

Chemistry, Manufacturing and Controls (CMC) Considerations for Xenotransplantation Products

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Learning Objectives

- FDA Centers with regulatory oversight for xenotransplantation products
- Types of xenotransplantation products
- Chemistry, Manufacturing and Controls (CMC) considerations for xenotransplantation products
 - Characterization of xenotransplantation products
 - Safety testing for xenotransplantation products

Presentation Outline

- Brief description of xenotransplantation products
- Three types of xenotransplantation products
- CMC expectations for xenotransplantation products
 - Identity, purity, potency and safety testing

Definition of Xenotransplantation

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.

2016 FDA Guidance on Xenotransplantation:

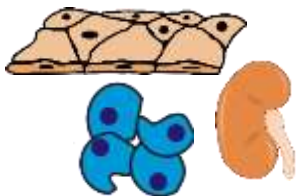
<https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/UCM533036.pdf>

Examples of Xenotransplantation Products



- Whole nonhuman organ transplantation into humans
- Implantation of nonhuman cells or tissues into humans
- Products with ex vivo contact with animal cells, tissues or organs
 - Extracorporeal perfusion of human blood over/through nonhuman cells or organ(s)
 - Administration to a human recipient of human cells previously cultured ex vivo with nonhuman cells

FDA Centers with Regulatory Oversight for Xenotransplantation Products



Regulated Articles



Center for Biologics Evaluation and Research (CBER)

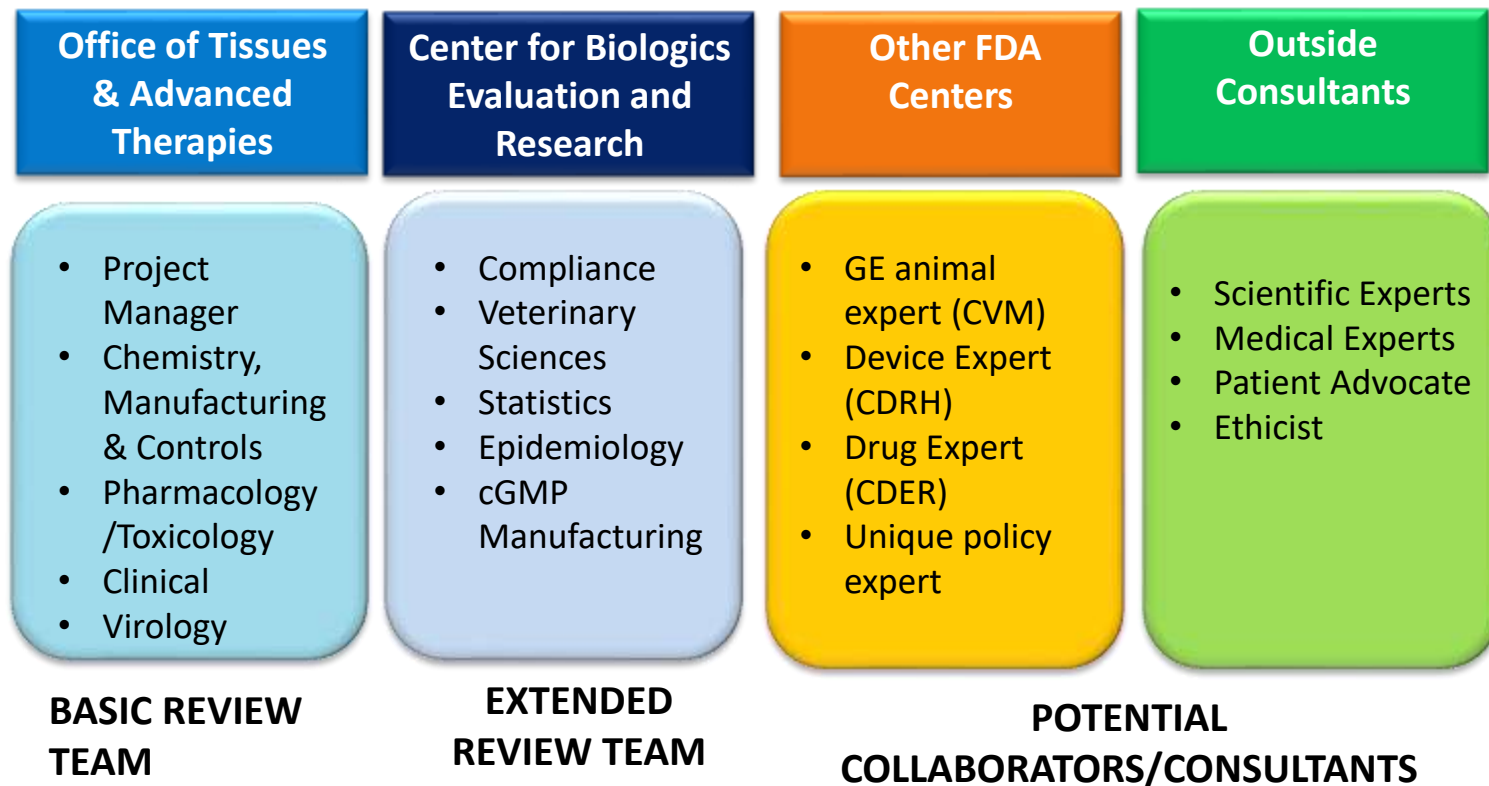
- Xenotransplantation products for clinical use
- Follow-up on patient post-transplant
 - Function of the transplant
 - Monitoring for infectious disease transmission

Center for Veterinary Medicine (CVM)

- Animals with Intentional Genomic Alterations (IGAs)
- Follow-up on the stability of the genetic alteration in the herd

SMG 4102 “Inter-Center Coordination of Regulatory Activities for Genetically Engineered Animals and their Expression Products” [*https://www.fda.gov/media/92780/download](https://www.fda.gov/media/92780/download)

Review Teams for Xenotransplantation Products



Components of CBER's Evaluation for Xenotransplantation Products

Source Herd

- Appropriate breeding, maintenance of animal health, maintenance of animal facility

Source Animal

- Procedures to minimize infectious disease risk, screening, harvest and handling of tissues, cells, organs

Product Processing and Testing

- Process control (cGMP), product characterization, safety testing, lot release (both test method & specification)

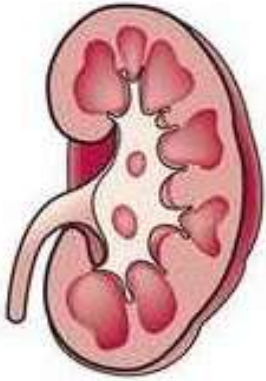
Preclinical Assessments

- Extrapolation of cross-species infections, immune reactions between source animal and recipient, function of xenotransplantation product, etc.

Clinical Issues

- Protocol review, informed consent, patient selection, follow-up screening, etc.

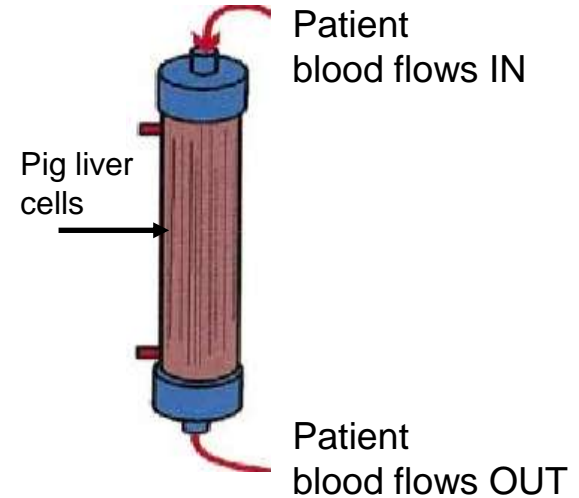
Xenotransplantation Products



Whole Organs



Cells and Tissues



Combination Products
(xeno product and device)

CMC Considerations

Process control (CGMPs)

- Appropriate procedures, reagents, and test methods
- Appropriate controls for tracking, labeling, and cross-contamination
- Appropriate conditions for processing, storage, and shipping

Product characterization

- Identity, purity, potency and safety

Safety testing

- Microbiological testing
- Infectious disease testing
- Virus inactivation or removal if possible

Considerations for Manufacturing and Process Controls

- Manufacturing process controls should be in adherence to cGMP regulations as per 21 CFR Parts 210 and 211
- Precautions should be taken to prevent contamination/cross-contamination
 - Ex: aseptic techniques for harvesting organs, tissues or cells, cleaning, sanitization, and sterilization of surgical suites and holding areas for animals
- Inactivation/removal of adventitious agents if possible

CMC Considerations

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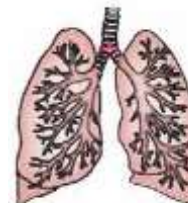
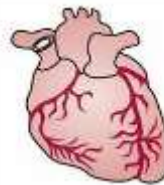
Product Testing Scheme for Xenotransplantation Products



- Testing Requirements same as other biologics:
 - potency (21 CFR 610.10)
 - sterility (21 CFR 610.12)
 - purity (21 CFR 610.13)
 - identity (21 CFR 610.14)
- Safety testing designed to detect infectious agents or other microbiological contaminants
- Verification of inactivation/removal of adventitious agents
- Approaches for meeting testing requirements depend on the type of xenotransplantation product.

Testing of Whole Organs

- Certain testing results may not be available before use
 - Testing of source animal prior to organ harvest is recommended
- Test sample recommendations
 - Whole organ biopsy or surrogate sample such as adjacent tissue sample can be used for identity, sterility, viral testing
- Potency testing
 - Organ function ex: hormone secretion, mechanical function, maintenance of blood pressure prior to harvest of the organ



Testing of Cells and Tissues that are Processed and/or Stored



- Testing for safety, identity, purity and potency
- When possible, testing results should be available before transplantation



Identity Testing of Cells and Tissues that are Processed and/or Stored



- To assess identity of the active component of the xenotransplantation product
- Identification of relevant cell or tissue types
- Histological evaluation, when possible
- Verification of the species/strain identity of the final product if animal facility handles more than one strain or species of animal

Purity Testing Recommendations for Stored/Processed Products



- For a heterogeneous products
 - Quantitative methods to assess the presence of the active cell type and contaminating cell types in the final product
 - Product with selected population of cells should be tested for purity



Endotoxin Testing Recommendations for Stored/Processed Products



- For any product that is cultured, stored, or processed
- Data for the assay specificity and sensitivity should be provided
- Bacterial endotoxin test in lieu of the rabbit pyrogen test can be performed during product development phase
- If endotoxin assay is performed in lieu of the rabbit pyrogen test after licensure, equivalency with the pyrogen test at or before the time of license application should be demonstrated

Potency Assay Considerations

- Potency assays should be performed to measure intended biological activity of the final product
 - Cytokines
 - Hormones
 - Neurotransmitters
- Development of potency assays may proceed with product development
- Cell viability as part of potency matrix



CMC Considerations

Process control (CGMPs)

- Appropriate procedures, reagents, and test methods
- Appropriate controls for tracking, labeling, and cross-contamination
- Appropriate conditions for processing, storage, and shipping

Product characterization

- Identity, purity, potency and safety

Safety testing

- Microbiological testing
- Infectious disease testing
- Virus inactivation or removal if possible

Testing for the Detection of Infectious Agents



- Safety testing should include fungal and bacterial sterility, mycoplasma and virus testing
- Methods used should be qualified for INDs and validated for BLAs
- Selected infectious agents should be appropriate to the source animal species, geographic origin, and the cells, tissues, or organ

Testing for the Detection of Infectious Agents



- Viral Testing
 - Culture Assays
 - Co-culture with a panel of appropriate indicator cells to amplify potential viral contaminants
 - Should include a cell line representative of the source animal species, animal tissue(s) type used in the manufacture, and a human cell line
 - Activation of Latent Viruses
 - Activation of viruses due to immunosuppression, transplantation and ex vivo culture of cells
 - Experiments appropriate to detect latent viruses depends on the tissue type and the virus in question
 - In Vivo Assays for the Detection of Viruses
 - In vivo assays for detection of viruses that may not be found by culture methods in vitro

Potential Zoonotic Viruses

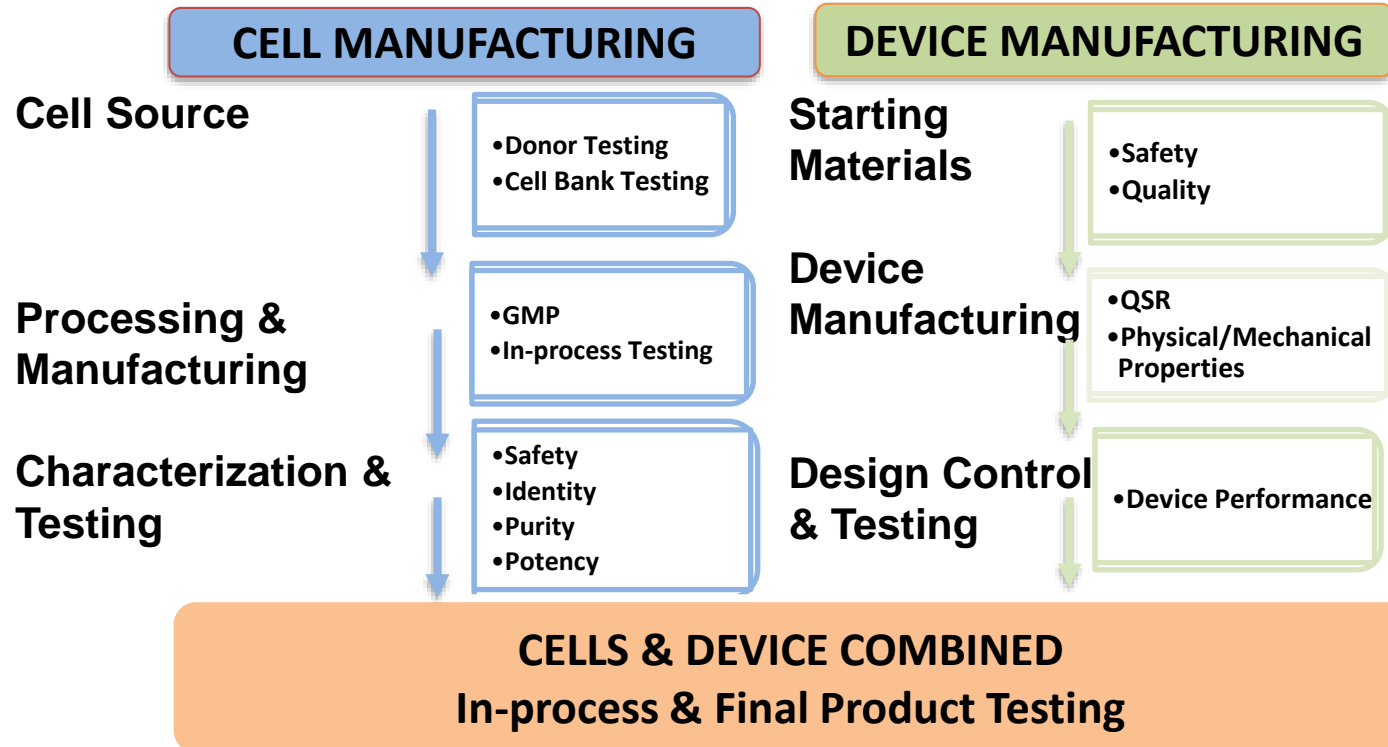
- Porcine Endogenous Retrovirus (PERV)
 - Type A and B infect human cells
 - Type C only infect pig cells
 - Type A/C recombinants are highly infective
- Porcine Circovirus 3
 - Deleterious effects on pig organ systems
 - Transmissible to humans??
- Porcine lymphotropic herpesvirus-1
 - Causes post-transplant lymphoproliferative disease (PTLV) in mini-pigs
 - Similar to human EBV that causes PTLV in humans post-transplant

Testing for the Detection of Infectious Agents



- Assays for the Detection of Porcine Endogenous Retroviruses (PERV)
 - Direct Methods: PCR, RT-PCR, q-PCR, dd-PCR, RT assay, electron microscopy, Western blot of cells/viral lysates, immunohistochemistry, immunofluorescence, Immunoperoxidase assay, and infection assays
 - Indirect methods: Western blot and ELISA
 - Xenotransplantation products should be tested by coculture with appropriate indicator cells to amplify any infectious retrovirus(es).
 - Evidence for virus production will not necessarily indicate that the xenotransplantation product is unsuitable for clinical use

Testing Considerations for Combination Products



Testing Considerations for Combination Products



- If a physical barrier is used to lower the risk of infectious agents transmission
 - Demonstration of the inhibition of transmission
 - Maintenance of device/barrier integrity
- Physical barriers used to protect the xeno product from the host immune system
 - In vitro or animal studies to demonstrate the effectiveness of the barrier system and maintenance of the integrity of the barrier
- Parameters for consideration to include in validation studies
 - Conditions under which the combination product is subjected to physical and biological stress
 - Use of infectious agents that are representative of those potentially present in the xenotransplantation product
 - Use of agents that would demonstrate the physical properties of the barrier

Challenge Question #1



What are the currently existing types of xenotransplantation products?

- A. Whole nonhuman organs
- B. Animal cells or tissues that are stored, processed, or expanded ex vivo
- C. Combination products containing cells from (or in contact with) nonhuman sources and a device
- D. All of the above

Challenge Question #2



Which of the following is considered as part of the safety testing of a xenotransplantation product

- A. Genotyping
- B. Evaluation of the presence of active cell types
- C. Testing for Porcine Endogenous Retroviruses (PERV)
- D. None of the above

Summary



- CMC considerations for xenotransplantation include process controls, product characterization and safety testing
- Xenotransplantation products should meet manufacturing requirements in 21 CFR Parts 210 and 211
- Product characterization includes testing for safety, identity, purity, and potency
- Selected infectious agents as part of safety testing should be appropriate to the source animal species, geographic origin, and the cells, tissues, or organ



FDA Resources for Regulation of Xenotransplantation Products

- Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans
<https://www.fda.gov/media/102126/download>
- PHS Guideline on Infectious Disease Issues in Xenotransplantation
<https://www.fda.gov/media/73803/download>
- CVM GFI #187 Regulation of Intentionally Altered Genomic DNA in Animals
<https://www.fda.gov/media/74614/download>
- Coordination of Regulatory Activities for Genetically Engineered Animals and their Expression Products
<https://www.fda.gov/media/92780/download>

Contact Information



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- **OTAT Learn Webinar Series:**

<http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/ucm232821.htm>

- **CBER website:** www.fda.gov/BiologicsBloodVaccines/default.htm

- **Phone:** 1-800-835-4709 or 240-402-8010

- **Consumer Affairs Branch:** ocod@fda.hhs.gov

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