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Directors
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Ministry of Health, Labour and Welfare

Public Health Guidelines on Infectious Disease Issues in Xenotransplantation

It goes without saying that clinical research should be conducted in an appropriate manner including obtaining an approval from an institutional ethical committee on the dignity of man and ethical matters. For xenotransplantation, cautions should also be paid to infectious diseases derived from a non-human animal source. For this purpose, Kouka No. 575 and Kenhatsu No. 21 “Notes for clinical research on xenotransplantation” have been published on October 31, 2000.
An attached document entitled “Public Health Guidelines on Infectious Disease Issues in Xenotransplantation” has just been completed as a part of the Health Science Special Research Program funded by the Health Science Grant. It will be appreciated if you understand the intent of the attached document and ensure that all facilities under your jurisdiction implement appropriate measures in xenotransplantation in order to prevent infection and transmission of infectious diseases derived from non-human species to recipients and medical professionals.

Attachment
Public Health Guidelines on Infectious Disease Issues in Xenotransplantation

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Preface

While allotransplantation has been established with the technological

advancement including immunosuppressive medicines, the number of patients on the waiting list has been increasing, and the shortage of donated organs has always been of a problem. In combination with the recent advancement in biotechnology and related fields, the fact has driven the development of a new treatment, xenotransplantation. For example, newly developed external perfusion devices using non-human cells, that are expected to be a bridge to allotransplantation or a treatment for sudden deterioration of underlying diseases, have been reported in many cases abroad. In addition, pigs inserted with human complement suppressive genes have been created by genetic modification. It has also been attempted to create pigs without an expression of heteroantigen. Swine cells, tissues, or organs can possibly be transplanted to human in the future.

For infectious diseases caused by cells, tissues, and organs from a non-human animal source used for xenotransplantation, however, there have been reports indicating the occurrence of infectious diseases derived form non-human animals, such as a new variant of Creutzfeldt-Jakob disease (vCJD) originated from Bovine Spongeform Encephalopathy (BSE), and the infection of swine endogenous retrovirus (PERV) to human cells co-cultured with swine cells. Thus, it cannot be assured that xenotransplantation will cause no occurrence or transmission of emerging infectious diseases at present, and there have been unpredictable aspects that are different from allotransplantation. Several countries, such as the U.S.A. and the U.K., developed guidelines on public health measures against emerging infectious diseases derived from xenotransplantation. International organizations have insisted on the necessity of international communication and surveillance system. Thus, infectious disease issues in xenotransplantation have raised international concerns.

In medical facilities, clinical research is usually conducted after technical and ethical review by their own Institutional Review Board (IRB). However, since certain public health guidelines are essential in xenotransplantation concerning the aforementioned matters and from the viewpoint of international harmonization, the present guidelines have been developed. According to the guidelines, xenotransplantation facilities and other organizations should establish the system enabling appropriate response to problems involved with xenotransplantation, both home and abroad, while making maximum efforts against infectious diseases.

An international surveillance system has been discussed by international

organizations. Each nation has just begun discussing it in cooperation with each other. In Japan, it is also necessary to appropriately deal with matters, as information on the implementation of xenotransplantation in keeping with the international trend.

And also, since several organizations with an appropriate IRB that can efficiently review technical and ethical matters, and with sufficient technology have just started to consider xenotransplantation studies, xenotransplantation will not rapidly increase in the near future. When various medical facilities are prepared to implement xenotransplantation with regulatory changes and technical developments, it will be necessary to consider new measures including revision of guidelines, registration and information gathering at academic meetings, inspection before the implementation, and inspection of xenotransplantation facilities concerning measures against infectious diseases.

The scope of the present guidelines is public health issues of infectious diseases related to xenotransplantation. In other words, the aim is to prevent overlooking the occurrence and expansion of potential infectious diseases caused by xenotransplantation. Therefore, it is not intended to ensure the effectiveness or morality of xenotransplantation itself, or to prevent infectious diseases in general in recipients. In addition, guidelines on clinical research, such as the “Guidelines on Clinical Research of Gene Therapy (Announcement of the Ministry of Health and Welfare No. 23, February, 1994) should also be applied, as appropriate.

1. General rules

1.1 Objective

The objective is to prevent infection and expansion of emerging infectious diseases caused by xenotransplantation from the public health viewpoint. Measures that are not indicated in the present guidelines could be adopted, only if it is shown to be more scientifically appropriate than the indicated ones from the viewpoint of preventing infectious diseases.

1.2 Definition

1.2.1 Xenotransplantation

(1) For the purpose of the present guidelines, xenotransplantation is any procedure that involves transplantation, implantation, or infusion into a human recipient of either

a. Live cells, tissues, or organs from a non-human animal source, or
b. Human body fluids, cells, tissues or organs that have had ex vivo contact with live non-human animal cells, tissues, or organs (contact includes indirect contact by co-culture)
(2) Therefore, it is not called xenotransplantation to use non-live materials or medicines, such as cardiac valves, insulin, and serum albumin in humans, even if they are derived from non-human animals.

1.2.2 Xenografts
Xenografts include live cells, tissues or organs derived from a non-human animal source transplanted, implanted, or infused into a human recipient, or having contact with human body fluids, cells, tissues, or organs in xenotransplantation.

1.2.3 Donor animals
Donor animals refer to animals donating xenografts.

1.2.4 Donor screening
Donor screening is to qualify a donor animal by diagnosis and examination to determine whether or not it is appropriate as a donor.

1.2.5 Microbiological monitoring
It means a continuous examination to determine the presence of infectious agents in donor animals, recipients or medical professionals by appropriate measures such as serological tests.

1.2.6 Medical Records
Medical Records include documents showing the health condition and results of microbiological monitoring before and after xenotransplantation in recipients.

1.2.7 Health control records
Health control records include records of the origin of an individual, population or colony (including breeding), breed, history of medicines, breeding condition, results of microbiological monitoring (including inspection) and other tests of donor animals.

1.3 Basic principles

1.3.1 Prerequisites for xenotransplantation

Although allotransplantation in which human cells, tissues, or organs are transplanted to patients, has already been established in clinical settings, the demand far exceeds the supply. The fact has driven the development of xenotransplantation studies.

Since the use of xenotransplantation raises public health concerns regarding the potential infection or transmission of agents derived form donor animals to recipients with current medical techniques, however, it should be assumed to implement sufficient measures against infectious diseases including surveillance.

1.3.2 Relationship with Pharmaceutical Affairs Law

It is necessary to comply with Pharmaceutical Affairs Law, if applicable. For aspects that are not subject to it, see the present guidelines.

1.3.3 Gene Therapy

Among transplantation of xenografts introducing genes, clinical research on gene therapy should comply with the “Guidelines for Clinical Research on Gene Therapy (Announcement of the Ministry of Health and Welfare No. 23, February, 1994). See also the present guidelines to prevent infection or transmission of infectious diseases derived from donor animals.

1.3.4 Other guidelines

Guidelines other than the Pharmaceutical Affairs Law and the Guidelines for Clinical Research on Gene Therapy should be complied with, if applicable.

1.3.5 Protection of personal information

Individuals who are involved with xenotransplantation should not disclose personal information of recipients acquired in the process of transplantation even after they have performed their duties.

1.4 Revision of the guidelines

The present guidelines should be revised in accordance with scientific development, changes of social situation concerning xenografts and other conditions, as necessary.

2. Implementation and review system of xenotransplantation

2.1 Xenotransplantation team

2.1.1 Duties of a xenotransplantation team

It is responsible for developing a xenotransplantation protocol, implementing xenotransplantation according to it under the control of the director, and conducting post-transplantation works such as surveillance.

2.1.2 Composition of a xenotransplantation team

Sufficient expertise and technique are required to detect infectious agents in donor animals and recipients in xenotransplantation. Therefore, it should be conducted by a team comprised of an attendant doctor and several specialists (hereinafter referred to as “a Team”). A Team should meet with the following conditions.

(1) To function under the director who administers the Team

(2) To include a doctor in charge of the xenotransplantation operation

(3) To include the following specialists

a. Infectious disease specialist who is familiar with zoonotic infection and microbiology

b. Veterinarian who is familiar with animal science of the donor animals and infectious diseases (especially zoonotic infection)

c. Specialist on in-hospital infection or anti-infection

d. Specialist on clinical microbiological tests

2.2 Director

A director is responsible for the following.

(1) To develop and submit a xenotransplantation protocol to the manager of the medical facility implementing xenotransplantation (hereinafter referred to as “a Facility”) for approval

(2) To obtain an approval from the manager of the Facility for revisions of a xenotransplantation protocol

(3) To control a Team, and to give instructions to the specialists, as necessary

(4) To report to the manager of the Facility as necessary

2.3 Manager of a xenotransplantation Facility

A manager of a Facility is responsible for the following.

(1) To establish an IRB to judge whether xenotransplantation can be conducted or not.

(2) To give instructions to the director based on comments from the IRB when the director asks for an approval for implementation or revision of xenotransplantation, and to judge the appropriateness of xenotransplantation based on the protocol reflecting the instructions. If the IRB judges that the implementation is not acceptable, it should not be approved.
(3) To inform the director of the judgement on acceptability in writing
(4) To give instructions to the director if necessary after receiving reports from the director, and to report to the Minister of Health, Labour, and Welfare
(5) To keep essential records including IRB's records and samples appropriately, and to cooperate with the public health authorities by disclosing them in response to its request.

2.4 Institutional Review Board
2.4.1 Duties of an IRB
To review and comment on ethical aspects, measures against infection from recipients to their intimate contacts, and from donor animals to recipients, and control of donor animals, when the Facility manager seeks advice on the implementation of xenotransplantation.

2.4.2 Establishment of an IRB
The following review board can replace an IRB established by a Facility manager.
(1) A review board established by a Facility manager in cooperation with a manager of other medical facilities
(2) A review board established by a corporation established according to requirements of Article 34 of Civil Law (Law No. 89, 1896).
(3) A review board established by an academic organization, comprised of medical professionals
(4) A review board established by a manager of another medical facility (excluding those listed in (1)).

2.4.3 Composition of an IRB
The following conditions should be met in addition to existing conditions for an IRB for clinical studies.
(1) To include a specialist who can evaluate potential risks of infection in recipients and their intimate contacts (e.g., medical professionals, family

members, friends, and neighbors), in order to discuss measures against infectious diseases among them
(2) To include a specialist who is familiar with microbiology, clinical test diagnostics, epidemiology, and zoonotic infection including risk assessment methods in order to discuss measures against infectious diseases from donor animals to recipients
(3) To include a specialist who is familiar with epidemiological matters concerning conditions for donor animal control including the frequency of screening and inspection in order to inspect donor animals
(4) Member researchers of a xenotransplantation team should not be included in IRB members
(5) Appropriate management procedures should be established to ensure the freedom and independence of its activities. Rules for procedures required for a review process including member composition, organization, management and publication should be established and kept transparent.

2.5 Xenotransplantation facility
A Facility should comply with the following conditions.
(1) A Facility should be positively cooperative with laboratories that can identify agents derived from a human or non-human animal source that do not exist in a normal condition.
(2) A Facility should be able to care for recipients, and to store and control samples even after xenotransplantation.
(3) A Facility should have experiences, expertise and equipment of allotransplantation related to xenotransplantation in question, if appropriate.

3. Contents and review of a xenotransplantation protocol
3.1 Contents of a xenotransplantation protocol
A xenotransplantation protocol should include the following.
(1) Quality control of donor animal population and colony (See 4.1, 4.2, 4.3, and 4.4)
(2) Quality control and screening of individual donor animals (See 4.1, 4.2, 4.3, and 4.5)
(3) Collection and preparation of xenografts (See 4.6)
(4) Screening of xenografts (See4.6)
(5) How to obtain informed consent from recipients and its contents (See3.3)
(6) Follow-up and monitoring of recipients (See 5.1)

(7) Sufficient explanation to recipients' intimate contacts (See 3.3, 5.2)
(8) Anti-infection measures in a Facility (See 5.3)
(9) Records and samples of donor animals and recipients (See 4.7, 5.4)

3.2 Review of a xenotransplantation protocol
A xenotransplantation protocol should be submitted to the IRB for reviewing, after internal reviewing by all members of the Team followed by an approval from the Facility manager.

3.3 How to obtain informed consent from recipients and its contents
3.3.1 How to obtain informed consent
In xenotransplantation, full explanation on expected medical benefits and risk, medical records and control of recipients, and protection of personal information should be given to recipients based on the Helsinki Declaration (Revised in Edinburgh, October, 2000), using written materials including those listed in the next section and the present guidelines, and written informed consent on the implementation and related matters should be obtained.

3.3.2 Contents of informed consent
Explanation to recipients should include the following concerning infectious diseases derived from non-human animal sources
(1) Possible infection of agents that are shown to be derived from donor animals
(2) Possible infection of emerging agents derived from non-human species, and the fact that the risks, occurrence timing, and symptoms are unpredictable.
(3) It cannot be denied that agents derived from non-human animals are transmissible to family members or sexual partners who have contact with recipients.
(4) Information on prevention of infection, for example, risks of infection to recipients' contacts listed in (3) above can be reduced by preventive measures such as barrier devices against body fluids in sex, and may increase in infants and children, pregnant women, aged, and patients with chronic diseases or under immunosuppressive condition.
(5) Recipients have responsibility to give sufficient explanation to their contacts on the possibility of infection of agents derived from non-human

animals.

Explanation to recipients' contacts should include the following. A Facility should prepare explanation materials for recipients to explain it.

a. Risks of infection of agents derived from non-human animals are unclear

b. Information on and measures to minimize the risks of behavior with possible infection of agents from human to human (contact with blood or body fluids including unprotected sex, breast feeding, drug infusions with shared needles)

c. Symptoms due to unknown causes in recipients or their contacts should immediately be reported to the attendant doctor of the Facility.

(6) Necessity of quarantine in the period of admission (expected quarantine period) and special cautions after discharge (e.g., diet and/or travel)

(7) Care should be paid to minimize potential biological risk factors between donor animals and recipients after discharge.[1]

(8) Since long-term microbiological monitoring is required, recipients should take regular and irregular examinations by collecting tissues and/or serum as necessary. In addition, visiting schedules after xenotransplantation, including tests aimed at microbiological monitoring, should be made as clear as possible. Visiting schedules should be determined after discussion by the Team, and recipients should be notified in advance.

If there are serious diseases or diseases for unknown reasons in recipients or their contacts, they should be reported to the attending doctor of the Facility.

(9) Collected samples and medical records should be kept for 50 years after xenotransplantation, and used for diagnosis, studies, and searching for causes of infectious diseases.

(10) Recipients should defer from donation of whole blood, serum, blood cells, bone marrow fluid, cord blood, organs, tissues, milk, ovum, sperm, and other components for use in humans.

(11) It cannot be denied that infectious diseases derived from non-human animals can occur in the offspring from conception through development, delivery and breast feeding.

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[1] For example, if donor animals and recipients come into contact with each other, xenografts or recipients have increasingly been exposed to infectious agents of the donor animals. At the same time, for other healthy donor animals, recipients with xenografts can become vectors if infected.

(12) For long-term health control, recipients should inform the attendant doctor of the Facility of changes of addresses or telephone numbers.
(13) Postmortem examination should be conducted to collect and store organs to be used for studies and seeking for the causes of infectious diseases. The necessity of autopsy should be explained to family members.
(14) All medical records should be disclosed to related public health organizations (the Ministry of Health, Labour, and Welfare, National Institutes of Infectious Diseases, health care centers, interested medical research facilities). It should be noted that privacy of recipients should be protected.
(15) Even if xenografts are rejected or extracted, all of the above items should be applied.

4. Donor Animals

In immunosuppressive patients after receiving xenotransplantation, not only existing zoonotic agents, but also indigenous microorganisms, either commensal or parasitic, of donor animals could cause diseases. Therefore, agents in xenografts should be thoroughly examined, but it is technically difficult to conduct thorough examinations of xenografts in themselves. Thus, it is essential to minimize risks of transmission of infectious agents to recipients by implementing the following in order to prevent public health infection.

4.1 Requirements for donor animals

4.1.1 Principles

Xenografts should be obtained from animals with the identified origin that are bred in closed environment.
Animals should be derived from closed population or colonies without infectious agents with potential risks of transmission to donor animals or recipients as much as possible.
Additionally, only individuals that are surely suitable for xenotransplantation should be selected among those derived from population or colonies bred in well-controlled closed environment.

4.1.2 Selection of species

In selecting species, microbiological characteristics by species including endogenous retrovirus should be considered.

4.1.3 Cautions

From the aforementioned viewpoints, the following should be considered.

(1) Wild animals or animals having lived in the open air should not be used as donors.

(2) Imported animals or their next generation can be used as donors only if they cannot be obtained domestically. Imported animals can be used only when the characteristics and regulations as donors have been met and are certified by the breeding center.

(3) Species for which prion diseases (e.g., transmittable spongeform encephalopathy) have been reported can be used as donors only when they are certified to be derived from a closed population without cranial nerve disorders and fed with well-controlled food. For those species, cells, tissues, or organs of animals originated from nations where prion diseases have been reported or likely to occur, should not be used as xenografts.

(4) Live cells, tissues or organs of animals obtained from slaughter houses should not be used as xenografts because they are not bred in a closed environment and health control records cannot generally be obtained.

(5) At present, non-human primates are not suitable as donors because of microbiological uncertainties.

(6) Animals bred in open environments (e.g., range-fed animals) should not be used as donors because they could be infected with infectious agents by occasional contact with arthropods or other living things.

(7) Vaccinated animals should not be used as donors because they may confuse results of microbiological monitoring such as antibody tests. Also, it is because vaccination is necessary when quality control of population or colonies is not appropriate.

(8) For species in which technology for generating and maintaining individual animals that are delivered by Caesarian section and bred in isolators without having contact with extrinsic agents (germ-free animals), or individual animals derived from colonies originated from those kinds of animals and bred in closed environments (SPF animals) has been established, such animals should be used.

4.2 Breeding facility

4.2.1 Requirements for a breeding facility

A facility that breeds donor animals (hereinafter referred to as “a Breeding Facility”) should meet the following conditions.

(1) A Breeding Facility should comply with “the Law Concerning Animal Protection and Control (Law No. 105, 1973)” and “Requirements in Breeding and Keeping Experimental Animals (Announcement of the Prime Minister’s Office No. 6, 1980).”
(2) A Breeding Facility should be inspected by an audit committee comprised of appropriate members of a Team (hereinafter referred to as “an Audit Committee”).
(3) A Breeding Facility should have a standard operating procedure (SOP), a written registry of animal health control, and microbiological monitoring system.
(4) A Breeding Facility should have a veterinarian who is familiar with infectious diseases of the species, and maintain positive cooperative system with a microbiological laboratory.

4.2.2 Standard operating procedure
(1) A SOP of a Breeding Facility should include details on the following matters.
a. Standards for introduction of animals
Except for animals delivered in a Breeding Facility, all animals to be introduced into breeding colonies should be quarantined in predefined quarantine units for a certain period. From the viewpoint of appropriate breeding reproduction, measures, such as artificial fertilization, embryo transplanting, early weaning, hysterotomy, hysterectomy, adoptive parents can be applied to minimize risks of infectious agents.
b. Standards for diseases and microbiological monitoring
For microbiological monitoring, appropriate measures should be adopted. See 4.3, 4.4 and 4.5.
c. Standards for quarantine and exclusion of affected animals
d. Standards for physical examination and microbiological monitoring of those who are introduced into a Breeding Facility
e. Control measures of access to a Breeding Facility
f. Cleaning methods of a Breeding Facility
g. Origin and supply of food, water, equipment and medicines
All food ingredients including medicines and additives should be recorded at least from the previous generation of donor animals. Pasteurized milk can be used. A description indicating that no animal-derived materials (except for pasteurized milk) are used in food is of special importance, because it is

essential in preventing prion-related diseases, the latent expression of viral infections and infections of agents. Since prion infections are characteristic for a prolonged clinical incubation period, seriousness, and a difficulty in early detection with current tests, so it is important to exclude risk factors.
h. Extermination measures against arthropods and other living things
i. Cautions in transporting animals
In-house transportation of animals during final screening and quality control of individual donor animals and harvesting and preparation of xenografts should be conducted by batch or all at once at every stage, rather than one by one, in order to reduce the possibility of contact with infectious agents. Areas for quarantine and graft preparation in a Breeding Facility should be washed and sterilized after shipping a batch of animals before accepting the next batch.
j. Disposition of Corps
k. Recording methods of supplied donor animals
A recording system of the supply of donor animals, that contains individual animals to be supplied, the type and amount of cells, tissues or organs transformed into xenografts, and names of xenotransplantation facilities to accept them should be maintained.
l. Range of stored samples and storing methods
m. Records to be kept and storing methods
The following records should be kept, and identification methods, such as a numbering system to link each piece of information, should be implemented for easy, accurate and immediate access to those records.
(a) Health control records of supplied animals from birth
(b) Health control records of animal population (including food records)
(c) Records of standard operating procedures in collecting and preparing xenografts from animals or transporting them
(2) If a Breeding Facility stops xenotransplantation related works, they should transfer samples and animal health control records defined in l and m of (1) to an appropriate Facility or a facility that succeeds storing duties.

4.3 Concept of donor screening
Screening methods for existing infectious agents in original populations of donor animals, individual donor animals and xenografts should meet the following conditions or even stricter conditions should be adopted.

4.3.1 Non-clinical studies in the development of xenografts

In developing clinical treatment using xenografts, non-clinical studies should be conducted. In non-clinical studies, identification of microorganism in xenografts should be specific to the donor species. Also, it should be reviewed as to whether or not the identified microorganism could cause diseases in human. It is of special importance to identify the pathogenicity of endogenous retrovirus or durable viral infections existing in cells, tissues or organs of donor animals for human beings. Screening methods suitable for quality control of xenografts for clinical use should be defined by those non-clinical studies.

See attachment 1 for swine agents to be considered in determining screening methods at present. Tests could be simplified only with evidence.

4.3.2 Revision of test methods

Since a Team is responsible for appropriate screening methods, test methods of existing infectious agents in animal population or colonies, individual donor animals and xenografts should be established by donor species and clinical use, and revised in accordance with the advancement of knowledge of infectious diseases, as necessary.

4.3.3 Evaluation of test methods before adoption

All the test methods to detect infectious agents in animal populations or colonies, individual animals or xenografts should be sufficiently verified for their sensitivity, specificity, and accuracy. Test methods under development should be evaluated for the results of supplemental screening.

4.3.4 Specific test methods

In non-clinical studies, samples from xenografts should be co-cultured with marker cells that can detect a series of appropriate virus including human peripheral blood mononuclear cells in order to attempt proliferation and detection of endogenous retrovirus and other viruses derived from non-human animals with the possibility of infection. The series of marker cells in co-culture should be determined based on xenografts and their clinical use. For example, for xenotransplantation related with human CNS, co-culture of samples collected from xenografts with the cell strain that is likely to proliferate neurotropic virus to detect virus in question, randomized subculture, observation of cell disorder and focal formation, determination of

reverse transcriptase activities and observation by electron microscope are considered to be suitable. In addition to those culture, immunological or gene engineering methods (e.g., immunoenzymatic technique, immunofluorescent antibody method, the southern blot method, the PCR method, and the RT-PCR method), or in vivo culture techniques using related species are efficient. It is sometimes easier to detect masked virus through activation by chemicals or radiation. For detection of potential bacteria, an established PCR primer can be used in screening of xenografts.

4.4 Quality control of animal populations or colonies

4.4.1 Basic factors in quality control

Basic factors in quality control of animal population or colonies as origin of donor animals for xenotransplantation include:

(1) Closed population or colonies

(2) There is an appropriate microbiological monitoring program for infectious agents.

Health control and microbiological monitoring program of animal population or colonies should be specific to its objective, and stated clearly in the SOPs of a Breeding Facility. The Audit Committee or a corresponding organization should approve those procedures. Health control records of animal population or colonies and individual animals supplied for xenotransplantation should be kept for 50 years from the implementation.

4.4.2 Control records

In a Breeding Facility, health control of animal population or colonies should be conducted and recorded based on veterinary medicine (e.g., extermination of parasites) using standard methods including biologically-clean methods using sterilized equipment in conducting parental treatment, such as blood collection and biopsy. In addition, events that could affect health of animal populations or colonies should be recorded (e.g., destruction of environmental maintenance device, occurrence of diseases, and /or the sudden death of animals in the facility). The details of screening test schedules should especially be described because they are necessary for the translation of test results of serum responses in screening.

4.4.3 Agent test schedule

In addition to standard veterinary control, invasion of infectious agents that

express no clinical symptoms in animal populations or colonies should be monitored. The SOP should contain monitoring schedules including physical observations, and types and schedules of clinical tests to detect infectious agents.

4.4.4 Infectious diseases to be tested

In conducting tests of animal populations or colonies, attention should be paid to zoonotic diseases that have been shown to exist in the same domestic species. For agents that are not found domestically, or found only in wild animals, a broad range of tests are not necessary for individual donor animals if tests or maintenance of closed populations or colonies in the Breeding Facility are appropriate. The geographic origin of populations or colonies is valuable information when considering the potential existence of agents in the populations or colonies in question. For local infectious agents pandemic in the origin or breeding location of donor animals, it is necessary to consult a veterinarian with sufficient knowledge.

Attention should be paid to the following aspects in testing.

(1) Serum samples should be collected regularly from animals extracted randomly from populations or colonies as a part of the microbiological monitoring program. Those samples should be tested for infectious agents that can affect the species or for agents to which the species can be exposed. If clinical symptoms are observed in individual animals, further serological tests or agent-separation culture tests should be conducted. Even if only one individual is affected among a population, the remaining animals should be tested clinically and epidemiologically.

In preparation for unexpected events in populations, colonies or individual donor animals, recipients and their contacts, serum samples should be kept in a Breeding Facility for 50 years after the xenotransplantation.

(2) If agents causing acute infections are detected, the animals should be excluded from population or colonies, and tested, while biological monitoring scheme should be established to monitor the presence of infections in parent populations for the period over the incubation period of the agents for the populations or colonies.

(3) For death with undefined causes, including stillbirth and abortion, sufficient tests including postmortem examinations should be conducted to determine whether it is caused by an infection or not, and the results should be recorded.

(4) Some of the animals in populations or colonies should be bred until their death for the purpose of monitoring. Life-long observation of these animals would expand the possibility for detecting diseases, such as prion diseases that can cause infection with masked or latent expression with unclear clinical symptoms.

4.5 Quality control and screening of individual animals

For quality control of individual animals, health control records should contain breed, origin, and history of medicines. During the inspection period, donor animals should be screened for every infectious agent that can affect clinical application of xenografts. Specifically, the following requirements should be met.

4.5.1 Inspection period

Before collecting xenografts, at least a 3-week inspection period should be set for individual animals. That is because acute diseases, caused by infectious agents to which donor animals are exposed just before shipping from populations or colonies, are thought to develop in this period. The period should be changed according to the features of the donor animals' populations or colonies, results of microbiological monitoring, and clinical emergency, as appropriate. If the period is shortened, the reason should be described in documents, and potential increase of infection risks should be stated on informed consent forms.

Cautions during the inspection period include:

(1) In order to identify the presence of infectious agents (bacteria, fungus, parasite, or virus), potential donor animals should have serological tests, agent separation culture tests, blood cell count tests, peripheral blood smear tests, and fecal examination for parasites conducted by veterinarians. For viral agents that have reportedly been transmitted in vivo or in vitro to cells of humans or non-human primates, careful attention should be paid, even if they are not recognized as being zoonotic. Cautions should also be taken for those whose pseudotyping has been shown by recombination, complementation, or replacing envelops by those of viruses that can affect different types of cells. Those tests should be conducted immediately before xenotransplantation, or as soon as possible, and the results should be obtained at least before clinical use.

(2) When agents causing acute infectious diseases are detected, the animals

should not be used as donors. For populations or colonies from which the animals are derived, inspections should be conducted for a longer period than the incubation period of the agents, and a microbiological monitoring scheme should be established to monitor the presence of infections in the parent populations.
(3) More than 3 months after the first screening and quality control (e.g., when the planned graft is not used, or when the second graft is harvested from the same donor), or when potential donors have come into contact with non-inspected animals between the time of inspection and harvesting of the xenograft, screening of the potential donor animals should be conducted again.
(4) Transportation of donor animals can damage non-infection condition secured in closed colonies. In transporting donor animals, full attention should be paid to minimize exposure to agents in order to secure microbiological quarantine of donor animals. After the arrival of donor animals, long-term inspection (at least 3 weeks, which can be shortened with appropriate reasons), and strict screening should be conducted. It is recommended that xenografts should be harvested and prepared in a Breeding Facility and shipped as final products.

4.5.2 Securing abacterial xenografts
All the prepared xenografts should be as abacterial as possible. Donor animals in which bacteria (e.g., virus) are detected in the incubation period should not be used. However, animals in which bacteria are detected in anatomically specific organs, such as the bronchial pathways or gastrointestinal tracts, can be used if it is shown that the agent is absent in the xenografts.

4.5.3 Access to and storing of records
Health control records of individuals, populations or colonies of donor animals should be available to the Audit Committee. Health control records of individuals and populations or colonies of donor animals should be accurately referenced to each other. Those records should be accessible before selection of donors, and collection and preparation of xenografts, and stored for 50 years after xenotransplantation for follow-back studies. A copy of records for individual donor animals should accompany xenographs and be stored for 50 years after xenotransplantation as a part of the medical records of recipients.

4.5.4 Measures in response to the detection of agents after extraction of xenografts

If infectious agents are detected in individual animals or their parent population after supplying xenografts (e.g., latent expression of prion diseases in animals for monitoring), the Breeding Facility should inform the Facility manager immediately.

4.6 Collection, preparation and screening of xenografts

4.6.1 Areas for collection and preparation

Necessary measures should be adopted for collection and preparation of xenografts under the sterile conditions to minimize contamination. For example, facilities and equipment required for that work should be separated from other areas.

In order to minimize risks of mix-up or infection of bacteria, fungus, or virus, it should be avoided to deal with several xenografts derived from different donor animals at the same time in the same room, or to adopt a storing method with potential risks of cross-contamination.

4.6.2 Screening

Biopsies of xenografts should be conducted so as not to damage their functions. Appropriate screening and histologic tests related to infections should be conducted before xenotransplantation, and the results should be recorded. All the results should be verified by the manager of the Breeding Facility before clinical use of xenografts.

4.6.3 Development of standard operating procedures

SOPs should be developed for every operation in collection, preparation and screening. They should be evaluated by preliminary operation in order to verify operations of harvesting, preparations, and accuracy control of screening.

4.6.4 Temporary storing of xenografts before xenotransplantation

When xenografts are temporarily stored by pretransplantation culture, maintenance of sterile conditions should be verified by screening tests including identification of viruses and mycoplasmas. If contamination of grafts by agents is suspected, and the suspicion cannot be excluded, the

xenografts should not be used for transplantation.

4.6.5 Donor animals after harvesting of xenografts

For animals from which xenografts are harvested, general autopsies including macro- and micro-pathological tests, and microbiological tests should be conducted. When xenografts are obtained while donor animals survive, the animals should be monitored throughout their life. At the time of death, including simple deaths, a general autopsy of the animals should be conducted, regardless of the period between the harvesting and preparation of grafts and the death. The autopsy results should be recorded in the health control records of the animals, and stored for 50 years after xenotransplantation. If an infection that may be related with the recipients' health is observed in an autopsy (e.g., prion diseases), all managers of Facilities from which the xenografts were harvested should be informed immediately.

4.7 Records and samples of donor animals

Health control records (especially on tests) and samples of donor animals are essential for public health surveillance in the occurrence of infection derived from non-human animals and microbiological monitoring. The following conditions should be met, and medical records of recipients and health control records and samples of donor animals should be systematically stored for immediate and accurate reference.

4.7.1 Storing manager

A Breeding Facility should be responsible for storing records and samples, and designate a manager responsible for controlling and handling them in the xenotransplantation protocol. When storing spaces are changed because of abolition of the Breeding Facility or other reasons, a new manager should be appointed with the approval of the Facility manager for appropriate storing.

4.7.2 Keeping records

Health control records of origin populations or colonies, health-control records of individual donor animals, and screening records of xenografts should be stored for 50 years after xenotransplantation.

Summaries of health control records of individual donor animals and copies of screening records of xenografts should be stored in a Facility as a part of

medical records of recipients.

4.7.3 Storing samples
For retro-analytical public health studies, samples of donor animals collected in the process of collection and preparation of xenografts should be stored for 50 years after xenotransplantation. Stored samples of donor animals should be available at any time, and easily referenced to health control records of donor animals and medical records of recipients.

4.7.4 Samples to be stored
For serum and plasma of individual donor animals, 5 vials of 0.5 cc aliquot should be stored. For white blood cells, 3 samples of at least 1×10^7cells should be frozen and stored. DNA and RNA should be extracted from white blood cells, and separated and stored, if possible. In addition, major organs (e.g., kidneys, livers, bone marrow, and central nerve systems) wrapped in wax paper or preserved in formalin, or frozen tissue samples should be prepared from donor animals in the process of harvesting and preparation of xenografts.

4.8 Other requirements
For equipment of facilities or quality control, see also “Regulations for Facilities and Equipment including Pharmacy (Regulations of the Ministry of Health and Welfare No. 2, 1961),” “Regulations for Production Control and Quality Control of Medicines and Quasidrugs (Regulations of the Ministry of Health and Welfare No. 16, 1999),” “Regulations for Production Control and Quality Control of Medical Devices (Regulations of the Ministry of Health and Welfare No. 40, 1995)” in addition to the above requirements.

5. Anti-infection measures after xenotransplantation
5.1 Recipients
5.1.1 Microbiological monitoring of recipients
Microbiological monitoring of recipients after xenotransplantation is essential in monitoring infection or genetic transmission of infectious agents derived from non-human animals to the public at large. A Facility manager is responsible for implementing and recording microbiological monitoring, and it should be continued for the rest of recipients’ life. Appropriate monitoring methods should include:

(1) Agents possibly related to infectious diseases derived from non-human animals should be regularly examined after xenotransplantation.
(2) Appropriate types and volume of test samples should be reviewed, collected and stored according to the types of xenotransplantation for retro-analytical examination of infectious diseases derived from non-human animals. It should be stated that they are used for public health studies.

In general, serum, plasma and peripheral mononuclear cells are collected and stored. 3-5 aliquots of 0.5cc coagulation-protected plasma with citrate acid or EDTA are stored at the following time points. In addition, at least 2 samples of live white blood cells (approximately 1×10^7 cells) should be frozen and stored. DNA and RNA extracted from white blood cells (approximately 1×10^7 cells) are separated and stored. Tissue samples of collected xenografts (e.g., after xenograft rejection or a recipient has deceased) should be stored.

a. Twice with one-month interval before xenotransplantation–if impossible, it should be collected with as long an interval as possible.
b. Just after xenotransplantation, and within one and six months
c. Within one and two years after xenotransplantation
d. Then it should be collected every 5 years as long as recipients survive. If necessary, more frequent collection should be conducted based on the xenotransplantation protocol or the clinical process of recipients.

(3) When recipients decease, samples including quick frozen solid samples, paraffin-wrapped samples, and samples for electromicroscopy should at least be collected from xenografts during the autopsies. In addition, samples of major organs related to clinical symptoms causing death should be collected. Those samples should be stored for 50 years from xenotransplantation for public health studies. Organs to be collected should be determined by the Team based on the cause of death.
(4) Since a Facility manager is responsible for continuous and accurate storing of records and samples, he/she should store them using appropriate devices (e.g., freezer with an alarm, storing divided samples in separate freezers) to enable immediate retrieval of and reference to the medical records of recipients and the data from donor animals.
(5) When infectious agents derived from non-human animals are detected or suspected in xenografts before or after xenotransplantation by non-clinical or other studies, clinical examination of recipients should be conducted according to a microbiological monitoring program. The aim of the

examination is to detect masked infection in recipients before transmission to the public at large. Infectious agents derived from non-human animals that are detected in transplanted tissues should be regularly examined after xenotransplantation for serum, peripheral blood mononuclear cells and/or tissues of recipients. Although microbioligical monitoring should be frequently conducted just after the operation (e.g., at postoperative 2, 4 and 6 weeks), the frequency can be reduced if no clinical symptoms are observed. Gene detection is also valuable to detect emerging agents. Additional tests should be conducted to detect virus-causing, persistent, masked infection without clinical symptoms (e.g., herpes virus and retrovirus). If viruses equivalent to those derived from non-human animals exist in humans, test methods that can distinguish them should be adopted. When serological tests are unreliable, because of immunosupressive conditions in some recipients, appropriate test methods in combination with co-culture with cells should be considered. Sensitivity, specificity and accuracy of test methods that are planned in xenotransplantation should be evaluated in advance and recorded in the xenotransplantation protocol.

(6) To prevent infections derived from non-human animals that are expected to derive from clinical symptoms, epidemioligical studies should be conducted to evaluate public health significance simultaneously with tests of stored samples.

(7) When regular tests cannot be conducted in a Facility for some reason, they should be arranged in another medical facility. The successor should be comparable to the original facility for testing, sample storing, and anti-infection measures after xenotransplantation including in-house conditions.

5.2 Recipients' intimate contacts

As described in 3.3.2(5), recipients should give full explanation of infection to their contacts. The Facility should help the explanation adequately.

5.3 Anti-infection measures in a xenotransplantation facility

5.3.1 Anti-infection methods

(1) To ensure that medical professionals carry out standard anti-infection measures such as appropriate hand washing, protection by barrier, and cautions in using and disposing needles and sharp devices.

(2) Preventive quarantine (e.g., blockade of air transmission, droplets, and physical contact) should be conducted based on the judgement of infection

specialists of a Facility and a Team. Since preventive quarantine during hospitalization depends on the type of xenotransplantation, the extent of immunosuppression, and clinical condition of a recipient, anti-infection measures should be reviewed before xenotransplantation and at the time of deterioration, discharge, readmission, or establishing the diagnosis of infection. Preventive quarantine should be continued until suspected infection derived from non-human animals is identified and cured, or suspicion is excluded.
(3) A Team should develop a written procedure for usage of medical devices, disinfecting and sterilizing methods and disposal of infectious wastes based on full consideration of characteristics of xenotransplantation and comply with it to the letter.

5.3.2 Procedures in response to acute infection
Agents are not often identified in acute transmissible viral infections. Recipients of xenotransplantations are exposed to risks of acute viral infections, and they are often observed in recipients of allotransplantation as well. From this viewpoint, if causes of the pathologic condition of recipients are not identified by standard diagnostic methods, body fluid or tissue samples should be further examined. An infection specialist in a Facility should be responsible for diagnosis and appropriate prevention of infection, based on consultation with the Team, epidemiology specialists, veterinarians, and clinical microbiologists, while paying attention to the following matters.
(1) In recipients under immunosuppressive therapy, infection sometimes cannot be identified by serological tests for detection of antibodies. In this case, other techniques such as co-culture tests and gene detection tests can be available for diagnosis. Therefore, a Facility should be equipped with devices for *in vitro*, *in vivo*, and *ex vivo* detection of viral agents. For emerging agents derived from non-human animals, detection should be conducted based on consultation with infection specialists both in medicine and veterinary medicine, tests of emerging infectious agents, and the biological safety of the virus in question.
(2) When infection derived from non-human animals, that cannot be expected in allotransplantation, or that can become a public health concern, is suspected in recipients, serum samples both in the acute and recovery phases should be stored based on the judgement of infection specialists or in-house epidemiology specialists. This will allow retro-analytical studies to

diagnose causes of clinical symptoms.
(3) In the case of (2) above, the Director should inform the Facility manager.

5.3.3 Health professionals
A health-control plan should be developed for educating medical professionals on risks in xenotransplantation and microbiological monitoring of infection. If a safety control is conducted according to safety control standards adopted by a Breeding Facility, animal hospital, or slaughter house, based on rules of National Personnel Authority 10-4 (health and security of employees) complying with the Industrial Safety and Health Law and the National Civil Service Law, infection risks to medical professionals handling tissues and organs of animals before transplantation will not exceed those of the past. However, the risks to medical professionals who diagnose or care for recipients either directly or indirectly cannot be stipulated. A Facility manager should decide the limitation of work and allocation of employees with reduced immunity. Health control planning should contain the following.
(1) Education of medical professionals
Every Facility should develop appropriate teaching materials for employees according to their duty. Those materials should state xenotransplantation processes and risks of existing and emerging infection related to the processes. In order to minimize exposure and transmission of zoonotic agents and in-house infection agents among recipients and medical professionals, medical activities with potential high risks should especially be stressed. Standard preventive measures should also be stated. Usage of personal barrier devices (e.g., gloves, gowns, and masks) should be encouraged and promoted, and it should also be stated that even if wearing gloves, it is important to wash hands before and after any contacts with recipients. Risks of transmission of infectious agents to the public at large should also be mentioned.
(2) Microbiological monitoring of medical professionals
Control serum (i.e., before contact with xenografts or recipients) collected from medical professionals of a Team, those who diagnose or care for recipients, technicians who handle xenografts or living samples of recipients after xenotransplantation should be stored. Stored serum is used for comparison with those collected when exposed to infectious agents.
(3) Evaluation and control of medical professionals after exposure to infectious agents

Records of exposure to infectious agents of medical professionals, including events such as needle sticking, should be kept. Medical professionals should be instructed to inform the Team's infection specialists of the occurrence of exposure to infectious agents immediately. The records should contain the date, types of exposure, related xenotransplantation processes, recipients' information, post-exposure measures (e.g., counseling, post-exposure control, follow-up), and the successive processes. The records should be stored for 50 years after xenotransplantation even if the person in question leaves the facility or the facility ceases xenotransplantation.

Medical professionals, who are exposed to agents, should report unexpected clinical events to infection specialists of the Team for medical evaluation, and be prepared to accept appropriate instructions. Then the Team should give the appropriate instructions and control after reviewing such reports.

5.4 Records of recipients and others

5.4.1 Storing records

A Facility manager should keep the following records for 50 years after xenotransplantation. Those records should be updated and be available for accurate cross-reference. Systematic maintenance of data is valuable in epidemiologic searching for causes of adverse events.

(1) Xenotransplantation records stating all xenotransplantation processes

It should contain information on the manager, individual donor animals and their breeding, collecting and preparing facilities, dates and methods of xenotransplantation, summary of recipients and their clinical history, recipients' contacts, and medical professionals involved in individual operations.

(2) Records of exposure to agents

It should contain dates, people concerned, and situation of all exposure events with potential risks of infection derived from non-human animals, related with the xenotransplantation protocol.

(3) Medical records of recipients

Medical records should contain copies of health control records of individual donor animals stipulated in 4.5.3.

5.4.2 Changes of a storing facility

If the aforementioned records cannot be stored in a Facility for some reason,

another facility should be arranged to store them. The successor should be comparable to the original Facility for storing records.

6. Public health control

6.1 Reporting system

6.1.1 Reports on implementation

Recognizing that international organizations propose the necessity of a surveillance system in response to existing and emerging infection derived from non-human animals that can possibly occur in xenotransplantation, public health information required to prevent expansion of infection should be supplied to the national authority when xenotransplantation is implemented.

(1) A Facility manager should report it.

(2) It should be reported within 7 days from the xenotransplantation.

(3) It should be reported to the Research and Development Division, Health Policy Bureau, Ministry of Health, Labour and Welfare.

(4) Contents of reports

a. The name, address and correspondence of the Facility, and the name of the Team director

b. The sex and age of the recipient

c. The name of the disease for which the xenotransplantation was required, the species of the donor animal, and the kind of transplanted cells, tissues and/or organs

d. The name of and correspondence with the facility keeping records of a recipient and a donor animal and samples (changes should be reported immediately)

6.1.2 Report of infection

When infection derived from non-human animals that cannot be expected in allotransplantation, or that can become a public health problem is suspected, diagnosis and appropriate preventive measures should be adopted and immediately reported to the division mentioned in 6.1.1(3) above.

If a report is also required in the Law Concerning Prevention of Infection and Medical Care for Infected Patients (Law No. 114, 1998), it should be reported according to the requirements.

6.1.3 Protection of privacy in reporting

Attention should be paid to protect privacy of recipients as much as possible. Reported details should not be disclosed to the public, as long as no exposure to the risks exists.

6.2 Reference on samples and other items
Records and samples defined in each item should be filed for immediate verification in response to queries from the Ministry of Health, Labour and Welfare, Health Care Centers, or other organizations.

Attachment 1: Onions D., Cooper DK, Yamanouchi K. et. al. An approach to the control of disease transmission in pig-to-human xenotransplantation. Xenotransplantation 7(2), 143-55(2000)

Lists of agents for which the risk of transmission from donor pigs to humans should be excluded (Zoonoses are underlined)
[Virus]
porcine parvovirus, porcine herpesvirus, African swine cholera virus, swinepox virus, porcine enterovirus, swine vesicular disease virus, swine vesicular rash virus, vesicular stomatitis virus, swine cholera virus, Japanese B encephalitis virus, porcine transmissible gastroenteritis virus, swine influenza virus, foot-and-mouth disease virus, encephalomyocarditis virus, rabies virus, porcine adenovirus, astrovirus, getah virus, porcine reproductive and respiratory syndrome virus, swine epidemic diarrhea virus, Leon virus, swine cytomegalovirus, hemagglutinating encephalomyelitis virus, swine respiratory coronavirus, porcine rubella virus, calicivirus, swine lymphotrophic herpesvirus, swine hepatitis E virus, Menang virus, Nipa virus, Hantavirus, Eastern and Western equine encephalomyelitis virus, Venezuelan equine encephalitis virus, Borna virus, Aboi virus, polyoma virus, swine endogenous retrovirus
[Bacteria]
Yersinia, Bordetella bronchiseptica, clostridium, tuberculosis (Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium), Salmonella, E. coli, Bacillus anthracis, Erysipelothrix rhusiopathiae, Pasteurella, swine dysentery bacillus, Haemophilus, staphylococcus, Brucella, Haemophilus paragallinarum, Mycoplasma, Listeria, Actinobacillus, Streptococcus, Pseudomonas aeruginosa, swine actinomyces, Actinobacillus, campylobacteriosis, Chlamydia, Coxiella, Lawsonia, Leptospira,

Eperythrozoon suis

[Fungi]
fungi, dermatophyte including Trichophyton

[Protozoan]
toxoplasma, coccidium, balantidium, cryptosporidium, Sarcocystis, babesia, trypanosomatid, swine ascarid, Toxocara, Echinococcosis, red Trichostrongylus, fasciola Echinorhynchus, metastrongylus elongatus, Taenia solium, armed tapeworm, ciliate, Trichostrongylus, swine whipworm, other ectoparasite

[microorganism that can possibly remain in strictly controlled miniature pigs including those introduced from abroad]
swine cytomegalovirus, swine ganmaherpesvirus, porcine circovirus, swine endogenous retrovirus 1.2, swine spumavirus

Note 1: The present list shows agents that should be considered in determining screening methods at present. Tests on listed agents can be neglected only with evidence.
Note 2: It is necessary to review agents listed in the present list in order to protect safety of recipients. It also is of significance in case a serious unexpected infection occurs in recipients. Especially for an unexpected infection, exclusion of the infection risks by review and tests of the listed agents in advance will lead to determine that the infection in question is likely to be an emerging one, and be helpful to immediately decide the appropriate public health responses.

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