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**Xenotransplantation: Guidance on Infectious Disease
Prevention and Management**

World Health Organization
Emerging and other Communicable Diseases,
Surveillance and Control

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Table of Contents

| | Page |
|--|------|
| 1. INTRODUCTION..... | 1 |
| 2. CONTAGIOUS DISEASE POTENTIAL OF XENOTRANSPLANTATION..... | 1 |
| 2.1 Risk of exposure..... | 1 |
| 2.2 Establishment of an agent in individual recipients..... | 2 |
| 2.3 Risk of dissemination to the general population..... | 3 |
| 2.4 Risk of disease production in the general population..... | 3 |
| 3. CONSIDERATION OF AGENTS FOR EXCLUSION..... | 3 |
| 3.1 Bacterial, fungal and parasitic agents..... | 4 |
| 3.2 Viral agents..... | 4 |
| 3.2.1 Known zoonotic viral agents..... | 4 |
| 3.2.2 Viral agents not recognized as zoonoses..... | 5 |
| 3.2.3 Virus with high recombination rates or mutation rates..... | 5 |
| 3.2.4 Retroviruses..... | 5 |
| 4. HOST AND CELLULAR SUSCEPTIBILITY..... | 6 |
| 5. UNKNOWN AGENTS..... | 6 |
| 6. MINIMIZING RISK TO PUBLIC HEALTH..... | 7 |
| 6.1 Source animal species selection..... | 7 |
| 6.2 Source animal selection requirements..... | 8 |
| 6.2.1 Gnotobiotic source animals..... | 8 |
| 6.2.2 Specified Pathogen Free (SPF) source animals..... | 8 |
| 6.2.3 Conventional source animals..... | 9 |
| 6.3 Source animal susceptibility to human agents..... | 9 |
| 6.3.1 Risk of human agent establishment in animal populations..... | 9 |
| 6.3.2 Source animal exposure to human agents..... | 9 |
| 6.4 Source animal surveillance..... | 10 |
| 6.4.1 Surveillance programme design..... | 10 |
| 6.4.2 Monitoring for unknown agents..... | 11 |
| 6.4.3 Diagnostic test reliability..... | 11 |
| 6.4.4 Diagnostic test interpretation..... | 11 |
| 6.5 Donor colony refinement..... | 12 |
| 6.6 Recipient follow-up..... | 12 |

| | |
|--|----|
| 6.6.1 Recipient infectious disease assessment programme..... | 12 |
| 6.6.2 Recipient monitoring..... | 13 |
| 6.6.3 Recipient restrictions..... | 13 |
| 7. RECIPIENT PROTECTION..... | 13 |
| 8. EVALUATION OF INFECTIOUS DISEASE MANAGEMENT MEASURES AND STRATEGIES..... | 13 |
| 9. CONCLUSION..... | 14 |
| Annex 1: Criteria for developing a xenotransplantation infectious agent exclusion list.... | 15 |
| Annex 2: Reference list..... | 17 |
| Annex 3: Reviewer list..... | 20 |

1. INTRODUCTION

Xenotransplantation, the transfer of animal cells, tissues or organs into human recipients, may become a biomedical reality. The development of protocols which modify the immune system to prevent both graft rejection and graft vs. host disease, and advances in surgical techniques may open the door to a field with the potential to save thousands of human lives each year. The field of xenotransplantation also brings with it the potential for introducing animal-origin infectious agents, both known and as yet unknown, into the human population. These agents could cause disease in their new host. Dissemination beyond the original recipient into the general population could lead to epidemics. Xenotransplantation, therefore, has the potential for being of both great benefit and detriment to humans.

Whether or not xenotransplantation should be done is beyond the scope of this paper. Therefore, this paper does not examine the important ethical issues surrounding this technology, nor does it evaluate its feasibility. Rather, making the assumption that this technology might be implemented, the authors¹ have focused their discussion on some of the infectious disease implications of xenotransplantation. The paper presents an evaluation of the risks involved and offers ideas on how to minimize them. By addressing the concepts presented in this paper, interested parties should be able to start developing appropriate guidelines for preventing or managing the infectious disease risks associated with this technology.

2. CONTAGIOUS DISEASE POTENTIAL OF XENOTRANSPLANTATION

2.1 Risk of exposure

There is a risk of disease transmission in any transplantation system. In allotransplantation, disease transmission is a major cause of morbidity and mortality in recipients. This is largely due to the required level of recipient immunosuppression, but is also attributable to the use of organs or tissues harboring latent microbial agents, usually from asymptomatic human donors. Cases of viral, bacterial, fungal and parasitic transmission are documented. In some cases, the presence of an infectious agent was known before transplantation. The decision was made to proceed because of the lack of alternative donors. This scenario is one reason offered for developing xenotransplantation as an alternative to allotransplantation. Totally infectious agent-free human donors are not available.

If it is decided that xenotransplantation should occur, the potential for introducing an

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infectious agent into a recipient must be recognized and assessed. The animal species currently under consideration as organ or tissue sources can carry and transmit infectious agents. The risk for zoonotic transmission depends on the presence of an infectious agent in the source animal and the exposure of the recipient to the agent. There are some zoonotic agents which, because of the route of exposure, do not represent a risk to the recipient. An example would be a gastrointestinal parasite like *Trichuris suis* (whipworm of swine) when a heart transplant is performed. There are, however, other agents which represent a higher risk for exposure to the recipient. For example, encephalomyocarditis virus (EMCV) or *Toxoplasma gondii*, with their cardiac muscle tropism, must be considered risk factors for heart transplantation. Other relevant infectious agents may be risk factors because of current technological inability to remove them from source animals, for example endogenous retroviruses.

2.2 Establishment of an agent in individual recipients

If a risk for exposure is present, then the potential for the establishment of an agent in the new host must be considered and evaluated. Establishment depends on the particular agent's ability to survive in the new host. Establishment may only require direct exposure. This would be typical of zoonoses like rabies virus. In these cases the risk for establishment after exposure would be relatively high. Establishment, however, may also require adaptation by the agent to its new environment. Theoretically such cases would represent a lesser risk, but are still quite possible. For example, human influenza viruses are thought to originate in migratory birds. By genetic reassortment in swine, they can become adapted to a mammalian internal environment and gain infectivity for humans. On the other hand, adaptation to the new host may not be possible. The required genetic alteration may not occur. Alteration may lead to non-viability. Sufficient numbers of altered agents may not be produced to permit establishment. In such instances, the actual risk might be low.

Theoretically, adaptation and establishment of source-animal agents in the human host might be easier if the agents were evolutionarily similar to analogous human agents. Also, the more phylogenetically related the source animal and recipient, the more potential the risk for the new host meeting the agent's environmental requirements. Along these lines, the development of host-recipient chimerisms or recipient immune suppression may provide a more permissive environment for any agent-restricting differences existing between donor and recipient species. These could represent serious risk factors for agent establishment.

On the other hand, another possible scenario is that establishment might be restricted to the transplanted tissue. It might not be able to disseminate through out the new host. This type of sequestration may have implications for the individual recipient and the success of the xenotransplant, but its implications for the general population may not be that great.

2.3 Risk of dissemination to the general population

If establishment in the recipient host is possible, the next issue to consider is the risk for dissemination to the wider human population. If the agent cannot disseminate from the individual recipient, then even if introduced and established in that individual, the agent's presence may not be viewed as a significant public health issue. The risk might be acceptable in cases where the consequences of infection in the recipient is less than the morbidity or mortality associated with organ failure. If dissemination into the general population is likely, however, a risk-benefit analysis may conclude that the risk is considered too great to proceed.

2.4 Risk of disease production in the general population

Ultimately, an assessment must be made of the risk for disease production in the general population as a result of xenotransplantation. For some agents the likelihood of disease production in the general population is high. (i.e., Marburg virus or other as yet unknown filoviruses). For other agents, like *Toxoplasma gondii*, the likelihood of disease production and transmission into the non-immune suppressed general population is considered absent or low. In such cases, the overall risk might be considered acceptable.

3. CONSIDERATION OF AGENTS FOR EXCLUSION

Attached is a list of suggested criteria for consideration when developing a xenotransplantation infectious-agent exclusion list. Examples are offered to demonstrate these criteria, but the generation of any specific agent list must be done with the exercise of sound professional judgement and cautious flexibility to assure that the generated list reflects the best possible integration of technical feasibility and risk acceptability (both for the individual recipient and for the general population). These two factors will to a large extent dictate the number and type of agents excluded from the xenotransplantation arena. Specific lists should be generated by panels of experts representing all scientific fields involved with xenotransplantation: infectious disease experts, public health and preventive medicine specialists, veterinarians, microbiologists, xenotransplantation scientists, and others as considered appropriate. The lists should reflect the host species variability of infectious agents, the origin of the source animals, their proposed transplantation usage, animal husbandry standards and potentials, and the detection capabilities of available diagnostic technology. Any generated lists should also reflect the characteristics of relevant regional and international geographic factors which influence the risk of importation or exposure to different agents. Finally these lists should undergo periodic review and updating to include newly recognized agents, and to account for any detected alterations in behavior or pathogenicity, and improvements in diagnostic technology. Obviously the generation of specific agent exclusion lists will be an enormous but necessary task.

3.1 Bacterial, fungal and parasitic agents

Bacterial and fungal agents probably do not present greater risks to the general population from xenotransplantation than from allotransplantation. The risk associated with these agents

is more a function of the immune status of the recipient than of the agent itself.

Parasites, both protozoa and helminths, not normally associated with humans, could produce aberrant infections in the xenotransplant recipient. They could pose some increased risk to the general population. The exact level of risk will depend on the parasite's tissue tropism and life cycle requirements. For most parasites not already possessing zoonotic potential, these factors should prevent establishment and dissemination in the general population.

The parasites presenting the most risk for establishment and dissemination, both within the recipient and the general population, are those with conducive tissue tropisms and direct life cycles, for example, ascarid nematode larvae. Parasites with non-conducive tissue tropisms and/or indirect life cycles should pose a lesser risk. The level of risk would be a function of the presence of an intermediate host in the new host's environment. In the case of parasites like *Hepaticystis kochi*, the parasite may also have to go through the process of adapting itself successfully to the new aberrant host. Because of the physical complexity of many parasites, this would be less likely to occur. The risk, therefore, would not be as great. Parasites found in the source animal, but not found in its transplanted tissue should not present a risk.

3.2 Viral agents

As a generality, the agents presenting the most risk for introduction and dissemination as a result of xenotransplantation are the viruses. (For the purpose of this discussion, prions will be considered with viruses, recognizing that the risk for prion disease may concern the individual patient primarily, whereas the risk for viral disease may equally concern the patient and the general population.) Not being a homogenous group, risk will vary with the individual characteristics of each virus. Some viruses pose a very significant risk, others do not. Certainly, the transmission of a persistent, but latent virus with the capacity to silently infect a large number of persons before recognition, would be of great public health significance.

3.2.1 Known zoonotic viral agents

The risk for dissemination of these viruses into the general population may not be greater with xenotransplantation than with other modes of transmission.

Viruses already possessing zoonotic capability are high risk factors for individual recipients. Here, only exposure to live virus is required to cause infection and disease, for example rabies, LCMV, EMCV, etc. During transplantation, if infective material is transmitted, establishment is likely to occur. The risk is high for both acute source-animal viremias and for chronic infections with viable viral material sequestered in the animal's transplanted tissues, for example, *Herpesvirus simiae* or prions. A truly resolved viral infection does not present a risk to the recipient or the general population.

3.2.2 Viral agents not recognized as zoonoses

Viruses not normally considered zoonotic, but which are so closely related to their human counterparts that a presumption could be made that only exposure is required for establishment in a new host, can also represent risk factors for xenotransplantation. Beta-herpesviruses, such

as the cytomegaloviruses of different animal species, and gamma-herpesviruses such as Epstein Barr virus and *Herpesvirus papio*, are examples of viruses not currently recognized as zoonoses, but which could have the potential of adapting to the new human-recipient environment. These viruses are thought to be extremely species specific. Theoretically, however, the events of transplant exposure, recipient immunosuppression, and/or host chimerism, may be sufficient to permit adoption by the new host species. These agents could become new recipient-associated viruses. Should adaptation take place such that infection of recipient cells occur, then recombination of the original host virus with an introduced virus could be possible as are dual infections. What effect such events would have on virulence and pathogenicity cannot be predicted, but attempts to research the potential tropisms of these viruses and to assess the risk to the general population should be made.

3.2.3 Viruses with high recombination rates or mutation rates

Viruses which tend to be highly mutable can be major risk factors if present in the xenotransplanted tissue, organ or accompanying hematopoietic cells. RNA viruses such as retroviruses, or influenza viruses are good examples of this. Because of what we know of their genetic plasticity and their demonstrated ability to adapt to new environments for continued survival, these viruses could pose a significant risk for establishment and dissemination in a new host species. Under xenotransplantation conditions, these viruses might face selective pressure to adapt. If the adaptation process were quick enough, the life span of the recipient, an organism could integrate into its new host species. Successful adaption to new host environments similar to their original host environment may be more probable than adaption to more disparate environments. Under favorable conditions, however, both must be considered possible.

3.2.4 Retroviruses

Many Retroviruses are thought capable of adapting to new host species. Examples of this are the believed relationships between STLV-1 and HTLV-1, and between SIV and HIV-II. In the case of STLV-1/HTLV-1, a progenitor virus underwent mutation to permit establishment in both host species. This crossing over did not produce a change in virulence or disease expression, however, both produce lymphomas. With SIV, changes in disease expression and virulence occurred with crossover and establishment. In the African Green monkey and some other *Cercopithecus* species, SIV is not considered pathogenic. In the crossover to macaques, pathogenicity developed. High genetic homology suggests a link between SIV (sooty mangabey) and HIV-II. What factors permitted establishment in the new host remains speculative. The important point is that exposure and successful adaption leading to establishment and dissemination in a new species occurred. In the xenotransplant environment, there is no reason to believe that this could not occur again.

Foamy (Spuma) viruses are not currently known to cause disease in their natural hosts. They can however, replicate in host-species' and novel species' cell cultures. Traditionally, they are not considered zoonotic, but there are at least two published accounts of monkey to human transmission. Under favorable conditions, foamy viruses could prove pathogenic. Prudence would dictate that, they should be considered risk factors for xenotransplantation.

The endogenous Retroviruses are another potential risk factor. Under natural conditions they are strongly integrated in their host's cell genome. Until now they are believed latent and non-pathogenic. That latency could be disturbed by the stimulus of xenotransplantation and new host exposure. An *in vitro* study has suggested that separation from an endogenous virus' host genome and subsequent reinsertion into a recipient's may be possible. A reinsertion may or may not be random depending on the presence of any associated, specific insertion sequences. Depending on where it occurred, latency might be reestablished, or viral expression may occur. Lacking its historical constraints, the endogenous virus could mutate, enhance its lodgement in the new host, and increase its virulence.

4. HOST AND CELLULAR SUSCEPTIBILITY

Factors other than the inherent properties of specific viruses also need to be considered when assessing the risk of xenotransplantation to the recipient and the general population. Hypothetically, xenotransplantation could provide the opportunity for exposure to receptive host tissues not normally available for contact in the recipient's species. A species-specific protective mechanism at the body or organ system level might be circumvented. Since cellular susceptibility is a function of appropriate cellular receptors and internal biochemical conditions, once exposure is accomplished, crossing species lines may only mean minor adjustments for the virus. What was once known as "inability to cross species lines" may only be "inability to come into contact with susceptible host cells". This is suggested by some viruses' ability to grow in cell cultures of non-host species origin. Once in a new species, the virus could behave as in its natural host or develop new characteristics unique to its new environment. This could mean increased virulence and dissemination.

5. UNKNOWN AGENTS

One must consider the possibility of existing yet undiscovered agents. It is thought that many new and emerging viruses that infect humans are of animal origin; given the opportunity because of alterations in their ecosystems, to enter the human population. In this sense, xenotransplantation might best be considered as just another human activity with the potential to facilitate cross-species transmission.

Also, advances in diagnostic technology have created a perception of new and emerging infectious agents. For example, hepatitis C virus was identified only recently as the etiologic agent of non-A non-B hepatitis, a disease which has been recognized for years. Past experience with transmitting previously unknown human pathogens through human blood products and allotransplantations, (i.e. HIV), demonstrates the need for caution when assessing the risk of unknown, but potentially problematic agents.

6. MINIMIZING RISK TO PUBLIC HEALTH

Despite the concerns and difficulties listed above, xenotransplantation could have the potential for relatively safe implementation. Since we do not have a complete understanding of all potentially pertinent infectious agents, a complete elimination of risk for both the individual recipient and for the general population will probably not be achievable. If precautions are taken however, the risks could be reduced to that which may be considered acceptable and outweighed

by the benefits of the technology.

6.1 Source animal species selection

Steps must be undertaken to minimize the risk of infectious disease transmission as a result of xenotransplantation. This desire to minimize risk will affect the selection of source-animal species. Availability, animal welfare considerations and ease of maintaining the animals will also affect source-animal selection.

Since 1963 the choice of source species has focused mainly on baboons and swine. Previous attempts were made using a variety of species including rabbit, goat and sheep. In the early 1960's, studies were conducted using the chimpanzee as a source-animal species. At that time, the chimpanzee was selected because of its close phylogenetic relationship to man, the size of its transplantable organs, and the existence of its A and O blood groups. Chimpanzee use was discontinued however, because of that species' designation as endangered under CITES. Baboons are now favored as the non-human primate organ source of choice because of the appropriate size of some of their organs and, relative to the chimpanzee, their larger population. They were also selected because of their apparent non-susceptibility to hepatitis B and HIV infections. Other monkey species are considered physically too small for practical use.

Domestic pigs have also been considered source candidates for xenotransplantation. As with baboons, some of their organs are considered appropriately sized. The advantages of the pig over the baboon are its much higher reproductive rate and relative simplicity of husbandry requirements. After 6 months of age, pigs can produce multiple young in a year. Baboons are limited to an average of one infant per reproducing female per year after sexual maturity at 5 to 6 years of age. This higher reproductive rate for pigs can facilitate a more rapid and economic development of genetically engineered, immunologically suitable source animals. Transgenic technology is already being explored in pigs. In the future, a transgenic pig could be used to reduce the immunologic disparity between a porcine source and human recipient. The application of this technology to primates will be much more difficult and time consuming. Therefore, the availability of required production techniques along with the level of certainty wanted for infectious agent-free status for transplanted tissues and organs will be, in the end, the determining factors in the choice of xenotransplant source species.

6.2 Source animal selection requirements

Risk minimization will require the establishment and implementation of stringent selection requirements for prospective tissue and organ source-animals. Ideally, since infectious agent-free status is a key to avoiding subsequent transmission of agents to human recipients, every effort must be made to prevent source animal contamination. This will require the establishment and maintenance of xenotransplant-dedicated animal colonies. It will probably necessitate the licensing and close monitoring of these colonies as is done for other biologics.

6.2.1 Gnotobiotic source animals

Theoretically, source animals should be gnotobiotic (free of all associated microbial flora except those non-pathogens purposely introduced for the biologic requirements of the animal). This would require the surgical delivery of offspring under aseptic conditions to prevent the exposure of offspring to microbes. The offspring must then be raised in an isolation type barrier, devoid of contact with any material harboring undesired bacteria, viruses or parasites. While this technology is possible, its practicality (husbandry requirements, ease of eliminating all contaminations) or desirability (welfare issues) for most potential xenotransplantation source-animal species is open to question.

6.2.2 Specified Pathogen Free (SPF) source animals

Given the difficulties associated with maintaining animals under gnotobiotic conditions, as an alternative, at a minimum, only animals which are well documented to be free of specified pathologic agents, demonstrated or hypothesized as significant in the xenotransplant setting, should be used. These animals should come only from closed, microbiologically well-defined and controlled colonies.

Animals initially selected to found these SPF colonies should be subjected to a stringent quarantine and microbial screening process. This process must detect all known or suspected transmittable agents of concern in the xenotransplantation environment (see attached criteria list). In the case of positive findings, treatments and procedures must be undertaken during the initial quarantine and conditioning period to completely clear the prospective colony member of the agent. No specified-agent positive animals should be allowed entrance into the colony.

The specific definition of which organisms should be excluded from these colonies should be based on up-to-date knowledge and understanding of known infectious agents, reasoned expectations about unknown or unrecognized agents, and on realistic expectations for the discovery of new agents. The expansion of what is known about xenotransplant relevant agents must be the focus of ongoing research as xenotransplant technology evolves.

6.2.3 Conventional source animals

Conventional animals, those with an unknown or minimally defined microbial status should never be used for xenotransplantation. This would include wild caught and conditioned animals which appear free of active clinical disease, and which have undergone some diagnostic screening and quarantine procedures. These animals can carry latent, subclinical, or otherwise undetected pathogenic agents. Their infectious disease status is too unpredictable for appropriate xenotransplantation use.

6.3 Source animal susceptibility to human agents

Source-animal susceptibility to human infectious agents must be addressed in the development and maintenance of source-animal colonies. Humans coming in contact with source animals can transmit zoonotic agents (i.e., *Mycobacterium* spp., measles virus). Humans could also transmit infectious agents not normally considered zoonotic, but which could be transmittable to genetically manipulated, or otherwise receptive source animals (for example, CMV, other herpes viruses, enteroviruses). Under favorable circumstances, these contaminated animals could subsequently transmit these agents to other colony animals or to transplant recipients.

6.3.1 Risk of human agent establishment in animal populations

Along these same lines, if human agents can be transmitted into manipulated and therefore, susceptible source animals, consideration must be given to the potential for these agents to undergo mutational events and gain infectivity for non-source, non-manipulated animals as well. Just as with agents originating in animal hosts, human agents could gain access and become established in the new host species. The potential for, and implications of such an event must be assessed.

6.3.2 Source animal exposure to human agents

Given animal size requirements, it may not be practical to maintain xenotransplant animal colonies in barriers that exclude all human contact with source animals. Small flexible film isolators, as used in gnotobiotic rodent facilities, are not practical for the long-term housing of large animals. The transportation of source animals to xenotransplantation sites may also require some human-animal contact. It will be necessary, therefore, to monitor humans coming in direct contact with source animals for the presence of those transmittable agents, potentially detrimental to the xenotransplant source colony. Contact with source animals must be limited, as far as technically possible, to those persons demonstrated free of agents, both human and animal, which could jeopardize the specified-agent-free status of xenotransplant source animals. Procedures to protect source animals from exposure to surveillance-system negative, but infectious-agent positive and therefore communicable humans, should be implemented.

6.4 Source animal surveillance

Once established, all source-animal colonies, must be subjected to a surveillance programme designed to detect infectious agents appearing in the colony. In the case of an outbreak, immediate measures must be taken to contain the contamination and eradicate the agent. This may require the destruction of economically and scientifically valuable animals.

6.4.1 Surveillance programme design

The exact design of the surveillance programme will depend on the natural history of the

agents being sought, the type, availability, and use of appropriate test systems, and the past history of the colony.

First, a surveillance programme should include an active basic research component designed to identify and characterize the microbial flora of source-animal species. This should include research into the infectivity, pathogenicity, and human-to-human transmission potential of such agents.

Secondly, flexibility must be built into the surveillance program to allow for advancement and improvements in diagnostic technology. New tests with heightened sensitivities such as PCR should be integrated into the programme as soon as practical to improve and refine diagnostic capabilities.

Thirdly, the surveillance programme must have flexibility to allow for diversity in the global distribution of different agents. Testing for Chagas' disease might be required in South America. It might be unnecessary for colonies originating and maintained in Africa. Likewise consideration must be given to the potential for the inadvertent importation of foreign or exotic agents not normally resident in the geographic area where the colony is maintained. The likelihood of exposure to an agent in one country or region may be very different from that in another.

Finally, the surveillance system should also include examination of source animals for diseases and infectious processes not normally considered contagious, but which could adversely affect the outcome of the xenotransplant process, for example an animal with hepatic abscesses or pyelonephritis. Affected animals should be culled from the colony and not used for xenotransplantation.

Ideally, all possible agents would be tested for at a frequent enough interval to allow for their rapid detection and elimination. This can become very expensive and labor intensive. As an alternative, in closed well-defined colonies, often indicator agents can be identified and used to detect breaks in colony management and health status. Monitoring for these agents should be done frequently. A more inclusive testing can then be done at less frequent intervals or when clinically required to define the microbial status of the colony. This type of layered surveillance should only be used in well-documented, infectious-agent free colonies. It is less useful in colonies of questionable microbial status.

To supplement surveillance testing, source-animal biologic sample archives should be established to create baseline information banks for retrograde investigations of emergent disease occurrences in both source-animal colonies and in xenotransplant recipients.

6.4.2 Monitoring for unknown agents

A major limitation for all surveillance programmes is the difficulty in testing for unknown agents. This necessitates the coupling of the surveillance programme with a continuous monitoring programme for detecting unknown but clinically emerging agents in the source-animal population. New diagnostic tests must be developed and incorporated into the overall programme as new infectious agents are discovered. Research is needed to develop systems for

identifying unknown agents. One approach could be the identification and application of "group-specific" markers or indicators unique to certain groups of agents. When detected, they signal the presence of a member of that group. An example of this would be the AMP RT technique which detects reverse transcriptase (RT), a common feature of Retroviruses. It can be used to signal the presence of a retrovirus, known or unknown in the source species. Such approaches could accelerate the pace of detecting "new" agents. While it is expected that most new agents will be viruses, new bacterial (i.e., *Helicobacter* spp.), fungal or parasitic agents are possible. Their potential for discovery should not be ignored.

6.4.3 Diagnostic test reliability

Basic research is needed to improve diagnostic assay specificity, sensitivity, and validity for many infectious agents of concern to xenotransplantation. Any surveillance system used to define source animals must include a description of the test system and its properties, in order to ensure the validity of the colony's SPF status claim.

Diagnostic tests must be able to reliably distinguish truly agent-negative animals from agent-positive ones. False-negative animals can result from anergy, for example as seen in some advanced cases of tuberculosis. They can also result from agent sequestration and protection from the host's immune system. Such a mechanism is suggested for some simian type D retrovirus infected but seronegative primates. Because of the ultimate use of the animals, the diagnostic tests used must possess a very high degree of sensitivity.

False-positive test results are also undesirable. They may be due to too high a test sensitivity and/or low agent prevalence in a truly negative colony. Confirmation of positive finding is necessary to prevent the unnecessary culling of false-positive animals.

6.4.4 Diagnostic test interpretation

Conservative interpretation of test results is required. A false negative reading due to an uninvestigated tissue culture reaction or other technical problem could have devastating results. Such conservatism will be costly in terms of source-animal production and maintenance, but is necessary for the safety of the recipient and the general population. Along these same lines, however, it should never be assumed that a positive finding is false because of the colony's past negative history. Breaks in infectious agent status can and do occur even in the best run facilities.

6.5 Donor colony refinement

Finally, the longer a colony is maintained without disease occurrences, the more confidence one might be tempted to have about the absence of undetected or unknown agents. This is an assumption, however, not a certainty. Risk can be considered decreased, but it must never be considered eliminated. Here the assistance of molecular virologists and geneticists is important. Genetic mapping of source-animals could help detect endogenous viruses. Ultimately, the development of genetically engineered animals, which are defined and specified pathogen free, may help to minimize the risk of infectious disease transmission.

6.6 Recipient follow-up

The risk of infectious agent transmission during xenotransplantation can be reduced

significantly by the source animal selection and screening procedures outlined above. These procedures can significantly decrease the infectious disease risk, both to the individual recipient and to the general population. They should be complemented, however, by follow-up procedures designed to detect and contain unrecognized or emerging infectious agents in the xenotransplant recipient. The establishment and implementation of these procedures are aimed at reducing risk in the general population. Recipient follow-up must be practical and clinically feasible. A total elimination of all risk is probably not possible.

6.6.1 Recipient infectious disease assessment programme

Before xenotransplantation, the potential recipient must be informed of the potential risks and inherent degree of uncertainty with regard to infectious agent transmission for both himself and others. It is in the interest of the general population that the recipient agree to participate in an infectious disease assessment programme designed to detect the emergence of xenotransplant associated agents.

A post-xenotransplant infectious disease assessment programme should be designed for the periodic surveillance and monitoring for all known associated risk agents. The use of diagnostic technology capable of detecting agents in an immune suppressed or altered host is required. Standard antibody detection tests while useful, if used alone will not be sufficient. Test systems based on antigen detection, or agent recovery are necessary and should be used to complement antibody testing. Test systems should also be able to determine the animal species of origin of agents detected. These requirements will necessitate continuing research and development efforts.

Infectious disease assessments of the recipient should continue long-term if not life-long. Periodic examinations should include the archiving of appropriate biological samples for future examination and research. Cohort studies may be required. Infectious disease assessment surveillance and monitoring procedures may also be required of recipient contacts such as health care providers and family members. These measures could help expand the epidemiologic understanding of xenotransplantation and should eventually lead to more accurate risk determinations.

6.6.2 Recipient monitoring

Recipients should be monitored for the emergence of unknown or new agents. This should include the investigation and diagnostic resolution of otherwise unexplained clinical symptomatologies expressed in the recipient. Novel clinical symptoms may be the result of a new agent, a change in behavior or expression of a known agent, or the result of an altered host response. All are possible in the xenotransplant environment. There should be a heightened awareness of these potential developments, and therefore, an increased attempt to define symptom etiologies. Sound clinical judgement should be employed in deciding what and how far to diagnostically pursue a problem.

6.6.3 Recipient restrictions

Recipient follow-up is a precautionary measure for containing infectious agents and

preventing their spread to the general population. Any containment steps undertaken, however, must be proportionate to the risk of dissemination into the general population assigned to the particular agent of interest.

When implemented, containment steps should be initiated immediately, and continued until a determination is made that there is no further risk to the general population. These steps may require quarantine or other physical restrictions on the individual recipient. Adopted procedures should be periodically reevaluated to assure applicability and appropriateness.

7. RECIPIENT PROTECTION

Recipients must be informed about and agree to follow-up infectious disease assessment procedures, and if necessary any personal restrictions that might be required in the event of a xenotransplant-generated public health problem. The recipient must agree to undergo such measures if required. This agreement must imply that every effort will be made to protect recipients from the initiation or continuation of unreasonable measures. Review boards with expertise in xenotransplantation, infectious diseases, public health principles, bioethics, and civil rights should be established to decide when such measures are necessary and how long they should be practiced. Review boards should be empowered to resolve disputes generated from the initiation and maintenance of infectious disease assessment and containment measures. Recipients should be informed about the function of these boards and how to access them expeditiously. Follow-up infectious disease assessment and containment procedures must be consistent with basic human rights principles.

8. EVALUATION OF INFECTIOUS DISEASE MANAGEMENT MEASURES AND STRATEGIES

Periodic evaluations should be made of the effectiveness and appropriateness of implemented animal selection and infectious disease management measures. These evaluations should include recommendations for research and development needs. Occurrences of infectious agent introduction and/or dissemination must be evaluated to determine the cause of the event and to develop or refine further prevention strategies.

9. CONCLUSION

As it is assumed that xenotransplantation will become medical practice, a consensus is needed on how best to balance the benefits associated with xenotransplantation with the infectious disease risks to the recipient and to the general population. Measures designed to minimize risk need to be agreed upon and implemented before problems arise. This will require input from many sectors, researchers, clinicians, veterinarians, infectious disease experts, public health proponents, patient advocates, ethicists and lawyers. Without a pre-accepted and adhered-to mechanism for protecting individuals and populations from the adverse potentials of xenotransplantation, the risks of the technology to the general population may be viewed as outweighing its benefits.

Criteria for developing a xenotransplantation infectious agent exclusion list

1. Source animals should be physically and physiologically healthy and free from signs of clinical disease.
2. Source animals should be free of recognized zoonotic agents transmissible to man outside the xenotransplant environment. For example: rabies virus, monkey pox virus, *Brucella suis*, *Mycobacterium* spp., etc.
3. Source animals should be free of zoonotic agents with transmission potential that is enhanced by the xenotransplantation application. For example *Trypanosoma cruzi*, *Ascaris* spp. larvae, *Toxoplasma gondii*, EMCV, etc.
 - a. agent transmission facilitated by direct tissue exposure.
 - b. agent transmission facilitated by recipient immune suppression.
 - c. agent transmission facilitated by other altered internal environments in the recipient, i.e. chimerism or other manipulation.
4. Source animals should be free of recognized human-origin infectious agents. For example: measles virus, Rubella virus, etc.
5. Source animals should be free of infectious agents not normally considered zoonotic but whose transmission is achieved by the xenotransplantation procedure. For example: *Hepaticystis kochi*, etc.
 - a. agent transmission facilitated by direct tissue exposure.
 - b. agent transmission facilitated by recipient immune suppression.
 - c. agent transmission facilitated by other altered internal environments in the recipient, i.e. chimerism or other manipulation.
6. Source animals should be free of infectious agents possessing high mutation or recombination potential which could lead to pathogenicity in the new human host. For example: influenza viruses, rota viruses, parvo viruses etc.
7. Source animals should be free of infectious agents known to be resistant, non-amenable or refractory to therapeutic treatment, and be free of agents for which no effective therapeutic intervention has been defined. For example: antibiotic resistant bacteria, drug resistant parasites, etc.
8. Source animals should be free of infectious agents of geographic relevance, both domestic and exotic. For example: *Trypanosoma cruzi*, African Swine Fever, Swine Vesicular Disease virus, etc.
 - a. agents endemic to the geographic origin of source animals.
 - b. agents considered exotic to an area, but which have importation potential.
9. Source animals should be free, as far as technically possible, from newly recognized agents before their pathogenicity and transmission potentials are defined, and before it is determined that they do not present an unacceptable* risk to public health.

* unacceptable must be defined within the context of developed agent lists, and should reflect a consensus of both scientific understanding and reasoned public opinion. Its definition must be dependent on sound professional judgement. Unacceptable may equate to a high potential for disease transmission, pathogenic capability, and/or likelihood of exposure. It might, however, equate to moderate or low potentials combined with low public tolerance for a transmission event.

References list

- Allan J.S. (1995): Xenograft Transplantation and the Infectious Disease Conundrum. *ILAR Journal*, 37(1):37-48.
- Allan J.S. (1992): Viral evolution and AIDS. *J. NIH Res.* 4:51-54.
- Allan J.S., Short M.S., Taylor M.E. Su S., Hirsch, V.M., Johnson P.R., Shaw G.M., Hahn B.H. (1991): Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J. Virol.* 65:2816-2828.
- CDC (1997): Nonhuman primate spumavirus infection among persons with occupational exposures - United States, 1996. *Morbidity and Mortality Weekly Report.* 46:129-130.
- Chapman L.E., Folks T.M., Salomon D.R., Patterson A.P., Eggerman T.E., Noguchi P.D. (1995): Xenotransplantation and Xenogeneic Infections. *New. Engl. J. Med.* 333(22):1498-1501.
- Domingo E., Holland J.J. (1994): Mutation Rates and Rapid Evolution of RNA Viruses. In: *The Evolutionary Biology of Viruses*, S. Morse, ed. Raven Press, New York.
- Essex M. (1994): Simian immunodeficiency virus in people. *New Engl. J. Med.* 330:209-210.
- Fishman J.A. (1995): Preventing Infections in Xenotransplantation: Xenosis from Miniature Swine. *Xeno.* 3(4):72-77.
- Flugel R.M. (1991): Spumaviruses: A group of complex Retro viruses. *J. AIDS* 4:739-750.
- Heneine W. (1996): Strategies for diagnosis of xenotransplant-associated retroviral infections. *Molecular Diagnosis* 1: 255-260.
- Heneine W., Switzer W.M. (1996): Highly sensitive and specific polymerase chain reaction assays for detection of baboon and pig cells following xenotransplantation in humans. *Transplantation* 62:1360-1362.
- Hirsch V.M., Olmsted R.A., Murphey-Corb M., Purcell R.H., Johnson P.R. (1989): An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature(London)* 339:389-392.
- Homma T., Kanki P.J., King N.W., Hunt R.D., O'Connell M.J., Letvin N.L., Daniel M.D., Desrosiers R.C., Yang C.S., Essex M. (1984): Lymphoma in macaques: association with human T lymphotropic family. *Science* 225:716-718.
- Huang L.H., Silberman J., Rothschild H. Cohen J.G. (1989): Replication of baboon endogenous virus in human cells. *J. Biol. Chem.* 264:8811-8814.
- Hubbard G.B., Soike K.F., Butler T.M. Carey K.D., Davis H., Butcher W.I., Gaunt C.J. (1992):

An encephalomyocarditis virus epizootic in a baboon colony. *Lab. Anim. Sci.* 42:233-239.

Institute of Medicine, Committee on Xenograft Transplantation: Ethical Issues and Public Policy (1996): *Xenotransplantation Science, Ethics, and Public Policy*. National Academy Press, Washington, D. C.

Kalter S.S., Heberling R.L. (1995): *Xenotransplantation and Infectious Diseases*. *ILAR Journal*, vol 37(1):31-37.

Khabbaz R.F., Heneine W., George J.R., Parekh B., Towe T. Woods T. Switzer W.M., McClure H.M., Murphey-Corb M. Folks T.M. (1994): Brief Report: Infection of a laboratory worker with simian immunodeficiency virus. *New. Engl. J. Med.* 330:172-177.

Lecatsas G., Neething F.A., De Klerk W.S., Gridelli B. (1992): Filovirus seropositivity in prospective organ donor baboons. *Transplant. Proc.* 24:617-618.

Lerche N.W., Yee J.L., Jennings M.B., (1994): Establishing specific retrovirus-free breeding colonies of macaques: An approach to primary screening and surveillance. *Lab. Anim. Sci.* 44:217-221.

Michaels M.G., Lanford R., Demetris A.J., Chavez D., Brasky K., Fung J., Starzl T.E. (1996): Lack of susceptibility of baboons to infection with hepatitis B virus. *Transplantation* 61:350-351.

Michaels M.G., McMichael J.P., Brasky K., Kalter S., Peters R.L., Sstarzl T.E., Simmons R.L. (1994): Screening donors for xenotransplantation: The Potential for Xenozoonoses. *Transplantation* 57:1462-1465.

Michaels M.G., Simmons R.L. (1994): Xenotransplant-associated zoonoses: strategies for prevention. *Transplantation* 57:1-7.

Morse S.S.(1994): The viruses of the future? Emerging viruses and evolution. Pp. 325-335 In: *The Evolutionary Biology of Viruses*, S. Morse, ed. Raven Press, New York

Morse S.S.(1994): Crossing Over: The Interspecies Traffic of Emerging Infections. *J. NIH Res.* 6:52-56.

Nowak R. (1993): One Baboon Liver, Two Baboon Livers.... *J. NIH Res.* 5:36-38.

Nuffield Council on Bioethics (1996): *Animal-to-Human Transplants. the ethics of xenotransplantation*. Nuffield Council on Bioethics, London.

Patience C., Takeuchi Y., Weiss R.A. (1997): Infection of human cells by an endogenous retrovirus of pigs. *Nature Medicine* 3:282-286.

Reemtsma K. (1995): *Xenotransplantation: A Historical Perspective*. *ILAR Journal*, vol 37(1):9-12.

Steele D.J.R., Auchincloss H. (1995): The Application of Xenotransplantation in Humans, Reasons to Delay. *ILAR Journal*, 37(1):13-15.

Starzl T.E. Tzakis A., Fung J.J., Todo S., Demetris A.J., Manex R., Marino I.R., Valdivia L., Murase N. (1994): Prospects of clinical xenotransplantation. *Transplant. Proc.* 26:1082-1088.

Schweizer M. (1995): Markers of Foamy Virus Infections in Monkeys, Apes, and Accidentally Infected Humans. *AIDS Res. Hum. Retrovir.* 11:161-170.

Van der Riet F. deSt. J., Human P.A., Cooper D. J.C., Reichart B., Fincham J. E., Kalter S.S., Kanki P.J., Essex M., Madden D. L., Tai-Tung M. T., Chalton D. Sever J. L. (1987): Virological implications of the use of primates in xenotransplantation. *Transplantation Proc.* XIX 4068-4069.

Weiss R.A. (1996): Foamy Viruses Bubble On. *Nature (London)*. 380:201.

Yamamoto S., Folks T.M., Heneine W. (1996): Highly sensitive qualitative and quantitative detection of reverse transcriptase activity: Optimization, validation and comparative analysis with other detection systems. *J. Virological Methods* 61:135-143