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Xenotransplantation-associated infectious risk: a WHO consultation

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Abstract

Xenotransplantation carries the potential risk of the transmission of infection with the cells or tissues of the graft. The degree of risk is unknown in the absence of clinical trials. The clinical application of xenotransplantation has important implications for infectious disease surveillance, both at the national and international levels. Preclinical data indicate that infectious disease events associated with clinical xenotransplantation from swine, should they occur, will be rare; data in human trials are limited but have demonstrated no transmission of porcine microorganisms including porcine endogenous retrovirus. Xenotransplantation will necessitate the development of surveillance programs to detect known infectious agents and, potentially, previously unknown or unexpected pathogens. The development of surveillance and safety programs for clinical trials in xenotransplantation is guided by a "Precautionary Principle," with the deployment of appropriate screening procedures and assays for source animals and xenograft recipients even in the absence of data suggesting infectious risk. All assays require training, standardization and validation, and sharing of laboratory methods and expertise to optimize the quality of the surveillance and diagnostic testing. Investigation of suspected xenogeneic infection events (xenosis, xenozoonosis) should be performed in collaboration with an expert data safety review panel and the appropriate public health and competent authorities. It should be considered an obligation of performance of xenotransplantation trials to report outcomes, including any infectious disease transmissions, in the scientific literature. Repositories of samples from source animals and from recipients prior to, and following xenograft transplantation are essential to the investigation of possible infectious disease events. Concerns over any potential hazards associated with xenotransplantation may overshadow potential benefits. Careful microbiological screening of source animals used as xenotransplant donors may enhance the safety of transplantation beyond that of allotransplant procedures. Xenogeneic tissues may be relatively resistant to infection by some human pathogens. Moreover, xenotransplantation may be made available at the time when patients require organ replacement on a clinical basis. Insights gained in studies of the microbiology and immunology of xenotransplantation will benefit transplant recipients in the future. This document summarizes approaches to disease surveillance in individual recipients of nonhuman tissues.

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Keywords

Clinical assays; clinical trials; donor; derived infection; infectious disease; porcine endogenous retrovirus; safety; xenotransplantation

Overview

Xenotransplantation is any procedure that involves the transplantation, implantation, or infusion into a human recipient of live cells, tissues, or organs from an animal source. This definition may include human bodily fluids, cells, tissues, or organs that have had ex vivo contact with live non-human animal cells, tissues, fluids, or organs. The definition may also include non-living or acellular biomaterials (e.g., heart valves, blood vessels, tendons) derived from non-human species. These latter non-viable tissues have a much reduced potential for the spread of infection but may elicit immune or other host responses. As with any form of transplantation, xenotransplantation carries the potential risk of the transmission of infection with the cells or tissues of the graft [1–8]. In xenotransplantation, there is the unique potential risk for the transmission of both known and unknown zoonotic infectious agents of animal origin into human recipients and into the wider human population. Thus, the term “xenosis” (also “direct zoonosis” or “xenozoonosis”) was coined to reflect both the unique epidemiology of infection of source animals used for xenotransplantation and experience with immunocompromised patients that indicates that novel pathogens may emerge as a cause of infection, including organisms not normally associated with human disease [2,6]. The degree of risk is unknown in the absence of clinical trials. The clinical application of xenotransplantation has important implications for infectious disease surveillance, both at the national and international levels. As a result, World Health Organization Resolution WHA57.18 emphasizes that members states should “allow xenogeneic transplantation only when effective national regulatory control and surveillance mechanisms overseen by national health authorities are in place [9].” This issue was further defined in the Changsha Communiqué of the First WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials (Changsha, China, 19–21 November 2008) [10]. The Changsha Communiqué stated that, in light of potential risks to xenograft recipients and to the broader community, “there should be no xenotransplantation in the absence of effective regulation by the government of the country.” A series of useful guidance for xenotransplantation have been issued by the U.S. Food and Drug Administration and other national authorities [11–14]. WHO has also previously provided a series of guidance documents related to xenotransplantation [15,16].

Pre-clinical data indicate that infectious disease events associated with clinical xenotransplantation from swine, should they occur, will be rare; data in human trials are limited but have demonstrated no transmission of porcine microorganisms including porcine endogenous retrovirus (PERV) [17–20]. This document will summarize approaches to disease surveillance in individual recipients of non-human tissues. Some general concepts may be useful:

1. The risk for infection is related to the properties of the specific organism, the quantity of the organism transmitted, the availability of appropriate machinery (e.g., receptors, nutrients) in the host, and the immune competence of the host. It is not possible to predict the precise behavior of unidentified, animal-derived pathogens in human hosts or the range of clinical manifestations that may occur.
2. Investigation into suspected xenogeneic infection events (xenosis, xenozoonosis) should be performed in collaboration with an expert data safety review panel and the appropriate public health and competent authorities. Expertise in the diagnosis

and management of infections in immunocompromised hosts should be available for trials using immuno-suppression. Reporting of such events should be expedited to allow optimal care of the recipients and investigation into their contacts so as to reduce the possibility of dissemination of infection.

3. It should be considered an obligation of performance of xenotransplantation trials to report outcomes, including any infectious disease transmissions, in the scientific literature while protecting the confidentiality of individual patients and investigators.

4. Xenotransplantation will necessitate the development of surveillance programs to detect known infectious agents as well as previously unknown or unexpected pathogens in the absence of recognizable clinical syndromes. This may include assays for known infectious agents, probes for classes of infectious agents (e.g., common genes or antigens of herpesviruses), and assays for unknown pathogens in a variety of tissues.

5. Microbiological assays will require standardization of procedures and validation by expert and/or reference laboratories. Such validation may necessitate the use of regional or reference laboratories and shared reagents, laboratory practices and methods. Such reference laboratories may require international collaboration.

6. Repositories of samples from source animals and from recipients prior to, and following xenograft transplantation are essential to the investigation of possible infectious disease events. As a result, such repositories will need to be maintained for prolonged periods of time (i.e., many years).

7. An ideal data repository for xenotransplantation data requires standardized definitions of terms and allows the international sharing of de-identified microbiological data via secure web-based applications.

8. The public should be engaged in discussions regarding infectious risks given recent public reactions to some innovations in biotechnology (e.g., in the application of genetic technologies to the agriculture and food industries).

In general, it is likely that the development of surveillance techniques might be guided by the “Precautionary Principle.” That is, the risk of xenogeneic infection is generally thought to be low but the deployment of appropriate procedures and assays should not wait until a risk is confirmed [21]. This concept includes implementation of appropriate assays and emergency protocols in advance of clinical trials as well as further improvement of surveillance technologies to facilitate future trials. All assays require training, standardization, and validation, and given the relatively small number of clinical samples, sharing of laboratory methods and expertise will be needed to optimize the quality of the surveillance and diagnostic services provided.

Concerns over the potential hazards associated with xenotransplant procedures tend to overshadow the potential benefits. Careful microbiological screening of source animals used as xenotransplant donors may enhance the safety of transplantation beyond that of allotransplant procedures. Xenogeneic tissues may be relatively resistant to infection by human pathogens such as HIV, HTLV, and the hepatitis viruses, which could be beneficial to individuals undergoing xenotransplantation [4]. Moreover, xenotransplantation may be made available at the time when patients require organ replacement on a clinical basis, possibly on a more timely basis than organs from deceased human donors. The insights gained in basic studies of microbiology and immunology in xenotransplantation will benefit many individuals in years to come.

Targets of surveillance activities

1. Recipients of xenotransplantation products.
2. Sexual or close contacts of xenograft recipients.
3. Source animals, animal handlers, and facilities.
4. Medical care providers.

Donor-derived infections have been detected in multiple clusters of allotransplant recipients receiving grafts from a single, infected donor [22–25]. Experience in this area has allowed the evaluation of such recipients to be divided into three general categories: common infections known to be transmitted frequently with viable cells or organs (e.g., cytomegalovirus [CMV]), uncommon infections (e.g., lymphocytic choriomeningitis virus [LCMV]), or unknown pathogens, presenting as an infectious syndrome (e.g., fever) or as asymptomatic infection (e.g., resulting in positive serology). A similar approach will be needed for the evaluation of xenograft recipients. Investigations will be based on a number of categories that reflect differing urgency and implications for clinical trials of xenotransplantation:

1. Routine surveillance of healthy source animals (screening).
2. Routine surveillance of recipients (screening) pre- and post-transplantation {e.g., microbiologic testing for specific agents (e.g., PERV by serology and/or nucleic acid testing [NAT] as dictated by the monitoring protocol such as every 3 months for 5 years) following the transplant, then at appropriate intervals for the life of the xenograft recipient}. Microbial assays that are performed in the absence of clinical symptoms or other abnormalities may provide epidemiologic data useful in the assessment of safety in clinical trials.
3. Routine evaluation of social and sexual contacts of xenograft recipients, possibly including household pets.
4. Evaluation of infectious syndromes (e.g., fever of unknown origin [FUO], leukocytosis, leukopenia, graft dysfunction, pneumonia, hepatitis, abscess formation) in xenograft recipients, including,
    - Exclusion of syndromes commonly associated with allotransplantation (e.g., CMV) or due to immunosuppressive drugs or of technical/surgical adverse events.
    - Evaluation of PERV infection by serologic and NAT testing.
    - Assessment of other recipients of xenografts derived from the same herd or source of swine.
    - Evaluation of sexual and close social contacts of recipient (and medical care providers as appropriate) after identification of infectious syndrome in the recipient.
    - Investigation of recipients for unknown pathogens or organisms not previously associated with clinical syndromes in humans.

Any indication that the infectious syndrome is related to the xenotransplantation procedure would place a temporary hold on further trials using the source animal herd until the identification and source of the presumed infection is determined. Consideration may be given to hospital admission and isolation of the individual based on the clinical protocol, the level of perceived risk to the community and local regulations.

Investigations would require testing of the source animal (archived specimens and/or herd) and recipient including, but not limited to (see details below):

1. Cultures and examination of blood, urine, sputum, stool, cerebrospinal fluids (as appropriate) for bacteria, fungi, viruses, parasites.
2. Testing for common human pathogens of immunocompromised hosts depending on the clinical syndrome (CMV, Epstein–Barr virus [EBV], *Cryptococcus neoformans*, mycobacteria, *Nocardia* species, *Pneumocystis jirovecii*).
3. Specific porcine pathogen testing (PERV, porcine cytomegalovirus [PCMV], porcine lymphotropic herpesvirus [PLHV], circovirus, hepatitis E virus, and others —see Tables 1 and 2).
4. High-throughput sequencing of nucleic acids derived from sera or cell samples using non-biased random or degenerate primers (search for unknown pathogens) [25].
5. Cocultures on permissive cell lines.

The clinical trial would resume with approval from the institution and public health authorities if the causal agent (or other etiology) is identified and found to be treatable, and there is no evidence of transmission to contacts of the xenograft recipient. Decisions regarding the subsequent use of the herd of source animals would be based on whether the infectious agent is present in the herd and whether it can be excluded from the herd. In the absence of a specific diagnosis, resumption of the trial would be assessed based on review of clinical data by experts external to the trial and by the appropriate competent authority.

“Certainty of diagnosis” in surveillance

Early investigation, diagnosis/detection, and reporting are essential features of any surveillance system developed for xenotransplantation and for the optimal care of xenograft recipients. Confirmation of a microbiological diagnosis may require levels of sophistication in clinical laboratories not available to all clinical centers. Laboratories should perform validated assays in facilities accredited according to national standards. International reference laboratories may serve as highly specialized resources for national research programs. Such inter-laboratory collaboration may provide an additional degree of certainty regarding microbiological assays. Any “in-house” (non-commercial) assays require validation. The use of highly sensitive assays risks generation of false-positive results and misinformation for patient and public health authorities. Confirmation of laboratory data should be consistent with optimal clinical care while protecting public interests. The infectious and pathogenic potential in humans of various organisms derived from swine is generally unknown unless the same or similar organisms infect humans—in which instance derivation from source animals may be suspect. A confounding variable may be whether the recipient has been in contact with or, in some cases, consuming animal-derived proteins or cells (e.g., porcine insulin, pancreatic lipases, heparin) that might affect certain assays (e.g., antibody assays). These may be potential sources of infection by specific viruses surviving manufacturing processes. The role of such products as a potential source of infection or false-positive assays merits clarification.

The presence of an organism in the xenograft itself, while undesirable, is not a clear predictor of the risk of infection for that individual or their contacts. Thus, if the organism cannot replicate in human cells or disseminate within the human host, the risk of infection is likely to be limited.

The need for accurate microbial diagnosis is emphasized by the need to share validated information with other patients who have received similar xenotransplantation products or grafts from animals of the same source herd and to test such individuals for infection.

Levels of responsibility

The performance of clinical trials in xenotransplantation and the initial responsibility for the recognition and investigation of possible infectious complications rests with the medical center performing clinical trials, the primary physicians providing clinical care, and local clinical laboratories. These individuals must be aware of the public health implications of possible infectious disease transmissions with xenografts and have a strategy in place for the initial collection, processing and storage of clinical samples, the potential need for isolation of the xenograft recipient, and the notification of public health and competent authorities.

Public health authorities must retain oversight for the maintenance of routine records and archiving of specimens, the implementation of proper investigations of xenogeneic infection events, and the communication of data to the appropriate competent authorities. The requirements placed on source herd development and laboratory testing will vary with local regulations. It is reasonable to consider “good manufacturing practices” and good laboratory practice in which the quality of manufacturing processes and laboratory techniques is clearly defined and controlled and critical processes carefully validated.

Specific pathogens-general considerations

Potential human pathogens derived from animals can be categorized according to the likely behavior of related organisms in allotransplant recipients (Table 1) [2,4,8]. Xenotransplantation may enhance the risk of graft-derived infection because recipients generally lack pre-formed immunity, clinical laboratory assays may not be available, incompatible major histocompatibility antigens may reduce the efficacy of host cellular immune responses, and because of unknown effects of genetic or other manipulations of source animals used to improve xenograft immune compatibility or to reduce physiologic (e.g., of the coagulation system) incompatibilities. For instance, human complement regulatory proteins introduced into swine to overcome hyperacute rejection may serve as receptors for human viruses.

The recognition of infection in immunocompromised hosts is more difficult than in normal individuals because signs of infection such as inflammation may be absent. In this setting, animal-derived infections may go undetected against the high background incidence of infection in immunosup-pressed transplant recipients. Difficulty in predicting which animal-derived organisms are likely to act as pathogens in human recipients is compounded when such organisms do not cause disease in native host species or acquire new characteristics (e.g., via genetic recombination or mutation) in a human host. The virulence of some organisms may increase with passage in a new host through adaptation.

Common pathogens in the immunocompromised host

Based on experience with immunocompromised human transplant recipients and with immunosuppressed swine and primate recipients of porcine xenografts, lists of microorganisms of swine that *could* be associated with human infection can be made (Table 2). Ideally, such organisms could be eliminated prospectively from source animals that could be considered “designated pathogen-free” for xenotransplantation purposes. Additionally, animals may be bred to exclude some porcine herpesviruses (PCMV) or porcine circoviruses (PCV1, PCV2). If not excluded from the donor herd, this list also provides some basis for the investigation of infectious syndromes in xenograft recipients. It

should be noted that most serological assays for viruses may not be species-specific and will not distinguish between porcine and human pathogens, for example, circovirus, hepatitis E virus, porcine parvovirus. Depending on the strategy developed for the screening, routine evaluation, and diagnostic testing of source animals and recipients, local regulatory bodies and competent authorities should require that these assays be validated in accredited clinical laboratories prior to the commencement of any xenotransplantation trials.

The list of microorganisms may vary with the use intended for various specific xenografts. Thus, encapsulated cells placed in the brain may pose a different risk from that posed by either a heart or liver xenograft. Although such lists provide a basis for screening source animals and recipients, these microbiological standards need to be dynamic and subject to frequent review and updating. To exclude infectious agents and to prevent their reintroduction or spread into animal herds, special facilities for housing source animals (e.g., barrier facilities) are needed. However, the precise manner for meeting these goals need not be uniform so long as microbiologic hazards are excluded or appropriately minimized.

Retroviruses

Concern about retroviral transmission in xenotransplantation relates to the potential for “silent” transmission, that is, unapparent infection that may cause altered gene regulation, oncogenesis, or recombination [1,3,4,7,26]. No exogenous viruses, equivalent to HTLV or HIV, have been found in pigs. However, endogenous retroviruses (part of the germ line DNA) have been demonstrated in all mammalian species studied to date. Endogenous retroviruses that are infectious for human cells in vitro have been detected in many species including baboons (BaEV), cats (RD114), mice (murine ERV), and pigs (PERV). Although the pig genome contains sequences closely related to mouse mammary tumor virus or Mason–Pfizer monkey virus (betaretrovirus) and murine leukemia virus (gammaretrovirus) sequences, only three subgroups of gammaretrovirus PERV (PERV-A, -B, -C) have been identified in swine that possess infectious potential [27–34]. Two of these, PERV-A and -B, can infect pig cells and several human cell lines and primary cell cultures in vitro [29,33,35,36]. The third subgroup, PERV-C, infects porcine cells only [29]. Infectious forms of the remaining PERV families have not been isolated and are unlikely to encode infectious virus due to disruptions in open reading frames [30]. PERV mRNAs are expressed in all pig tissues and in all breeds of swine tested to date; expression can be amplified by the stimulation of swine peripheral blood lymphocytes in vitro [32,33,36,37]. There is a variation between tissues in terms of the size and amount of PERV mRNA transcripts, consistent with in vivo recombination and/or processing [26,32]. High-titer human-tropic PERV (HTHT-PERV) are recombinants between PERV-A and PERV-C sequences. Although the site of recombination varies, viral sequences are derived from the recombination of PERV-A elements with the post-VRA (envelope) region of PERV-C [33,36–38]. Therefore, although PERV-C is not capable of infecting human cells, it appears to be an essential component of HTHT-PERV and important in the assessment of infectious risk associated with PERV in xenotransplantation [33,36,39]. The source of these recombinants in vivo is unknown. However, recombinant PERV-AC sequences have been found in the cellular DNA of some miniature swine capable of infection of human cell lines in vitro [26]. It is not known whether these elements result from autoinfection following exogenous viral recombination or are pre-existing proviral elements. No evidence of PERV infection has been demonstrated for human cells in vivo, and no disease due to this family of viruses has been described in swine or humans to date. PERV appears to be susceptible to certain currently available antiviral agents [40,41].

Assays related to PERV

Assays for PERV may be used prior to xenotransplantation in the selection of the “safest” animal donor and after the procedure to detect evidence of PERV transmission in the recipient. Although it is extremely difficult, if not impossible, to eliminate all PERV from the genomes of pigs, it is possible to select source animals with phenotypes consistent with reduced capacity for PERV transmission to human cells. Current terminology includes a “non-transmitter” animal that transmits PERV to pig, but not human cells; a “null” animal does not transmit PERV to either human or pig cells in vitro [33,37,42]. It has been observed that the non-transmitter or “null” phenotype is not stable and that transmission can be demonstrated at subsequent testing [33]. The use of source animals free of PERV (none identified to date) or free of PERV-C could, in theory, prevent PERV transmission. Therefore, pigs with a null phenotype may or may not represent a reduced risk for PERV transmission; this remains to be tested in vivo. Such phenotypic differences in PERV transmission are partly directed by genotypic differences in PERV integration patterns. The PERV integration pattern is highly polymorphic between various swine [28,31,35]. Pigs without active PERV loci may be found by genomic analyses in the future. In this regard, next-generation sequencing technologies and the swine whole genome sequence [43] will be useful in addressing these issues.

To determine the transmission phenotype of pigs, PERV transmission methods were employed based on the cocultivation of activated PBMC derived from candidate source animals with human or porcine cells [33,36,37,42,44,45]. These protocols are considered the “gold standard” for analyzing the potential for PERV transmission. These assays are complex and time-consuming, but no alternatives are currently available.

Xenograft recipients can be monitored for both circulating pig cells (a potential source of complication forassays termed “microchimerism”)and for any potential PERV infection. It is notable in this regard that xenograft procedures to induce immunologic tolerance may involve intentional exposure to porcine hematopoietic cells (“mixed chimerism”) [46,47].

Prior PERV infection or exposure in recipients can be assessed using serologic assays seeking the presence of anti-PERV antibodies using an ELISA system [18,48,49]. Neutralization assays measuring virus-neutralizing antibodies in vitro may be more specific than Western blots [7,50]. However, in the absence of consistent control samples (i.e., to exclude exposure to pig cells or pig-derived products previously), the interpretation of such assays in regard to the presence of active infection may be difficult. Individual recipients may serve as their own controls for such assays; rising titers (usually at least four-fold) or an antibody class switch (IgM to IgG) may be taken as evidence of likely infection. Immunosuppressed patients may not generate timely serologic responses.

The most sensitive methods for PERV detection are PCR-based NAT. Use of accurate primer sets to identify PERV subtypes and the ability to distinguish true infection from contamination with porcine cellular material is essential. For PCR amplification, nucleic acids should be prepared from blood, sera, cerebrospinal fluid, and other bodily fluids. The use of validated, quantitative methods using human samples and with relevant controls and confirmed specificity and sensitivity measurements is paramount. Standard operating procedures should be provided to all participating laboratories, all equipment calibrated, and assays validated prior to initiating the trials. Methods should be able to detect <5 copies of DNA or RNA per 300 000 cells or 3 ul of supernatant, respectively [7,17,18,48,51]. The lessons learned from recent false-positive nucleic acid assays from clinical samples (e.g., xenotropic murine leukemia virus-related virus) suggest the importance of meticulous laboratory maintenance against contamination and assay error.

Surveillance and search for novel pathogens

Familiar microbial agents account for many infectious syndromes in transplant recipients. Infection in such hosts is often asymptomatic. Thus, stored samples should be investigated for known pathogens at pre-established intervals as a basis for epidemiologic and safety studies. Etiologic agents causing disease in immunocompromised hosts are often unknown. With such uncertainty, investigators caring for xenograft recipients need to consider alternative approaches to surveillance and diagnosis in their immunocompromised xenograft recipients. This requires the routine collection and storage of frozen cells, serum samples, and tissue biopsies as well as donor sera and multiple tissue samples in advance of any adverse event. While these studies may be costly and considered as research investigations, possible approaches include:

1. Use of broad-range molecular probes or polymerase chain reaction (PCR) primers that identify common genes (e.g., ribosomal genes such as 16S rRNA) shared within organism subclasses to recognize novel pathogens similar to those already known to cause disease in immunosuppressed hosts.
2. Use of “chips” or microarrays carrying cDNAs for common classes of pathogens.
3. Developing additional molecular approaches for detecting novel *genetic material* (e.g., new DNA or mRNA species) present in recipients of xenograft tissues but not in normal individuals, including,
    - Cloning of differences between two complex genomes (representational difference analysis or RDA) can be used to isolate unusual mRNAs against a background of “normal” mRNAs. This technique was used to characterize pathogens such as Whipple's bacillus, hepatitis C and G viruses, and HHV8, the Kaposi's sarcoma-associated virus.
    - Next-generation sequencing or pyrosequencing allows sequencing of cDNA samples for the detection of unknown pathogens. This may utilize specific genetic targets such as hypervariable regions within bacterial 16S rRNA genes amplified by PCR and then subjected to DNA pyrosequencing. A new arenavirus has been identified from allotransplantation recipients by unbiased high-throughput sequencing [25]. Such approaches will enable clinical investigators and public health officials to begin to recognize and assess novel infections in xenotransplantation.

Routine monitoring for xenogeneic infection

In xenograft recipients, the risks of infection and rejection necessitate lifelong monitoring. Monitoring schemes have been proposed to test for known pathogens and archive specimens from source animals, and from patients, intimate contacts, and animal handlers on a routine basis for use in the event of unexplained infectious episode (see also the Changsha Communiqué and FDA guidances [11,13,14]). Archived aliquots are likely to be required to be stored at least two separate locations. These samples may be utilized as further microbiologic assays are developed against previously unrecognized pathogens. Routine samples of sera and leukocytes might be studied for the emergence of human and pig-derived pathogens (PERV, PCMV, HCMV, EBV, PLHV) and cocultivation of peripheral blood leukocytes with human and donor cell lines in the absence of clinical evidence of infection. In addition, it may be recommended for patients to annually complete a questionnaire regarding their health status to monitor any developing syndromes or issues that need attention.

Assessment of the xenograft recipient with an infectious syndrome

The recipient presenting with fever, elevated or depressed leukocyte counts, hepatitis, pneumonia, signs of central nervous system infection, abscess or other focal infections, or xenograft dysfunction will have standard microbiological evaluations performed. This includes blood, urine, sputum, stool, and/or cerebrospinal fluid, chest radiography, biopsy, or drainage of infected fluids in advance of any empiric antimicrobial therapy. Hospital admission and isolation may be required until the nature of the process is further defined. Special precautions (e.g., respiratory, secretions, neutropenia) will be dictated by the patients' clinical presentation. In the event of the recognition of a novel recombinant organism or severe infectious illness without explanation, strict isolation with HEPA filtration will be required. Empiric antimicrobial therapy should be provided based on the common causes of post-transplant infections.

If no etiologic agent or process can be established, special xenotransplant studies may be obtained on a regular basis (e.g., every 3 days) including cocultivation of lymphocytes on human and porcine cells, viral cultures of serum on permissive human and porcine cells, nucleic acid testing for HCMV, PCMV, EBV, PLHV, hepatitis E virus, porcine circovirus, PERV-A, -B, and -AC, human herpesvirus 6, and adenovirus. Specimens (serum, leukocytes) for archiving should also be obtained and processed. Any lesions (skin, tumors) should be biopsied for histopathology and cultures or nucleic acid testing.

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Table 1

Categories of potential pathogens resulting from xenotransplantation (examples and availability of validated microbiological assays)

Common Human Pathogens of Allotransplant Recipients (EBV, CMV, herpes simplex virus, varicella zoster virus, Apergillus species, *Listeria monocytogenes*, mycobacterial species, *Pneumocystis jirovecii*)
Specific microbiological assays are generally available
Traditional Zoonoses: well-characterized clinical syndromes of humans (*Toxoplasma gondii)*
Specific microbiological assays are generally available
Species-specific agents: organisms *generally* thought to be incapable of causing infection outside the xenograft (e.g., porcine CMV)
Some specific microbiological assays are available; few standardized assays available for use in humans
Potential pathogens: Organisms of broad “host range” which *may* spread beyond the xenograft (adenovirus)
Some specific microbiological assays are available for use in humans, may not be standardized for porcine strains
Unknown pathogens: Organisms not known to be human pathogens, not known to be present in the source animals, or for which clinical syndromes and microbiologic assays are poorly described or unknown
New pathogenicity within the new host, while not known to be present or pathogenic (e.g., protozoa or retroviruses)
Viral recombinants resulting from intentional genetic modification of donor diseases resulting from multiple simultaneous infections

Table 2

Common microorganisms of swine to be considered among potential causes of infection in immunocompromised swine and/or human xenograft recipients[a]

Bacteria	Viruses
Actinobacillus species (e.g., *pleuropneumoniae*)	*Adenovirus* sp.
Bordetella bronchoseptica	Encephalomyocarditis virus
Brucella suis	Influenza virus (swine, avian, human)
Campylobacter species (e.g., *coli, jejuni*)	Lymphocytic choriomeningitis virus (LCMV)
Chlamydia psittaci	Nipah (Hendra-like)
Clostridium difficile	Menangle virus
Corynebacterium species (i.e., *pyogenes, suis*)	Porcine circovirus
Haemophilus species (i.e., *parasuis, suis*)	Porcine cytomegalovirus (PCMV)
Klebsiella species (e.g., *pneumoniae*)	Porcine endogenous retrovirus (PERV)
Legionella pneumophila	Porcine hepatitis E virus
Leptospira species	Porcine lymphotropic herpesvirus (PLHV)
Listeria monocytogenes	Porcine parvovirus
Mycobacterium species (i.e., *bovis, tuberculosis*, non-tuberculous mycobacteria)	Porcine Reproductive and Respiratory Syndrome virus
Mycoplasma hyopneumoniae (lung transplant)	Pseudorabies virus
Nocardia species	Rabies virus
Pasteurella species (i.e., *haemolytica, multocida, pneumotropica*)	Rotavirus
Pseudomonas species (i.e., *aeruginosa, pseudomallei*)	Torque teno virus
Salmonella species (i.e., *typhi, typhimurium, cholerasuis*)	**Fungi**
Serpulina hyodysenteriae	*Aspergillus* species
Shigella species	*Candida* species
Staphylococcus species (i.e., *aureus, hyicus*)	*Cryptococcus* species
Streptococcus species (e.g., *pneumonia, suis*)	*Histoplasma capsulatum*
Strongyloides species (e.g., *ransomi*)	*Microsporum* species
Yersinia species (i.e., *enterocolitica, pseudotuberculosis*)	*Trichophytum* species
Parasites	
Ascaris species	
Cryptosporidium species (i.e., *parvum*)	
Echinococcus	
Isospora species	
Neospora	
Strongyloides stercoralis	
Toxoplasma gondii	
Trichinella spiralis	

[a] Many porcine organisms have not been associated with human infection or disease but are included as being similar to organisms associated with human infection, infect human cells (e.g., PERV) in vitro, or are important causes of infection in immunocompromised swine or non-human primate recipients of porcine xenografts.