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

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Guidance for Industry

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Contains Nonbinding Recommendations

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Guidance for Industry

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

The Food and Drug Administration (FDA) is issuing this guidance to provide sponsors and applicants of xenotransplantation products with updates concerning the production, testing, and evaluation of products, during protocol development and during the preparation of submissions to FDA, e.g., Investigational New Drug Applications (INDs) and Biologics License Applications (BLAs). This guidance also includes updated references and Agency practices intended to prevent the introduction and spread of infectious agents of animal origin into the human population. This guidance amends the guidance of the same title dated April 2003 (April 2003 guidance).

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. Also, for the purpose of this document, xenotransplantation products include live cells, tissues, or organs used in xenotransplantation. However, for the purpose of this guidance, xenotransplantation does not include transplantation, implantation, or other use of acellular animal tissues. These acellular products derived from animal tissue, often referred to as xenografts, are beyond the scope of this guidance (See Definitions in section III of this document).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended but not required.

II. BACKGROUND

In the mid 1990's, xenotransplantation was suggested as a mechanism for circumventing the shortage of human allographs for transplantation. Proposed xenotransplantation protocols included implantation into humans of live organs, tissues, and cells from nonhuman animal sources and procedures in which human cells have had ex vivo contact with nonhuman cells, tissues, or organs. Examples of procedures involving xenotransplantation products include transplantation of xenogeneic hearts, kidneys, or pancreatic tissue to treat organ failure, implantation of neural cells to ameliorate neurological degenerative diseases, administration 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 xenotransplantation products presents potential public health risks, such as:

1. Transmission of infectious agents 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 infectious agents, particularly viruses, with nonpathogenic or endogenous human infectious agents, to form new pathogenic entities.

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 due to 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 previously took place in the FDA Biological Response Modifiers Advisory Committee (BRMAC) - Subcommittee on Xenotransplantation; the Department of Health and Human Services (DHHS) Secretary's Advisory Committee on Xenotransplantation (SACX); and other public fora.[i]

[i] The BRMAC Subcommittee on Xenotransplantation (SACX) was initiated in 2001 and was discontinued in 2005. Further discussions on xenotransplantation have taken place in the Cellular, Tissue, and Gene Therapy Advisory Committee (CTGAC). In addition, a discussion took place in 2009 on the topic of "Animal Models for Porcine Xenotransplantation Products Intended to Treat Type 1 Diabetes or Acute Liver Failure."

In 1996, the Public Health Service (PHS) published a "Draft Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation" (61 FR 49920, September 23, 1996) that was revised and issued on January 29, 2001 (66 FR 8120), based on public comments received and advances in fields relating to xenotransplantation[1] (hereafter referred to as "PHS Guideline"). This FDA guidance document reiterates many of the concepts in the PHS Guideline but, in addition, includes specific advice, regarding all aspects of xenotransplantation product development and production and xenotransplantation clinical trials.

Since the publication of the PHS Guideline, the World Health Organization (WHO) published the Second Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials.[2]

FDA anticipated that its approach to regulation of xenotransplantation products would evolve as the scientific knowledge in the area of xenotransplantation continued to accumulate. Since the issuance of the final guidance in 2003, FDA has issued additional guidance that contains recommendation relevant to certain xenotransplantation products; see references.[3-8] FDA realizes that it may not be appropriate to 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 consisting of well-characterized, long-established animal cell culture lines or human cells co-cultured with such lines.

III. 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 infect, potentially could infect, or have an inadequately defined ability to infect humans.

ATCC: American Type Culture Collection.

BLA: Biologics license application. Approval of a biologics license application or issuance of a biologics license constitutes a determination that the establishment(s) and the product meet applicable requirements to ensure the continued safety, purity, and potency of such products.

BSL: BioSafety Level.

CDC: Centers for Disease Control and Prevention.

CDRH: Center for Devices and Radiological Health.

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 21 CFR Part 606. For devices, quality system regulations can be found at 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.

CVM: Center for Veterinary Medicine.

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 infectious agents, such as viruses.

FDA: Food and Drug Administration.

GE: Genetically engineered.

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

GVHD: Graft versus host disease.

HEPA: High-efficiency particulate air.

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 Public Health Service Policy on Humane Care and Use of Laboratory Animals, revised 2015.)

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 Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.)

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals For Human Use.

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 Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 360(g)), and the implementing regulations can be 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(i) of the Act (21 U.S.C. 355(i)) or to the licensure provisions of section 351 of the PHS Act (42 U.S.C. 262). The statutory requirement is at section 505(i) of the Act (21 U.S.C. 355(i)), and the IND regulations are found at 21 CFR Part 312.

INAD: Investigational new animal drug.

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.)

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 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.)

NADA: New animal drug application.

PBMC: Peripheral blood mononuclear cells.

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

PERV: Porcine endogenous retrovirus(es).

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.

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.

SAF: Source animal facility.

Sentinel animal: Those animals, usually of the same species as the resident herd or colony, housed in direct contact with the animals being monitored and tested for the purpose of detecting adventitious agents in the herd or colony, including viral, fungal, and bacterial diseases as well as endo- and ectoparasites.

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-unidentified viral agent may be necessary for transmission.

USDA: United States Department of Agriculture.

WFI: Water for Injection.

Xenograft product(s): Xenograft products are acellular products derived from animal tissue. These xenografts, are beyond the scope of this guidance. Please consult CDRH for guidance on xenograft products.[9]

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 (e.g., brucellosis, rabies) that may be transmitted to humans under natural conditions.

Zoonotic: Relating to diseases that arise from the transfer of infectious agents by normal contacts between animals and humans.

IV. REGULATORY RESPONSIBILITY

FDA has regulatory oversight of xenotransplantation products, including live organs, tissues, or cells from a nonhuman animal 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. If xenotransplantation products are to be used in clinical investigation, they require an appropriate investigational application be in place (e.g., 21 CFR Part 312). FDA will regulate most xenotransplantation products as biological products. 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*.). You will find regulations for drugs, biological products, and devices in Title 21 of the Code of Federal Regulations (e.g., 21 CFR Part 312 for regulations governing INDs, 21 CFR Part 812 for regulations governing IDEs, and 21 CFR Part 601 for regulations governing licensing of biological products). To assist developers of cell therapy products in preparing IND submissions to the Agency, FDA has published guidance on regulatory considerations in the development of somatic cell therapy and gene therapy and the content and review of chemistry, manufacturing, and control information for cell therapy product INDs.[3, 10] In addition, for products derived from GE animals, please refer to 21 CFR Part 511 and Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs[4] for recommendations regarding regulations governing investigational new animal drug that are regulated by FDA's Center for Veterinary Medicine (CVM). The primary responsibility for designing and monitoring the conduct of clinical trials rests with the sponsor (e.g., 21 CFR 312.23(a)(6)(iii)(*d*) and 312.50). In this document, "you" refers to the sponsor, the clinical investigator, or to any party designated by the sponsor to fulfill the recommended function (e.g., SAF staff, laboratory personnel, etc.).

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. Refer to 21 CFR Part 3 for issues regarding the regulation and assignment for premarket review of combination products.

V. SOURCE ANIMAL CHARACTERIZATION

A. General Considerations

The cross-species infectious potential of specific animal pathogens should be a major consideration in the selection of source animal species. Anatomic and physiologic considerations are also important. For example, whether an organ is of appropriate size

and will function adequately across species barriers are considerations, as are certain immunologic concerns, including the suitability of current regimens in prevention of rejection of the nonhuman animal live cells, tissues, or organs. Please note that the species you use may be subject to other federal laws and regulations (see, e.g., 16 U.S.C. 1538), such as those covering endangered or protected species. You 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"[11] before submitting an application to FDA that involves the use of nonhuman primates as sources of a xenotransplantation product. (The term "xenograft" as used in the above referenced document is synonymous with the term "xenotransplantation product" however, these terms are no longer synonymous in current use (see Definitions)).

Due to potential infectious disease risks associated with the use of xenotransplantation products, you should develop appropriate source animal qualifications. These qualifications 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, and 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 source animals. Procedures for animal husbandry, tissue harvesting, and 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. 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. 289(d)). We recommend that the SAF be accredited by the AAALAC. Standards for accredited facilities for when funds are received from the National Institutes of Health are provided in the National Research Council's Institute for Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals.

C. Animal Origin

1. Animal and Herd Qualification

You should derive source animals only 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, transmissible spongiform encephalopathies (TSEs), and parasites for which the herd is screened and supply this information to FDA as part of the application to FDA (e.g., IND). You should consider all infectious agents known to infect the source species. You should justify the rationale for omitting agents that are found in the source animal species from the herd screening program in the application to FDA requesting investigational use (e.g., IND). For example, the geographic location of the herd may allow exclusion of certain infectious agents. You should obtain source animals from TSE-susceptible species only from closed herds that are documented to be free from TSE diseases or TSE-associated agents[12] (see also section V.C.3.c.). You should not use source animals obtained from geographic areas in which TSEs are known to exist in the source species. In the application to FDA requesting investigational use of the xenotransplantation product (e.g., IND), you should describe and justify the frequency of the screening, the method of assay, and the method of identifying which and what proportion of animals are sampled. As data are accumulated that demonstrate product safety, you may modify the screening program in consultation with the FDA.

2. 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 TSEs or other adventitious or infectious agents of concern (see Definitions, section III.). Source animals should be bred and raised in captivity and be derived from closed herds. You may use artificial insemination, embryo transfer, cloning, or hysterotomy plus foster feeding to establish animal herds with fewer endemic pathogens. In particular, the PHS Guideline suggests that breeding programs use caesarean-derived animals, whenever possible.[1] You should document the use of these procedures in the animal history.

3. Source Animals from Outside the U.S.

a. You should not use animals from outside the U.S. or their first generation offspring 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, you should apply the same considerations for these animals as for source animals bred in the U.S. (e.g., see section V.D. for Animal Health and Husbandry). You should use a quarantine period of sufficient length to demonstrate the absence of infectious agents of concern, and you should perform extensive screening of the animals. In the application to FDA requesting investigational use of the xenotransplantation product (e.g., IND), you should submit thorough documentation 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. You should include in the screening agents that are endemic in the country of origin. In the application to FDA requesting investigational use of the xenotransplantation product (e.g., IND), you should describe methods and conditions of transport of imported animals. 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, you should use them as founders for a domestic herd that will be well-characterized for an extended period of time before use, using procedures sufficient to validate the herd's acceptability as source animals.

c. You should not import source animals from any country or geographic region where TSEs are known to be present in the source species. For these purposes, only countries that are listed by the World Organisation for Animal Health (OIE) as having "negligible" risk of BSE in cattle should be considered acceptable.[13] Use of the OIE BSE risk status for source animal importation is consistent with current USDA regulations (9 CFR Parts 92-96 and 98) with respect to importation of bovines and bovine products.[14]

d. You should consult the USDA and, when appropriate, CVM and CDC for their requirements regarding importation of animals or animal tissues.

4. Range and Wild Animals

You should not use source animals that are raised under free-ranging conditions. 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. You should not use wild-caught animals 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 are unknown. Therefore, you should not use such animals as source animals.

6. Semen Donors

You should apply the same source animal considerations to semen donors, whether or not they are members of the herd, including, for example, screening for infectious agents that may be transmitted by semen.

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.

You should obtain source animals exclusively from SAFs. Your application or submission to FDA should include detailed plans for maintaining source animals. These plans should include SOPs, 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.

1. Facilities

You should house the animals in facilities built and operated in accordance with recommendations described in the National Research Council's Institute for Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals and accredited by the AAALAC. You should not locate SAFs in geographic proximity to manufacturing or agricultural activities that could compromise the facility's 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 21 CFR 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.

You should include a detailed description of the facilities and procedures for housing source animals 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), lighting, temperature, 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. You should keep records of 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. You should include procedures for elimination of animal wastes. Include in the description how qualified source animals will be housed (for example, as a batch or as 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 a veterinarian with expertise in infectious diseases is not on staff, you should provide documentation in the investigational application (e.g., IND) 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., 21 CFR 600.10 and 600.11). Developers of xenotransplantation products should consider becoming ISO 9001:2015[15]certified.

2. Maintenance of Source Animals

a. General

You should maintain source animals in accordance with standard operating procedures appropriate to the species, xenotransplantation product, and the intended clinical application. SOPs should provide for admission of new animals to the SAF and source animal pool, for quarantine, and for removal, isolation, or elimination of diseased animals. You should provide this information to FDA in the application for investigational use (e.g., IND). You should not reintroduce animals that have been removed from the source animal pool due to illness or infection.

You should develop procedures 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. You should report such information to FDA as well as procedures to collect the information as part of the application for investigational use (e.g., IND).

b. Health Screening

i. You should maintain source animals 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 you should include a copy or a summary of the SOPs in the FDA submission requesting investigational use (e.g., IND). You may modify the frequency of testing as the reliability of the production system is established using data from earlier screens. You should consult appropriate experts, such as infectious disease consultants, virologists, microbiologists, accredited microbiological laboratories, and veterinarians, 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, you should use more general assays for detection of classes of agents. For example, you should examine feces from source animal herds 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. You should consult with CBER if the use of such animals is contemplated (see, for example, section VII.C.4.d.). Techniques for introducing new animals, such as artificial insemination, caesarean 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 sentinel animals should help minimize this problem and may help identify

infections in recipients, post transplantation. You should consider establishing a sentinel animal program that includes periodic necropsy and extensive histology and pathology evaluations. 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 be able to detect zoonoses known to exist in the species or geographical regions in which the source animals originate and are maintained. For example, a sentinel animal program for ruminants might specify that the program maintain some animals into old age and test all diseased animals eliminated from the herd as well as all sentinels at necropsy for evidence of BSE using USDA-approved postmortem diagnostic tests for BSE with brainstem tissue collected as specified by USDA.[16]

iii. You should quarantine and screen individual source animals before harvest of cells, tissues, or organs, as discussed in section V.D.4.b., below, and in the PHS Guideline[1].

c. Health Care

The herd health surveillance system should include comprehensive documentation of all veterinary care received by source animals. This includes documentation of all illnesses, medical care, procedures, drugs administered, vaccinations, routine physical exams, and any treatments received by each animal. You should carefully document use of antimicrobial agents due to potential risk to allergic recipients receiving unprocessed nonhuman animal live cells, tissues or organs. You should validate residual drug levels 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. You should use live vaccinations 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. You should describe the procedures to deal with illnesses or other incidents that affect the health of the herd in your application to FDA for investigational use. You should not use as source animals those animals requiring treatment with blood, blood products, or tissues obtained from animals outside the closed herd, and you should remove these animals from the herd. You should use aseptic techniques and sterile equipment 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), you should document the treatment in the application to FDA requesting investigational use (e.g., IND), and you should provide copies of package inserts or labeling. You should document and maintain records showing the treatment of animals with drugs for any reason, including the withdrawal period following drug treatment. You should develop procedures for disposal of dead animals (see section V.G.).

d. Feed

You should describe the storage and delivery of feed, water, and other consumables in the application to FDA for investigational use (e.g., IND). Records should include manufacturer, batch numbers, and other pertinent information, and you should describe recordkeeping procedures in an SOP. You should record in the individual source animal's records the vendor and contents of feed given to a source animal for at least two generations before use as a source for live cells, tissues, or organs used in xenotransplantation. You should not use feeds containing animal proteins or other cattle materials that are prohibited by the FDA feed ban as expanded in 2008 as source animals (21 CFR 589.2000).[17] You should not use feeds containing significant drug contamination or pesticide or herbicide residues for source animals (21 CFR 589.2001).[9] You should not use natural, non-sterile foods, such as hay, 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. You may include pasteurized milk products in feeds. You should feed newborn animals colostrum or milk from dams only if the dams have been specifically qualified by the same procedures used for herd qualification.

e. Caretakers

You should provide SOPs for animal caretakers in the FDA submission requesting investigational use (e.g., IND), and you 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., 21 CFR 600.11). There should be a documented training program for personnel as described in the cGMP regulations (21 CFR 211.25).

You should monitor the health of humans in contact with animals on a routine basis.[18] You should predetermine and customize the program for screening and monitoring of caretaker and other staff to maximize screening information, and you should describe the program in an SOP.

Health monitoring of humans who come into contact with the animals should include physical exams with baseline and, if indicated, either periodic sampling and storage of serum or plasma for individuals having frequent and close contact with source animals or less rigorous monitoring for those with occasional contact. You should obtain baseline samples from all caretakers.

3. Animal and Personnel Traffic through the Source Animal Facility

You should develop SOPs for entry and exit of animals, and they should include transportation of animals to and from the facility. You should subject all animals entering the facility to a defined quarantine period, allowing for completion of any screening procedures. The minimum quarantine period for animals used in manufacture is seven days (21 CFR 600.11(f)(2)). However, you should use longer quarantine periods that extend beyond the incubation period for infectious agents in the source animal species for animals entering a SAF. You should devise a tracking system that allows unique identification of each individual animal in the facility. You should minimize entry and exit of animals and human staff to avoid exposures to transmissible infectious agents. We encourage the use of an 'all in/all out' or batch approach for moving qualified source animals as a method of minimizing the potential for infectious agent transmission.

You should describe personnel traffic patterns 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

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

When feasible, you should examine a biopsy of the live animal cells, tissue, or organ or other relevant tissue (e.g., contra-lateral kidney as a surrogate for a kidney graft or thoracic tissue as a surrogate for a heart) by

histopathology and tested for infectious agents by appropriate assays. You should take care in performing biopsies to use fastidious sterile technique so as not to introduce new microbial contaminants. You should archive remaining biopsy tissue as described in section V.E.3.

You should perform all tests 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 before their use. If more than 3 months have elapsed since the initial testing or biopsy of the source animal, you should repeat tests before harvest.

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

b. Quarantine

You should generally quarantine individual source animals for a minimum of three weeks before 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 shorter, you should provide justification in the application to FDA for investigational use (e.g., IND). During the quarantine period, 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

You should describe the procedures and physical facilities used for harvesting of live cells, tissues, or organs from source animals in detail in the application to FDA requesting investigational use (e.g., IND). Qualified and controlled procedures for avoiding the introduction of infectious agents during harvesting should be in place. Qualification 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 anesthetic agents should not be harmful to the human recipient. A summary of the health records (e.g., health status and microbiological screening reports, results of lot release tests, and

anesthetic used, if relevant) regarding the source animal should accompany the xenotransplantation product, and you should incorporate them 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. Therefore, we recommend 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, you should maintain barriers, equivalent to or better than those in place at the SAF 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 you should document the method in the submission to FDA (e.g., in the IND). If there is any question regarding the effectiveness of the transportation and containment procedures, you should quarantine and rescreen animals in a fashion comparable to that used for entry of new animals into a closed herd.

You should develop and implement procedures for avoiding shipping errors, avoiding contamination, and documenting transfer of animal materials to the correct patient. You should describe in detail the method of transporting the live animal cells, tissues, or organ from harvest site to the clinical xenotransplantation site in the application to FDA requesting investigational use (e.g., IND).

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, you should conduct a full necropsy, including gross, histopathological, and microbiological evaluation, and you should obtain archival samples, including portions of the product for storage as described in section V.E.3.b.

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

When source animals die or are euthanatized, you should perform a full necropsy, and you should obtain archival samples for storage as described in section V.E.3.b.

b. Samples to be Archived and Storage Conditions

You should store archived samples of source animal tissues and body fluids at -70 degrees Celsius or lower temperatures, as appropriate for preserving the sample, or you should maintain fixed samples at room temperature. Section 3.7.1 of the PHS Guideline[1] recommends that you cryopreserve at least ten 0.5 cc aliquots of citrated- or EDTA-anticoagulated plasma and at least five aliquots of viable leukocytes ($1x10^7$/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). You should select the conditions of cryopreservation and storage for viable samples to maintain cell viability for the period of storage (see section V.E.3.c.). You should collect appropriate tissue samples for formalin fixation and paraffin-embedding and for cryopreservation from source animals at the time the live cells, tissues, or organs are procured. You should collect and cryopreserve tissue samples representative of major organ systems of source animals (e.g., spleen, liver, bone marrow, central nervous system, lung,) at necropsy. As appropriate to the xenotransplantation product, you should archive other body fluids, such as cerebrospinal fluid, at the time of procurement of the product and/or necropsy. If sentinel animals are used, you should also archive tissue samples and body fluids obtained at necropsy.

c. Archive Rationale, Duration, and Responsibility

The PHS Guideline[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,

ii. for use if needed for recipient diagnosis and care, and

iii. for use by the sponsor, as appropriate.

You should include detailed plans for obtaining and storing the archive samples in the application to FDA requesting investigational use (e.g., IND). The PHS Guideline[1] recommends that samples should be stored for 50 years from the time of sample acquisition. You should clearly designate responsibility for the archives and access to the specimens.

4. Herd Records

You should keep records pertaining to the source animals and facilities. These records are subject to inspection, and you should maintain the records for 50 years beyond the date of procurement of the nonhuman animal 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. You should make provisions for all records to be maintained for the requested period in the event that the establishment ceases operation. If you are a sponsor who plans to cease doing business, you should consult FDA 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. You should include the history of the cell line 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. You should include, whenever possible, information such as age and sex of the source animal, laboratory of derivation, date of derivation, and the immediate provider of the cell line. For short-term cultures, (e.g., derived less than one year previously), you should also include a description of the husbandry and health status of the particular source animal and herd or colony used to derive the cells. 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. Information regarding the commercial source and country of origin of any animal products used in deriving or maintaining the cell line should also be included in the history. You should characterize and test the final product as described in section VII. You may also consult the "Points to Consider in the Characterization of Cell Lines Used to Produce Biological Products"[19] for pertinent recommendations regarding the production, identification, and characterization of cell lines used in manufacture. Additional information may be found in relevant ICH Guideline.[20]

FDA has considered how this guidance relates to human embryonic stem (hES) cell lines that were in existence prior to August 9, 2001. These hES cell lines had used murine feeder layer cells and, thus, fit the definition of xenotransplantation used in this guidance and the PHS Guideline. FDA does not intend that the Agency's regulation of xenotransplantation will preclude the use of these hES cell lines. It may be necessary for a sponsor who wishes to investigate a stem cell product derived from existing hES cell lines in a clinical trial to demonstrate to FDA that the hES cell line is free from infectious agents, including murine infectious agents. Given current technology, a sponsor should

be able to do this without undue burden. For example, it will not be necessary for a sponsor to provide FDA with complete animal husbandry information for mice from which the mouse feeder layers were derived. If continued expansion on murine feeder cell layers is planned, FDA may request that a sponsor demonstrate that the mouse colonies, from which such murine feeder layers are to be obtained, are maintained appropriately to ensure safety. (See section V. of this guidance and section 3 of the PHS Guideline[1]). The same recommendations apply to other xenotransplantation products that contain human cells with a history of co-culture with nonhuman animal cells.

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, you should not use such animals as sources of human food via milk or meat or as ingredients of feed for other animals. You should not use such animals as pets or breeding animals because of the potential for them to enter the food/feed chain, either directly or through the rendering process. You should dispose of source animals in a manner consistent with the disposal of 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 you may contact CVM directly through the Division of Compliance, HFV-235, FDA, Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855, 240-276-9227.

VI. CHARACTERIZATION OF XENOTRANSPLANTATION PRODUCTS

A. General Considerations

In general, you should test the xenotransplantation product for safety, identity, purity, and potency. Part 610 (21 CFR Part 610) describes types of assays that are required for licensed biologics. You should use similar tests during investigational stages of product development. We discuss in more detail assays for safety testing, including infectious agent tests and tests for endotoxin, in section VII. 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.[20-22]

Additional recommendations and comments regarding microbiologic tests are found in section VII. 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 before use. However, you 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. You should also perform tests for endotoxin or pyrogen. Although we realize that results of these tests will not be available before transplantation, you should still complete assay or culture periods and record the results. As appropriate, you may use histology, performed on a retention sample or biopsy of the xenotransplantation product, 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, you should perform additional product characterization to measure identity, purity, and potency. As much as possible, results of these assays should be available before xenotransplantation and used for lot release. You should also apply these same product characterization steps 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.

a. Safety

We discuss tests for bacterial and fungal sterility, mycoplasma, and viral agents, generally considered safety tests, in detail in section VII. of this document.

b. Identity

You should develop a means to assess identity of the active component of the xenotransplantation product. This may include identification of relevant cell or tissue types, using immunological, immunohistological, or biochemical cell markers. In some cases, it may be possible to use histological evaluation. Depending on the manufacturing process, verification of the species or strain identity of the final product may be necessary, 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, the determination of the purity of the cell population is especially important. You 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. You may achieve this, for example, using morphologic, histologic, molecular genetics, 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, you should perform quantitative assays to assess the presence of nonhuman cells in the final product. You should validate purity assays. Purity assays are important for production of a consistent product. You should use results of such tests 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, you should still test the product for purity, and you should develop tests for identity of the cells.

You should measure endotoxin levels on the final product, and results should be available for use as a lot release specification. We also discuss tests for endotoxin in the context of tests for infectious agents (section VII.C.3.).

d. Potency

You should perform potency assays that measure and reflect the intended biological activity of the final product. 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, you should assay cell viability and use the results for lot release.[5]

VII. MICROBIOLOGICAL TESTING OF XENOTRANSPLANTATION PRODUCTS

A. General Considerations

1. Framework

This section of the guidance document provides a general framework for the microbiological testing of xenotransplantation products. We suggest some specific examples of tests and infectious agents. However, we encourage sponsors to consider all available up-to-date information, regarding potential pathogens and testing strategies, to evaluate their own systems, to perform experiments to identify potentially infectious agents, and to propose and validate appropriate tests in consultation with CBER. During the initial stages of investigations, complete validation for all assays may not be necessary, with the exception of standard sterility tests. However, you should establish the specificity, sensitivity, and reproducibility 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).[3, 10, 20-22]

You can also find additional guidance on these issues as they relate to xenotransplantation in section 3.3 of the PHS Guideline.[1]

3. Inactivation or Removal of Infectious Agents

Whenever possible, without compromising the integrity and effectiveness of the xenotransplantation product, we encourage you to develop and incorporate validated procedures for inactivation or removal of adventitious agents, infectious agents, or other microbiological contaminants into the manufacture of the xenotransplantation product.

4. Archiving

You should cryopreserve and archive for further testing, as needed, samples of all final xenotransplantation products (i.e., cells or tissues or biopsies of organs), whether fresh or from culture ex vivo. 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, you should archive tissues or organs that have been in contact ex vivo with live nonhuman animal cells, tissues, or organs and samples of the final product and the nonhuman animal cells, tissues, or

organs. As in the case of the animal source samples (see section V.E.3.c.), you should harvest and cryopreserve sufficient quantities and numbers of replicates of the xenotransplantation product for three different uses:

a. dedicated sample(s) for use only by PHS[1];

b. for use if needed for recipient diagnosis and care; and

c. for use by the sponsor, as appropriate.

You should include detailed plans for obtaining and storing archive samples in the application to FDA requesting investigational use (e.g., IND). You should store samples for 50 years from the time of manufacture of the xenotransplantation product. You should clearly describe responsibility for the archives and access to the specimens.

If you are the sponsor, you should provide 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 before its clinical use. In such cases, testing of the source animal, itself, may be all the testing that is possible before 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 before use of the xenotransplantation product because results may contribute to patient management. (See also section VI.B.1.)

2. Stored or Processed Xenotransplantation Products

For xenogeneic cells or tissues that are stored, processed, or expanded ex vivo, you should accomplish or, at a minimum, initiate testing for infectious agents before xenotransplantation. If cells or tissues are maintained in culture, you should validate cell culture procedures and reagents for maintenance of microbial sterility, including both xenogeneic infectious agents and other cell culture adventitious agents. You should perform testing periodically during the culture period. Performance of all tests at every time point may not be necessary, but you

should provide FDA, as part of the application for investigational use (e.g., IND), with a scientific rationale to support the selection of tests performed at each given time. As an example, you may test samples:

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 before 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, physical barriers may separate the xenogeneic component from human fluids or tissues and might prevent or reduce transmission of certain classes of infectious agents. If such claims are to be made or are implicit, or if the existence of the physical barrier is to be used in lieu of certain other precautions to lower the risk of transmission, you should provide to FDA, as part of the application for investigational use (e.g., IND), 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.[23] For example, if such claims are to be made in the patient informed consent document, you should provide the results of these studies in the application to FDA requesting investigational use; if such claims are to be made during marketing, you should provide the data in the marketing 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 infectious agents that are representative of those potentially present in the xenotransplantation product. Note that the size and plasticity of selected agents should be considered; and

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

Without supporting data obtained from such studies, you should not assume that xenotransplantation products contained within a barrier present lesser risk of infection to humans than xenotransplantation products not contained within a barrier.

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 type of tissue, the processing of tissue before use, and the proposed use or clinical indication. You should give special consideration to infectious agents known to infect the source animal and those known to cause zoonoses. You should base the list of infectious agents for which you will test on that used for individual source animal qualification. We encourage discussions with CBER. You should include data 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

You can find standards concerning the types of methods used for detection of bacteria, fungi, and mycoplasma in licensed biologics in 21 CFR Part 610. You may use alternative methods during product development, but you should support use of such methods by data on the sensitivity, specificity, and reproducibility of the method. For xenotransplantation products, you should obtain such data 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. You should submit these data to FDA in the application for investigational use.

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

3. Endotoxin Test

During the product development phase, you may perform a bacterial endotoxin test in lieu of the rabbit pyrogen test as described for licensed products (21 CFR 610.13(b)). You should describe the type of endotoxin assay and its specificity and sensitivity in the application for investigational use submitted to FDA. If you intend to use an endotoxin assay in lieu of the rabbit pyrogen test after licensure, you should demonstrate equivalency with the pyrogen test for the specific xenotransplantation product at or before the time of license application (21 CFR 610.9).

Since it is possible to perform an endotoxin assay within a few hours, you should select and perform an appropriate assay on the final product. Results should be available before use for any xenotransplantation product that has been cultured, stored, or processed for more than the few hours required to perform the assay. You should use these results as a lot release specification. Consult the USP <85> and the FDA "Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers"[6] for information on bacterial endotoxins testing.

4. Viruses

a. Culture Assays

You should test xenogeneic cells used for xenotransplantation (fresh or cultured) 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 Biological Products."[19] This reference provides especially useful information for testing rodent xenotransplantation products for viral adventitious agents. You may find additional information in "Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin Q5A(R1)."[23] When possible, you should also co-cultivate manipulated and/or unmanipulated source animal cells with recipient cells, such as peripheral blood cells. You should routinely observe co-cultivation cultures for CPE, focus formation, RT activity, and changes in cell growth or other unexpected changes. We recommend visualization of co-cultures by EM to identify morphologic changes or to recognize certain viruses. You should try to identify any viruses detected using immunoassay, PCR, or other assays using virus-specific probes. At the end of the culture period, you should test cultures for hemagglutination and hemadsorption with erythrocytes of three different species[19]. 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.

You should base lot release specifications on available data. You should use these specifications for release of xenotransplantation products for which results can be available before administration of the product to humans, such as for products that can be cryopreserved. For cells that are manipulated ex vivo, if time allows, you should perform viral tests during the period of culture or manipulation so that the results are available before delivery of the product to the recipient. If it is not possible to obtain the results prior to use, you should still test samples of each product

lot. In these instances, you should qualify assay procedures and obtain data on a number of representative final product lots before beginning human trials.

b. Activation of Latent Viruses

You should give special consideration 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.[24] Manipulation or culture of cells ex vivo may also activate latent viruses (see references).[25, 26] 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:

i. the expression of endogenous retroviruses is induced by culturing in vitro; or

ii. by treatment with iododeoxyuridine or demethylating agents, such as 5-aza-cytidine[27]; and

iii. cultivation in vitro of ganglia latently infected with Herpes simplex virus results in the production of infectious virus[28].

In certain cases, positive results may not necessarily preclude use of such tissue (see section VII.C.4.d. for information regarding xenotransplantation products containing porcine endogenous retrovirus(es) (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 X.F.3.).

You should attempt to evaluate the potential of the processing or clinical use of the xenotransplantation product to activate latent viruses (e.g., PERV) before use.

c. In Vivo Assays for the Detection of Viruses

You should test xenotransplantation products 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.[28] Therefore, we recommend that if there are no reliable in vitro assays, appropriate in vivo assays should be applied (see "Points to Consider in the Characterization of Cell Lines Used to Produce Biological Products"[19] and "Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin Q5A(R1)."[23] Certain in vivo assays, such as antibody production assays, may be particularly useful for testing for rodent viruses.[19, 23]

d. Assays Suitable for the Detection of Porcine Endogenous Retrovirus(es) (PERV)

All live cells, tissues, or organs derived from pigs contain sequences for PERV in their genome[28]. It has been demonstrated in some primary porcine cells these sequences are expressed, resulting in production of infectious retroviruses. In light of data demonstrating that PERV can infect human cell lines in vitro[19, 23] we recommend that all porcine-derived xenotransplantation products be evaluated using appropriate assays for the production of infectious retrovirus. You should assess several lots of each xenotransplantation product. Each time major manufacturing changes occur, such as use of a new source or herd or method of product procurement, you should repeat testing for infectious PERV.[29]

You should test 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) 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 Collection (ATCC CRL-1573)), mink lung fibroblasts (ATCC CCL-64), certain feline cell lines (such as PG-4, ATCC CRL-2032), and a swine testis (ST) cell line (ATCC CRL-1746). You should choose one or more of these cell lines for initial analysis of the porcine xenotransplantation product or appropriate relevant surrogate tissue. After co-culture for a period of at least 30 days or 10 cell passages, you should analyze the cells for the transfer of PERV from the porcine cells to the indicator cell by either an optimized RT assay or use of PERV-specific primers to amplify, by PCR, reverse-transcribed viral RNA or cellular RNA. Evidence for virus production will not necessarily result in the xenotransplantation product's being considered unsuitable for clinical use. Rather, you should pursue additional characterization of the virus in consultation with Center for Biologics Evaluation and Research (CBER) in order to ensure appropriate reagents are available for recipient follow-up (section X.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 X.F.).

VIII. MANUFACTURING AND PROCESS-RELATED GMP CONSIDERATIONS FOR HARVEST AND PROCESSING OF XENOTRANSPLANTATION PRODUCTS

A. General Considerations

You should use facilities for the harvest and/or processing of xenotransplantation products that have been 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, you should phase in the validation activities described in this section, during the investigational phase, as the clinical studies progress toward submission of an application for approval to market (e.g., BLA). The exception to this is sterility assurance validation, which you should complete 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

You should take precautions to prevent contamination/cross-contamination during harvest and manipulation of xenogeneic cells or tissues. You should give consideration to:

- personnel, animal, material and waste flow 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

Your facilities should be designed such that personnel, animal, material, product, and waste flows into and out of the facility 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, you may accomplish segregation of activities procedurally and/or temporally. In this case, you should take special care 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). You should ensure that animals are prepared in such a way as to exclude potential surface contaminants, which may be carried from the animal facility.

2. Cleaning and Sanitizing Agents

You should demonstrate that 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), are effective against facility isolates, viruses, and other potential adventitious agents. You should establish cleaning schedules which maintain acceptable control in relation to the activities performed in the specified area. We expect that validation studies, demonstrating the efficacy of the agents used, will be performed as the trial progresses toward submission of a marketing application

3. Environmental Monitoring

You should establish a program for monitoring the environment in the harvesting and processing areas, based on the criticality of the manufacturing process involved.

You should perform nonviable particulate monitoring to verify air cleanliness classifications in the harvesting and processing areas (see section VIII.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. After initial verification, you should perform nonviable particulate monitoring at established intervals to demonstrate maintenance of the assigned air cleanliness classification.[30]

You may monitor viable particulates, i.e., microbes, 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. You should establish quantitative methods as clinical trials progress toward submission of a marketing application. You should monitor surfaces, including those of personnel performing production activities (e.g., gloved hands), using contact plates or swabs to demonstrate the continued efficacy of the cleaning regimen, and maintenance of asepsis for personnel. We recommend that personnel engaged directly in harvesting and processing activities be monitored at the conclusion of each critical activity (e.g., surgery, aseptic surgery). Additionally, you may randomly sample operators performing cell expansion activities.

4. Changeover Procedures

You should establish, follow, and document changeover procedures designed to prevent contamination between harvests of xenotransplantation products. 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, you should address segregation procedures if multiple lots of xenogeneic cells or tissues are processed at the same time. 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. We recommend that only one lot of xenogeneic cells or tissues be centrifuged at a time. You should demonstrate integrity of centrifuge tubes or closed systems employed, when possible. You should adequately clean centrifuges between each lot operation.

C. Validation and Qualification

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

1. Air Handling Systems

HVAC systems should be designed to provide adequate air quality for harvesting and processing of xenotransplantation products. You may use laminar flow units above the operating table to provide high quality air during harvesting operations. You may use biological safety cabinets to maintain aseptic conditions during processing. We expect that this equipment will be capable of producing Class 100 conditions for the most critical of processes although we understand 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 toward 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 should achieve the desired cleanliness level (see section VIII.B.3.). Testing of the HEPA filters contained in the system should address integrity and efficiency.

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

2. Water

We expect 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<1231> monograph on Water for Pharmaceutical Purposes[31]. If WFI is purchased, you should perform lot specific testing and validate hold times for open containers. If WFI is generated at the facility, you should properly validate and routinely monitor the system to ensure continued quality.

3. Equipment

You should adequately calibrate and qualify equipment used for harvesting and/or processing of xenogeneic cells and tissues. You should then routinely monitor temperature-controlled equipment, such as refrigerators/freezers and incubators, 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, you should use maintenance procedures for water quality. 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, you should perform media fills (substitution of media for product) 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[32]. You should adequately train and monitor personnel performing these functions to assure consistent performance during normal production.

All product contact equipment should be sterile and free of pyrogens when aseptically processing cells or tissues. You may use disposable labware 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 are to be sterilized, there should be evidence that the autoclave cycle(s) is validated to provide an acceptable level of sterility assurance. Minimally, you should establish and follow basic load configurations, and you should place biological indicators within each load to verify lethality. As studies progress, we expect that formal validation of all sterilizing/depyrogenating processes will be performed.

5. Process Validation

Ultimately, before licensure or product approval, you should validate all critical processes used to manufacture the product. We have previously defined process validation.[7, 32] We expect 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. We expect that information on the validation protocol(s) and summaries of data resulting from its execution will be included in the license application.

IX. PRECLINICAL CONSIDERATIONS FOR XENOTRANSPLANTATION

A. General Considerations

This section provides a general framework for the preclinical testing of xenotransplantation products before use in clinical trials. You may also apply the general principles as set forth in the document generated by the ICH on the safety of biotechnology-derived pharmaceuticals to these products (see reference[33]). 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 an FDA regulatory 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, you should design strong preclinical safety programs and also consult the FDA "Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products."[34]

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.

Since 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 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 planning the clinical testing program, including selecting an appropriate clinical indication, inclusion/exclusion criteria, recipient monitoring scheme, dose, and 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, you should consider the microbiologic burden carried by the xenotransplantation product as well as the immune status of the recipient 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).

To prevent the spread of demonstrable or potential infectious agents, you should care for animals 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. You may evaluate host immunocompetence 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

You should assess survival of the xenogeneic cells, tissues, or organs 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;

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. You should attempt to delineate toxicities due to immunosuppressive drugs from toxicities due to the xenotransplantation product.

You should consider and study, where appropriate, relative activity of immunosuppression on the source species of the live xenogeneic cells, tissues, or organs 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 IX.E.).

4. Cross-Species Compatibility of Bioactive Molecules

For xenotransplantation products for which you intend that the product synthesize and provide bioactive molecules, such as cytokines or hormones, you should provide to FDA, as part of the investigational application , data from preclinical experiments that support that the molecules produced will be active in humans. Experiments to address this issue should evaluate concentration-response issues. You should perform the experiments 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 (i.e., clinical signs, gross pathology, and histopathology) as well as markers of activity of the xenotransplantation product. You may use combination toxicity and activity studies to evaluate both potential therapeutic and constitutive functions of the xenotransplantation product. In some instances, the ability to biopsy xenotransplantation products periodically is potentially an available tool for evaluating the histopathologic status of the product and host immune response, especially when evaluated in conjunction with clinical chemistries. You might also perform control experiments 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. (Please note that this advice also pertains to heterogeneous xenotransplantation products as discussed in section IX.D.2.).

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 IX.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

You should apply the following principles 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, where relevant, activation or expansion of the xenotransplantation product, should mimic the procedures intended for use in clinical trials, and you should characterize cell types in the product being tested in an analogous fashion to the proposed clinical xenotransplantation product. See section VI.B.2.c., regarding recommendations for the evaluation of purity of heterogeneous xenotransplantation products.

2. Secretion of Biologically Active Molecules by Xenotransplantation Products

Uncharacterized cells or tissues present in the xenotransplantation product may produce biologically active molecules with unintended activities. You should perform experiments to identify released, bioactive substances of potential biologic significance and appropriate to the particular xenotransplantation product (e.g., neurotransmitters, hormones, cytokines), whether by intended or extraneous cell types in the xenotransplantation product. For example, you may maintain or culture in vitro samples of tissues being prepared for transplantation and test the

supernatants for activities or relevant bioactive substances. You should consult the ICH guidance document on safety preclinical evaluations in biotechnology for additional guidance on these studies.[33]

In addition to assessment in vitro, you should evaluate heterogeneous xenotransplantation products in appropriate animal models. (See section IX.C.4. for additional discussion of this topic).

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. You should 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, you may monitor cell viability, morphology, and functional endpoints (e.g., endocrine, behavioral, or immunological) over time to guide development of clinical monitoring regimens.

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

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.[3, 10, 33, 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, you should consider 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 exposure to certain infectious agents 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 you demonstrate that the tests have equivalent sensitivity in your hands.

   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 IX. 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 be studied in an animal species with blood volume and size and, possibly, anatomic structures, similar to those 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 addressed by review staff in CDRH. Additional device toxicity issues, also considered by staff at CDRH, are covered in the biocompatibility guidance published by the International Organization for Standardization.

Implanted devices may be intended for permanent or extended residence in the human body. Since these are considered chronic therapies, long-term risks (such as chronic inflammation, carcinogenicity, consequences of re-implantation, and local/systemic toxicities) of implanted xenotransplantation product/device combinations may necessitate evaluation before product premarket approval and, to some extent, before initiation of investigational studies in humans. Studies before initiation of clinical investigational studies would usually last a minimum of 3 months.

Membranes with pores may partially isolate xenogeneic tissue housed in devices from attack by host immune cells, 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. You should evaluate encapsulated xenotransplantation products intended to be permeable to bioactive substances (such as encapsulated islets) for preimplantation activity, and you should retrieve and assess the products for activity, capsule integrity, and tissue viability after various periods of time in the animals.

You should conduct extended animal studies (e.g., 12 to 24 months), using the clinical route of administration (e.g., implant site) and clinical grade materials. You should design studies 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. You should be aware that later changes in manufacture of the xenotransplantation product may necessitate new toxicology studies.

For devices used for extracorporeal hemoperfusion, you should conduct studies to evaluate the hemodynamic effects of establishing and discontinuing the extracorporeal circuit, the products released from the tissues housed in the device (e.g., proteins that could cause anaphylactic responses or stimulate unintended autoimmunity), the deposition of blood cells (such as platelets) on device tubing or other components, coagulation 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 you may replace the biologic component of the device at appropriate intervals to maintain life-supporting pharmacologic or metabolic activity.

In summary, you should design animal studies of xenotransplantation product/device combinations, as with other preclinical experiments, taking into consideration all aspects of the clinical trial and proposed patient population, the need to study both desired and undesired activities of the xenotransplantation product, and toxicities at the local and systemic levels.

X. CLINICAL ISSUES IN XENOTRANSPLANTATION

A. General Considerations

This section provides general principles rather than specific guidance. Since the available basic knowledge and clinical experience with xenotransplantation are 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).[1]

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

1. the potential risks of infection for the 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. adequacy of the proposal to address issues related to human and veterinary infectious diseases (including virology, laboratory diagnostics, epidemiology, and risk assessment).

C. Xenotransplantation Site

The PHS Guideline recommends 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.[1]

D. Criteria for Patient Selection

Due to the potentially serious public health risks of possible zoonotic infections, you should limit xenotransplantation to patients with serious or life-threatening diseases for whom adequately safe and effective alternative therapies are not available, except when very high assurance of safety can be demonstrated. You should limit candidates to those patients who have potential for a clinically significant improvement with increased quality of life, following the procedure. You should also consider the patient's ability to comply with public health measures as stated in the protocol, including long-term monitoring.

E. Risk/Benefit Analysis

We understand 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. You 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. You should describe and examine the likelihood and uncertainties associated with any potential recipient and public health consequences of each of the following events. 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. 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). You should describe and examine the possibilities and uncertainties associated with any other types of adverse events that could have potential, significant recipient or public health impact, arising from the xenotransplantation product. Similarly, you should describe any immunological risks, including rejection of the live xenogeneic cells, tissues, or organs, and, in some cases, GVHD.

F. Screening for Infectious Agents

Consult the PHS Guideline[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, you should individualize clinical tests for the specific xenotransplantation product in question. The categories of infectious agents of concern include bacteria (such as 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. You should have the capability to test for latent viruses or pathogens, and you should be prepared to develop and validate clinical tests for new pathogens that may not be recognized at the time of xenotransplantation. You should identify specific infectious agents for which tests will be performed. (See section X.F.3. for additional information on testing.)

2. Collection and Analysis of Clinical Samples

You should only perform xenotransplantation 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. You should place specimens into viral transport medium at the bedside, store at 4 degrees Celsius, and inoculate 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. In your investigational application to FDA (e.g., IND), you should describe tissue cell culture systems. These 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, you may examine tissue 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

In your investigational application to FDA, you should describe tests of clinical specimens from recipients for specific agents of concern. The tests may include, for example, serological and culture assays. You should also describe tests for latent agents known to be in the source animal species (e.g., retroviruses, herpesviruses). 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 versus human Cytomegalovirus (CMV)). Data should be available to demonstrate specificity, sensitivity, and reproducibility for all tests that are 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.

You should also describe the schedule for screening of recipients for infections in the application for investigational use.

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. We anticipate 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 you collect relevant data, during the clinical trial and for the lifetime of the recipient, and that investigation of acute infectious episodes includes 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, you should perform testing 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[35]. When there is a suspicion of a possible xenogeneic infection, you should notify FDA promptly if a non-xenogeneic causative infectious agent is *not* readily identified and notify FDA immediately if a potentially xenogeneic, causative infectious agent *is* identified.

b. Chronic Infections

An immunosuppressed recipient will be at risk for infection by the pathogens most commonly associated with allotransplantation. In addition, you should consider pathogens potentially derived from the source animal. 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.

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 persons, regardless of the presence or absence of symptoms, who shared a common or similar exposure to a xenotransplantation product. We recommend 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 PHS Guideline[1]. However, the time points identified by the 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:

- before xenotransplantation (two samples, one month apart);
- at the time of transplantation;
- in the immediate post-transplant period;
- at one month and six months after transplant;
- annually, for the first two years; and
- 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 X.H. for recommendations regarding the number, size, use, and duration of storage of collected samples.

ii. Active Screening

In addition to a passive screening program, you should consider an active screening program. Possible mechanisms of active screening range from testing of samples immediately after collection from recipients to periodic performance of specific additional laboratory tests on a subset of the samples collected in the passive screening program. In addition, you should consider implementation of centralized review of routinely collected clinical data to detect trends suggestive of emerging diseases. Section 4.1.1.2 of the PHS Guideline[1] suggests an active screening program for agents known to be in the xenotransplantation product with testing of samples acquired at 2, 4, and 6 weeks after the patient receives the xenotransplantation product.

A significant advantage of such a program is screening prospectively for evidence of infection in the absence of symptoms 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 before 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). If a xenotransplantation product known to harbor an infectious agent is used for xenotransplantation, you should implement active screening for that infectious agent. For example, you should assess all recipients of xenotransplantation products involving the use of porcine cells, tissues, or organs for evidence of infection by PERV.[29] Recipient screening for PERV should include analysis by multiple methods. Ideally, you should use all of the following detection methods:

- PCR of recipient's PBMC for PERV DNA sequence,
- serologic analysis for PERV-specific antibodies, and
- assays capable of detecting plasma virions, such as RT-PCR for detection of viral RNA or highly sensitive methods for detection of RT activity.[29]

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

d. Identification of Xenogeneic Retroviruses in Recipients

One particular concern is the potential transmission of xenogeneic retroviruses, such as PERV in the case of recipients of porcine products. If you are a sponsor of porcine xenotransplantation product clinical trials, you should 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 versus false positive). For example, in the case of porcine xenotransplantation products, we recommend PCR of DNA isolated from recipient PBMC for detection of PERV genetic sequences. However, if a positive result is obtained from this analysis, one possible explanation would be the presence of porcine cells. Therefore, you should perform additional DNA PCR for a repetitive porcine genetic element 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 and appropriate IRBs;

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; and

vi. Additional actions, if required, for the safety of the recipient and intimate contacts and to address possible public health risks.

e. Post-mortem Detection of Agents and Archiving of Autopsy Samples

You should request a complete postmortem examination, including histopathology and cultures of all recipients. At post-mortem, you should fix and embed samples of body tissue for examination by light and electron microscopy. You should obtain samples from the xenotransplantation product and, as appropriate, all major organs related to the product or to clinical syndromes that resulted in the recipient's death, were deemed to have been serious, or were of unexplained etiology. You should archive tissue and fluid samples at -70 degrees Celsius or lower, as appropriate for preserving the sample, for 50 years beyond the recipient's death as discussed in section X.H.1.

4. Infections in Recipient Contacts

We recommend that you develop a program to educate and monitor health care providers and to monitor other intimate 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 X.F.3.c.i.) may be appropriate. You should obtain baseline samples of plasma and archive them at -70 degrees Celsius, and you should obtain leukocytes and archive them in liquid nitrogen for health care personnel, for example, when they join the clinical teams. You should also ensure that such contacts are 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. 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 X.F.3.c.i.). 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. Before patients are treated, you should create 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.

    a. You should follow appropriate biosafety precautions in collection of clinical samples from recipients. You should follow standard

precautions in obtaining blood from recipients.[35] The PHS Guideline[1] recommends 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 X.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 PHS Guideline[1], including those obtained from patients during acute infectious episodes and from health care workers.

d. You should store patient blood and plasma samples in volumes and quantities, according to the recommendations for animal plasma and blood cell samples (see section V.E.3.b.).

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, you should also archive samples of such tissues. You should store samples at -70 degrees Celsius or lower as appropriate for preserving the sample.

2. Archive Samples

a. You should follow the recommendations in the PHS Guideline[1] and also section X.F.3.c.i. of this document, regarding archiving of plasma, blood, and other specimens. You should collect, archive, and reserve samples for use by PHS in the event that the need for a PHS-led investigation arises. The PHS Guideline[1] recommends that biologic specimens for PHS use be maintained for 50 years beyond the date of xenotransplantation, 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 recordkeeping 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 X.H.1.).

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

I. Health Records and Data Management

1. You 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, you should develop an appropriate tracking system for all recipients of their xenotransplantation products and use this tracking information to facilitate notification in the case of a serious adverse event related to a xenotransplantation product. You should collect information 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. We recommend that the information to be collected and tracked 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 - You should identify recipients by code number or other identifier to link the recipient to relevant information in the tracking system.

   c. Procedure information - You should record information about each xenotransplantation procedure. 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 must record adverse events 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 - You should periodically collect clinical status information for recipients of xenotransplantation products (see section X.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;

v. any hospitalizations since the recipient's last clinical follow-up examination.

f. Animal Health Events - Animal facilities should record animal health events. These events include but are not limited to:

i. breaks in environmental barriers of the secured animal facility;

ii. disease outbreaks; and

iii. sudden, unexplained or unexpected animal deaths.

The animal facility should report animal health events to the IND sponsor. The sponsor should include this information in its 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. You should record death certificate and autopsy information, if available. You should also report deaths to FDA.

2. You should maintain health records for at least 50 years beyond the date of transplantation.

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

J. Informed Consent

1. General Comments

The informed consent document must include the standard contents (see 21 CFR Part 50).

2. Specific Issues

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

a. Participation in the Study

i. Since the zoonotic, opportunistic, and xenogeneic infectious risks to the recipient may extend to the recipient's family or intimate contacts, the patient should consent to inform his or her current and future intimate contacts of their potential risks from the source animal species and of their deferral from blood donation. Intimate contacts of xenotransplantation product recipients include persons who have engaged repeatedly in activities that could result in intimate exchange of body fluids, including blood or saliva, with a xenotransplantation product recipient. Examples of intimate contacts include but are not limited to sexual partners, household members who share razors or toothbrushes, and health care workers or laboratory personnel with repeated percutaneous, mucosal or other direct exposures. Mere sharing of housing or

casual contact, such as hugging or kissing without the exchange of saliva, would not be interpreted as intimate contact. You should offer the recipient 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 individuals who may be at increased risk for zoonotic or opportunistic pathogens, such as infants, pregnant women, the elderly, and chronically ill or immunosuppressed individuals.

ii. Pending further information, clarifying risks, and further public consultation and discussion, xenotransplantation product recipients and their intimate contacts (see section X.J.2.a.i.) should be deferred 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. For more detailed information, please consult the FDA "Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)."[8]

iii. You should counsel the recipient, regarding other behavioral modifications. You should provide, as appropriate, advice on the use of barriers to transmission of infectious agents during sexual activity and the use of appropriate precautions for nonsexual contacts.

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. You should explain, to the extent possible, the schedule for such clinical and laboratory monitoring.

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 (e.g., FDA, CDC) to the recipient's medical records. To the extent permitted by applicable laws and/or regulations, you should maintain the confidentiality of medical records.

viii. You should include a request for autopsy in the informed consent document signed by the intended recipient or his/her appropriate representative.

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

i. The informed consent document should address the specific and known risks of all protocol-related activities not directly associated with source animal issues as well as the known and unknown zoonoses that may be associated with the source species. It should mention the uncertainty of the risks of infection or its transmission and of the risk of tumorigenesis. It should mention the possibility of a long latency period before detection of possible adverse effects. It should specify the need for and risks from prophylactic antimicrobial, antiviral, or other chemo- or immunotherapy. It should provide in an attachment for the recipient and the recipient's family the reasoning behind the use of any prophylactic treatments.

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

iii. It should include any known time course for the risks of disease development and transmission. It should discuss infectious diseases with protracted incubation periods, including TSEs and other unusual pathogens.

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

- PERV 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.
- 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 PERV, can cause neurological disorders and diseases, such as lymphomas and other malignancies, in certain animal models.

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. It should clearly convey the specific desired 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.

d. Alternative Treatments

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

e. Possible Consequences and Subsequent Treatment Options

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

f. Confidentiality Issues

The informed consent should inform the patient 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

You 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 become available or necessary. You 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. If you are the sponsor, you should ensure that the investigators are also willing to commit to providing new information to recipients and their families.

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