virus).

## **F. Screening for Infectious Agents**

Consult the revised PHS Guideline (reference 1) for additional guidance and information on testing recipients of xenotransplantation products.

### **1. Infectious Agents of Concern**

Infectious agents of concern will differ among source animal species and among cell or tissue types within each species. Therefore, clinical tests should be individualized for the specific xenotransplantation product in question. The categories of infectious agents of concern include bacteria (including the rickettsiae), fungi, mycoplasma, viruses, and the agent(s) causative for TSEs. Tests should be available for agents known potentially to be present, including those that are pathogenic in the source animal species and agents that are known to infect human cells *in vivo* or *in vitro*. The capability to test for latent viruses or pathogens should exist, and the sponsor should be prepared to develop and validate clinical tests for new pathogens that may not be recognized at the time of xenotransplantation. Specific infectious agents for which tests will be performed should be identified. (See section VIII.F.3. for additional information on testing.)

### **2. Collection and Analysis of Clinical Samples**

Xenotransplantation should be performed at clinical centers with available state-of-the-art virology and microbiology laboratories that include a staff with knowledge and experience in the isolation and identification of unusual pathogens. In addition, there should be access to laboratory facilities where viral cultures can be done *in vivo*, such as in embryonated eggs and suckling mice. Specimens should be placed into viral

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transport medium at the bedside, stored at 4°C, and inoculated into cell cultures as soon as possible and always within 24 hours of collection. The sample(s) selected for culture will depend upon clinical evaluation of the recipient. Tissue cell culture systems should be described and may include primary monkey, primary human embryonic kidney, semicontinuous human diploid, and continuous human heterodiploid cells. If isolation remains difficult then inoculation in vivo, e.g., into embryonated hen's eggs and/or suckling mice, may be necessary. In addition to culture, tissue can be examined by EM. Immunohistopathology, immunofluorescent antibody, radioimmunoassay, Enzyme-Linked Immunosorbent Assay, and PCR may be helpful when appropriate antibodies and probes are available.

### 3. Testing and Scheduling of Testing of Recipients for Infectious Agents

Tests of clinical specimens from recipients for specific agents of concern should be described, and may include, for example, serological and culture assays. Tests for latent agents known to be in the source animal species (e.g., retroviruses, herpesviruses) should also be described. Assays should be able to distinguish between an infectious agent derived from the source species and a related infectious agent present in humans (i.e., porcine vs. human Cytomegalovirus (CMV)). Data should be available to demonstrate specificity, sensitivity and reproducibility for all tests not in widespread use, or for newly developed tests. In some cases, completion of development of new tests, which have already demonstrated some level of utility, specificity and reproducibility, may proceed concurrently with the clinical trial.

Attention should be given to the tests and schedule for screening of recipients for infections. Infectious agents, tests, and schedules should be described in the submission to FDA.

#### a. Acute Infections

Recipients are at risk for the same infections that are common among individuals who have received allografts. In general, these infections will be related to the use of immunosuppression and will arise from the recipient's endogenous flora, reactivation of latent infections, and environmental sources. The detection methods useful for these diseases will not differ from the methods used to detect infections after allotransplantation.

In addition to being at risk for these infections, a recipient may be at risk for infection by agents contained in the xenotransplantation product. Little clinical experience exists with xenogeneic infectious agents infecting humans from xenotransplantation products. It is anticipated that a recipient will be at greatest risk for infection during the first few months after the procedure. However, there may be significant delay in the clinical manifestations of infection in some cases. The timing of occurrence of infectious episodes may vary depending on immunosuppression. It is important that relevant data are

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collected during the clinical trial and for the lifetime of the recipient and that investigation of acute infectious episodes include appropriate tests. It is difficult to predict the diagnostic symptoms and signs of such infections in the immunosuppressed patient. When the source of a recipient's post-transplant illness remains obscure, testing should be performed on appropriate fluid and tissue samples. Such testing should include the use of serology as well as various cell and microbial culture systems and in vivo systems. Culturing may detect infections that serologic testing has missed (for example, when immunosuppressed transplant recipients are unable to mount the usual immunological response to a pathogen).

Patient care workers who work with acutely ill recipients should follow recommended procedures for handling and disinfection/sterilization of medical instruments and disposal of infectious waste (references 22, 23, 24, and 48).

**When there is a suspicion of a possible xenogeneic infection, FDA should be notified promptly if a non-xenogeneic causative organism is *not* readily identified, and should be notified immediately if a potentially xenogeneic causative organism *is* identified.**

b. Chronic Infections

An immunosuppressed recipient will also be at risk for infection by the pathogens most commonly associated with allotransplantation. In addition, as above, pathogens potentially derived from the source animal should be considered. With adequate preclinical and xenotransplantation product testing before the procedure, the most likely chronic pathogens from the animal may be endogenous or exogenous viruses, although parasites such as *Toxoplasma* should be considered.

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c. Routine Screening for Clinically Inapparent Infections and Seroconversions

In addition to diagnostic testing when a recipient appears ill, it is important to establish ongoing recipient screening programs. Sponsors should describe and validate their screening programs, taking into consideration the source animal species and type of cell, tissue, or organ used.

i. Passive Screening Program

In passive screening programs, appropriate clinical samples, such as blood, plasma, urine, etc., are obtained periodically and archived for possible future testing. In the event of a diagnosed infection, or the onset of symptoms that may represent infection in one recipient, these samples are then available for retrospective screening of asymptomatic persons who shared a common or similar exposure to a xenotransplantation product. It is recommended that a passive screening program be accomplished through an established schedule for routine sample collection and storage of samples from asymptomatic recipients. Such a passive screening program would be in addition to the collection and archiving of biologic specimens designated for PHS use as described in the revised PHS Guideline (reference 1). However, the time points identified by the revised PHS Guideline as appropriate for archiving specimens designated for PHS use also provide guidance on the minimal frequency with which specimens should be obtained and stored as part of a passive screening program. These time points include:

- (a) prior to xenotransplantation (two samples, one month apart),
- (b) at the time of transplantation,
- (c) in the immediate post-transplant period,
- (d) at one month and six months after transplant,
- (e) annually for the first two years, and
- (f) every five years subsequently.

In certain cases, more frequent acquisition of samples may be appropriate. The sponsor should consider the animal source and type of product in proposing the schedule and tests to be used in the passive screening program.

See section VIII.H. for recommendations regarding the number, size, use, and duration of storage of collected samples.

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ii. Active Screening

In addition to a passive screening program, an active screening program, in which samples are tested immediately after collection from recipients, should be considered. A significant advantage of such a program is that by screening prospectively for evidence of infection in the absence of symptoms, it provides for a prospective understanding of the patterns of infection and disease that may be occurring in recipients. Active screening could allow potential detection of a novel infection in the asymptomatic recipient and enable implementation of infection control practices to contain it prior to secondary human to human transmission or widespread dissemination in the general public, even in the absence of manifestation of associated disease (which may be absent altogether or simply delayed in onset). Possible mechanisms of active screening range from centralized review of routinely collected clinical data to detect trends suggestive of emerging diseases, to periodic performance of specific additional laboratory tests on a subset of the samples collected in the passive screening program. Section 4.1.1.2 of the revised PHS Guideline (reference 1) suggests an active screening program for agents known to be in the xenotransplantation product at 2, 4, and 6 weeks after the patient receives the xenotransplantation product.

If a xenotransplantation product known to harbor an infectious agent is used for xenotransplantation, active screening for that infectious agent should be implemented. For example, all recipients of xenotransplantation products involving the use of porcine cells, tissues or organs should be assessed for evidence of infection by porcine endogenous retrovirus(es). Recipient screening for PERV should include analysis by multiple methods. Ideally, all of the following detection methods should be used:

- (a) PCR of recipient's PBMC for PERV DNA sequence,
- (b) serologic analysis for PERV-specific antibodies, and
- (c) assays capable of detecting plasma virions, such as RT-PCR for detection of viral RNA or highly sensitive methods for detection of RT activity (reference 47).

A sufficient quantity should be collected of each sample in the active screening program to permit archiving for future use should the need arise. See section VIII.H. for recommendations regarding the number, size, use, and duration of storage of collected samples.

d. Identification of Xenogeneic Retroviruses in Recipients

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One particular concern is the potential transmission of xenogeneic retroviruses, such as PERV in the case of recipients of porcine products. FDA recommends that sponsors of porcine xenotransplantation product clinical trials develop a plan to address the possibility that a recipient tests positive for the presence of PERV or other similar xenogeneic infectious agents. The plan should include the following:

- (i) Strategies to identify the source of a positive signal in the screening test (e.g., infection vs. false positive). For example, in the case of porcine xenotransplantation products, PCR of DNA isolated from recipient PBMC for detection of PERV genetic sequences is recommended. However, if a positive result is obtained from this analysis, one possible explanation would be the presence of porcine cells. Therefore, additional DNA PCR for a repetitive porcine genetic element should be performed, to determine whether the source of the positive result may be from microchimerism for pig cells, rather than from human cells infected with a pig retrovirus. If this analysis suggests the latter possibility, additional analysis should include an attempt to isolate the virus from relevant recipient specimens in an appropriate co-culture assay.
- (ii) Determination of infectivity of the agent using appropriate assays (e.g., co-cultivation) and additional characterization of the agent as necessary.
- (iii) A plan to notify FDA, and relevant sponsors and investigators.
- (iv) A contingency plan to modify the clinical trial (including suspension or termination of enrollment).
- (v) Provisions for acute and follow-up medical care and counseling of the patients in the study.
- (vi) Additional actions if required for the safety of the recipient and close contacts and to address possible public health risks.

### e. Postmortem Detection of Agents and Archiving of Autopsy Samples

A complete postmortem examination including histopathology and cultures should be requested of all recipients. At postmortem, samples of body tissue should be fixed and embedded for examination by light and electron microscopy. Samples should be obtained from the xenotransplantation product and, as appropriate, all major organs related to the product or to clinical syndromes that either resulted in the recipient's death, were deemed to have been serious, or were of unexplained etiology. Tissue and fluid samples should be archived at -70°C or lower, as appropriate for preserving the sample, for 50 years beyond the recipient's death as discussed in section VIII.H.1.

4. Infections in Recipient Contacts

It is recommended that a program be developed to monitor health care providers and other close contacts of recipients (e.g., persons with whom recipients repeatedly engage in activities that could result in intimate exchange of body fluids). In these groups, passive screening (see section VIII.F.3.c.i.) may be appropriate. Baseline samples of plasma should be obtained and archived at -70°C, and leukocytes should be obtained and archived in liquid nitrogen for example, for health care personnel when they join the clinical teams. It is recommended that such contacts also be advised and counseled regarding potential risks.

**G. Patient Follow-up**

The sponsor should propose and submit a plan for clinical follow-up of recipients in a xenotransplantation protocol in the FDA application requesting investigational use (e.g., IND). This plan should take into account the timetable for collection and storage of specimens for the passive screening program and should extend for the life of the recipient (see section VIII.F.3.c.i.). It is realized that the frequency of follow-up will decrease with time post-procedure. It is reasonable to plan for a tapering frequency of clinical monitoring and follow-up, with the flexibility to increase the frequency for individual recipients or trial participants as a whole, if events occur to make this appropriate.

**H. Archiving of Patient Plasma and Tissue Specimens**

1. Protocols or SOPs for archiving all samples of patient tissue and fluids, including samples archived as part of recipient screening, post-mortem samples, and samples for PHS use, should be in existence before patients are treated.
  - a. Appropriate biosafety precautions should be followed in collection of clinical samples from recipients. Standard precautions should be followed in obtaining blood from recipients (reference 48). The revised PHS Guideline (reference 1) has recommended the use of at least a BioSafety Level (BSL) -2 containment facility with BSL-3 practices for any manipulation of clinical samples.
  - b. For the schedule for archiving biological specimens recommended by PHS, see reference 1 and section VIII.F.3.c.i. of this document. The specific protocol or the recipient's medical course may indicate more frequent archiving.
  - c. Plans should exist to maintain all archived samples according to the procedures recommended in the revised PHS Guideline (reference 1), including those obtained from patients during acute infectious episodes, and from health care workers.

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d. Patient blood and plasma samples should be stored in volumes and quantities according to the recommendations for animal plasma and blood cell samples (see section III.E.3.b.).

e. In addition to recipient samples collected during screening programs or post mortem, when xenotransplantation recipient tissues are collected for any medical use, such as a biopsy for diagnostic purposes, samples of such tissues should also be archived. Samples should be stored at -70°C or lower as appropriate for preserving the sample.

### 2. Archive Samples

a. The revised PHS Guideline (see reference 1 and also section VIII.F.3.c.i. of this document) recommendations regarding archiving of plasma, blood, and other specimens should be followed. Samples should be collected, archived, and reserved for use by PHS should the need for a PHS-led investigation arise. The PHS Guideline (reference 1) recommends that biologic specimens for PHS use be maintained for 50 years, based on the latency periods of known human pathogenic persistent viruses and the precedents established by the U.S. Occupational Safety and Health Administration with respect to record-keeping requirements.

b. In addition to the designated PHS samples, the sponsor should archive separate samples of patient plasma, blood cells, xenotransplantation product, or other tissues for clinical follow-up and for storage as part of a passive screening program, as detailed above. (See section VIII.H.1).

c. Samples archived for use by PHS (see section VIII.H.2.a.) or for monitoring of the recipient through a passive screening program (see section VIII.H.2.b.) should not be used for other purposes, such as research.

## **I. Health Records and Data Management**

1. Sponsors should ensure that the recipient's medical record contains information on the recipient's health, and all xenotransplantation related information including procedures, a description of the xenotransplantation product, and any xenotransplantation product-related adverse events. In addition, sponsors should develop an appropriate tracking system for all recipients of their xenotransplantation products. Tracking information may be used to facilitate notification in the case of a serious adverse event related to a xenotransplantation product. Information should be collected when events occur, such as a xenotransplantation procedure or an adverse event, and at the time of clinical follow-up examinations.

Reporting forms should be uniform and include information relevant to the recipient. It is recommended that the information to be collected and tracked

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include, at a minimum, the following:

- a. Facility information - Sponsors should record information regarding their animal facilities, manufacturing facilities, and clinical centers associated with each source animal, xenotransplantation product, and recipient.
- b. Recipient information - Recipients should be identified by code number or other identifier to link the recipient to relevant information in the tracking system.
- c. Procedure information - Information about each xenotransplantation procedure should be recorded. This information should include, but is not limited to:
  - (i) recipient identifiers,
  - (ii) the date of the procedure,
  - (iii) the clinical center where the procedure was performed,
  - (iv) the physician or investigator who performed the procedure,
  - (v) the clinical indication for the xenotransplantation procedure,
  - (vi) medications and therapies administered at the time of the procedure,
  - (vii) a description of the xenotransplantation product(s),
  - (viii) identification of the animal source(s),
  - (ix) animal facilities for each animal source,
  - (x) xenotransplantation product manufacturing facilities, and
  - (xi) other pertinent clinical information
- d. Adverse Event Reports - A sponsor should record adverse event reports and report the events to FDA pursuant to existing regulation (21 CFR 312.32). Sponsors should keep records of each event.
- e. Recipient clinical follow-up examinations - Clinical status information for recipients of xenotransplantation products should be periodically collected (see section VIII.F.). This information should include, but is not limited to:
  - (i) the date of the clinical follow-up examination,
  - (ii) the location of the clinical follow-up examination,
  - (iii) the status of the xenotransplantation product in the recipient,
  - (iv) any new significant co-morbidities or inter-current conditions, and
  - (v) any hospitalizations since the recipients last clinical follow-

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up examination.

- f. Animal Health Events - Animal health events should be recorded by animal facilities. These events include, but are not limited to:
  - (i) breaks in the environmental barriers of the secured animal facility,
  - (ii) disease outbreaks, and
  - (iii) sudden, unexplained, or unexpected animal deaths.

Animal health events should be reported to the IND sponsor by the animal facility. This information should be included in the sponsor's tracking system for recipients and in reports to the FDA.

- g. Recipient Death Reports - Sponsors should maintain death reports on recipients. This information should include recipient identifying information, the date of death, and the cause of death. Death certificate and autopsy information should be recorded if available. Deaths should also be reported to FDA.

2. The FDA, together with other PHS agencies, is developing a computerized National Xenotransplantation Database intended to assist in data monitoring and tracking of recipients for Public Health Service needs. Sponsors may be requested to submit information to this database when it is mature.

3. Health records should be maintained for at least 50 years beyond the date of transplantation.

4. The sponsor should make provisions for all records and samples (including post-mortem samples) to be maintained for the requested period in the event that the establishment ceases operation.

**J. Informed Consent**

- 1. General Comments

The informed consent document should include the standard contents (see 21 CFR 50.25, Elements of Informed Consent).

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2. Specific Issues

Within the general outline of the informed consent document, certain specific issues should be addressed regarding recipients.

a. Requirements for Participation in the Study

i. Because the zoonotic, opportunistic, and xenogeneic infectious risks to the recipient may extend to the recipient's family or contacts (e.g., persons with whom recipients repeatedly engage in activities that could result in intimate exchange of body fluids and other contacts such as health care workers) the patient should consent to inform his current and future contacts of their potential risks from the source animal species, and of their deferral from blood donation.

The recipient should be offered assistance with this education process, if desired. This discussion should include the recipient's potential to transmit zoonotic or opportunistic infections if such an infection were to occur, and the possibly increased risk of such transmission to infants, pregnant women, the elderly, chronically ill or immunosuppressed individuals and others who may be at increased risk for zoonotic or opportunistic pathogens.

ii. As an interim precautionary measure, xenotransplantation product recipients and certain of their contacts should be deferred indefinitely from donation of Whole Blood, blood components, including Source Plasma and Source Leukocytes, tissues, breast milk, ova, sperm, or any other body parts for use in humans. Pending further clarification, contacts to be deferred from donations should include persons who have engaged repeatedly in activities that could result in intimate exchange of body fluids with a xenotransplantation product recipient. For example, such contacts may include sexual partners, household members who share razors or toothbrushes, and health care workers or laboratory personnel with repeated percutaneous, mucosal or other direct exposures. These recommendations may be revised based on ongoing surveillance of xenotransplantation product recipients and their contacts to clarify the actual risk of acquiring xenogeneic infections, and the outcome of deliberations between FDA and its advisors.

(See also FDA draft guidance document “Guidance for Industry: Precautionary Measures to Reduce the Possible Risk of Transmission of Zoonoses by Blood and Blood Products from

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Xenotransplantation Product Recipients and Their Contacts,” which has been published for public comment (reference 12). FDA will consult with its advisors to identify the range of xenotransplantation products for which recipients and/or certain of their contacts should be recommended for deferral from blood donation. Additionally, the range of contacts who should be deferred from blood donation will be clarified after further public discussion.)

iii. The recipient should be counseled regarding other behavioral modifications. Advice on the use of barriers to transmission of infectious agents during sexual activity and the use of appropriate precautions for nonsexual contacts should be provided as appropriate.

iv. The informed consent document should contain information about the proposed life-long surveillance for all recipients and the need for clinical and laboratory monitoring throughout. The schedule for such clinical and laboratory monitoring should be explained, to the extent possible.

v. The document should address the need for archiving plasma and tissue specimens from the source animal and the recipient for analysis in the case of xenogeneic disease concerns. The document should explain that such specimens may be tested in the future by the sponsor or PHS agencies as needed to evaluate concerns regarding xenogeneic infections.

vi. The document should inform the recipient of the responsibility to inform the investigator or his/her designee of any change in address or telephone number for the purpose of enabling life-long health surveillance.

vii. The document should inform the recipient of the long term need for access by the appropriate public health agencies to the recipient’s medical records. To the extent permitted by applicable laws and/or regulations, the confidentiality of medical records should be maintained.

viii. A request for autopsy should be included in the informed consent document signed by the intended recipient or his/her appropriate representative.

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b. Risks to the Recipient and his/her Close Contacts

i. The informed consent document should address the specific and known risks of the surgery, of the specific immunosuppressive agents, and of the known and unknown zoonoses that may be associated with the source species. The uncertainty of the risks of infection or its transmission, and of the risk of tumorigenesis, should be mentioned. The possibility of a long latency period before detection of possible adverse effects should be mentioned. The need for, and risks from, prophylactic antimicrobial, antiviral, or other chemo- or immunotherapy should be specified. The reasoning behind the use of any prophylactic treatments should be provided in an attachment for the recipient and the recipient's family.

ii. In addition, the possible need for confinement, reverse isolation or other specialized medical housing should be described, including the estimated duration of such confinement. Any specialized dietary, travel or other precautions should be described in as much detail as possible.

iii. Any known time course for the risks of disease development and transmission should be included. Discussion of infectious diseases with protracted incubation periods including TSEs and other unusual pathogens should be provided.

iv. In the specific case of xenotransplantation products from porcine sources, the informed consent document should include the following information:

- (a) Porcine endogenous retrovirus can be transmitted from pig cells to human cells in culture and this virus can be transmitted from a human cell line to other human cell lines in culture.
- (b) The clinical significance, if any, of this observation is unknown and is an area of active research; however, it is known that infection by certain type C retroviruses, similar in structure to porcine endogenous retroviruses, can cause neurological disorders and diseases, such as lymphomas and other malignancies, in certain animal models.

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c. Potential Benefits

It should be clear in the informed consent whether xenotransplantation is being studied as a first-line, second-line, or salvage therapy of the condition for which it is being proposed for the individual recipient. The specific anticipated benefits, e.g., limited prolongation of survival, improved specific organ function, xenotransplantation product support until allograft becomes available, or experimental use without known or anticipated benefit, should be clearly conveyed.

d. Alternative Treatments

The anticipated therapeutic options available to participants in the event of failure of the xenotransplantation product should also be explained in detail in the informed consent document.

A discussion of the possibility that additional therapies, prophylactic treatments or diagnostic tests may become available after xenotransplantation should be included.

e. Possible Consequences and Subsequent Treatment Options

The consequences to the patient should the product fail or undergo irreversible rejection should be explained to the extent possible, including clear and unambiguous statements about the options that will not be offered before xenotransplantation, or that may not be possible after rejection of the xenotransplantation product, e.g., allotransplantation.

f. Confidentiality Issues

The patient should be informed that all data, including data collected during the follow-up period, could be made available to PHS agencies.

**K. Responsibility of the Sponsor in Informing the Patient of New Scientific Information**

The sponsor should commit to providing recipients with updated information as soon as possible in the event that new data on risks, benefits or the need for additional treatments relevant to the recipient's clinical course becomes available or necessary. The sponsor should be willing to make a long-term commitment to provide information to the recipient's families in the event that a recipient has died and new safety information of relevance to their potential exposures becomes known. The sponsor should ensure that the investigators are also willing to commit to providing new information to recipients and their families.

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