

Terrestrial Animal Health Standards Commission Report

September 2009

CHAPTER 4.10.

**COLLECTION AND PROCESSING OF LABORATORY
RODENT AND RABBIT EMBRYOS / OVA**

Article 4.10.1.

Conditions applicable to the maintenance of laboratory animal colonies

Maintenance of laboratory animal colonies of specific genotypes requires intensive breeding management within specialised premises. They may be kept in a gnotobiotic environment, in either a 'germfree' system or a 'barrier' room (usually with defined flora), in a conventional colony, or under undefined conditions. In both the germfree and barrier systems, the animals are raised in a controlled environment according to protocols that attempt to eliminate potential sources of microbiological contamination. The primary difference is that the barrier maintained animals have been inoculated with known (defined) microbes¹ using a cocktail of non-pathogenic flora, whereas germfree animals are kept free from both pathogenic and non pathogenic microbes.

A second category is where laboratory animals are kept in closed, conventional colonies within which known pathogens may exist. Here, less rigid colony management protocols are used to control potential sources of contamination, but implementation of simple aseptic precautions (e.g. autoclaving of feed and bedding) should allow animals to be maintained in a microbiologically defined system. Finally, laboratory animals may live in environments with undefined microbiological conditions (e.g. non restricted colonies, free ranging animals).

Disease testing and donor animal/embryo handling requirements can therefore be considered as being of three distinct types, depending on the type of colony being dealt with, i.e. defined floral, conventional and undefined. The health status of all colonies should be confirmed quarterly by bacteriological, virological, parasitological, serological and immunohistochemical tests on pre designated sentinel animals or other representative animals of the colony (e.g. older breeding males which have sired multiple litters).

Microbial status of laboratory animal colonies

Colonies of the various species and genotypes of laboratory animals are usually kept within specialised premises and their microbial status depends largely on the system whereby the colony was formed and is maintained. In this Chapter the microbial status of colonies is considered to be of three main types: 'defined', 'conventional' and 'undefined'. Colonies of defined status are those where, at least initially, the animals are totally free of pathogenic and non-pathogenic micro-organisms (i.e. gnotobiotic), although sometimes a cocktail of known, non-pathogenic micro-organisms has been given subsequently. In either case defined colonies are kept in highly controlled environments in barrier maintained rooms, with strict protocols in place to exclude all potential sources of unwanted microbiological contamination. Colonies of conventional status are those where the animals are kept in closed colonies but where known ("specific") pathogens as well as non-pathogenic micro-organisms may exist. While management protocols for conventional colonies may be less rigid than those for defined colonies, they are designed to control potential sources of microbial

contamination. Simple aseptic precautions (e.g. the autoclaving of food and bedding) are taken to ensure that the animals do not become infected with any unwanted microflora. Finally, laboratory animals may be kept in microbiologically undefined colonies which are unrestricted and may include free ranging animals. Details of these different types of colony can be found in the FELASA Report¹.

The health status of defined and conventional colonies should be confirmed at least quarterly by bacteriological, virological, parasitological, serological and other tests on pre-designated sentinel animals or other representative members of the colony. Older breeding males which have sired multiple litters are often selected for this purpose.

The purpose of official sanitary control of laboratory rodent and rabbit embryos intended for movement internationally is to ensure that specific pathogenic micro-organisms, which could be associated with such embryos, are controlled and transmission of *infection* to recipient animals, progeny and colonies, is avoided. Requirements for the management of donors and processing of embryos vary depending on the microbial status of the colony, i.e. whether it is defined (including gnotobiotic), conventional, or undefined.

Article 4.10.2.

Conditions applicable to the embryo ~~production~~ collection team/~~laboratory~~

The embryo collection team is a group of competent technicians including at least one experienced professional to perform the collection, processing and storage of embryos/oocytes.

The following conditions should apply:

1. The embryo ~~production~~ team ~~must~~ should be ~~composed of competent technicians~~ supervised by an experienced embryologist team professional holding a graduate academic degree (e.g. M.S., Ph.D., D.V.M.).
2. The team professional is responsible for all team operations which include verification of colony and donor health status, sanitary handling and surgery of donors, *disinfection* and hygienic procedures. The team professional should be responsible to the institute *veterinarian*.
3. The institute *veterinarian* should be certified or accredited in laboratory animal care and should be specifically *approved* for the purpose of embryo collection for export. It is the responsibility of the institute *veterinarian* to ensure that required health profiling procedures appropriate for the colony status are implemented. He/she is responsible for certifying that the embryo handling procedures and laboratory facilities conform to the requirements laid down in this Chapter.
24. Team personnel should be adequately trained in the techniques and principles of *disease* control and in the use of aseptic techniques in embryo handling. ~~Laboratory sanitary procedures must conform with requirements in the IETS Manual²~~The zoonotic potential of specific pathogens affecting the various laboratory animal species should be identified and understood so as to avoid contamination of colonies via human vectors, and vice versa.
35. The embryo ~~production~~ team ~~must~~ should use all necessary precautions to protect the ~~animals, animal facilities, laboratory and equipment~~ against microbiological contamination. In particular, the zoonotic potential of specific pathogens should be identified and understood by staff members to avoid contamination of colonies via human vectors, or vice versa. High standards of hygiene should be practiced to preclude the introduction of *infection* to the donor animals, colonies, facilities, and equipment.

Restrictions should be established to prevent free access of personnel into the embryo collection and handling laboratory facilities especially after their exposure such personnel have been exposed to other animal facilities.

6. The team should have adequate facilities and equipment for:
 - a) collecting embryos;
 - b) processing and treatment of embryos at a permanent **site** or mobile laboratory;
 - c) storing embryos.

4. Proper records must ~~should~~ be maintained for inspection by the chief embryologist (i.e. supervisor).

~~Until standardised record sheets are developed for laboratory animals, it is the responsibility of each laboratory to maintain complete animal and embryo records (i.e. embryo collection, cryopreservation data). Information of the type shown in standard IETS record sheets² for livestock species should be incorporated, where applicable, and data such as embryo quality grading system, morphological stage at cryopreservation and genotypic identification of the donors should be clearly given in the records.~~

57. It is the responsibility of the ~~chief embryologist (i.e. laboratory supervisor)~~ *institute veterinarian* to ensure that ~~the complete animal and embryos are properly stored in sterile, sealed containers (e.g. ampules or straws)~~ records, including records of collection, processing and storage of embryos are maintained. In addition, the containers must be correctly identified using a standard format which includes embryo species/genotype, cryopreservation date, number and stage of embryos, container number and indication of any specialised procedure (e.g. *in vitro* fertilisation, micromanipulation) or condition (e.g. germfree, microbiologically defined) Record sheets of the type shown in the IETS Manual² for livestock species should be used where applicable, and data such as genotypic identification of the donors, embryo quality grading, morphological stage and should be given. If appropriate the embryo collection team should keep a record of its activities which should be maintained for inspection by the *Veterinary Authority* for at least 2 years after the embryos have been exported.
8. The embryo collection team, if involved in the *export* of embryos, should be subject to regular inspection, preferably annually, by an *Official Veterinarian* to ensure compliance with procedures for the sanitary collection, processing and storage of embryos.

Article 4.10.2bis.

Conditions applicable to the processing laboratory

A processing laboratory used by the embryo collection team is a facility in which embryos are recovered from donors (or from their excised reproductive tracts), and from the collection media. Here also the embryos are examined and subjected to any required treatments such as washing, cryopreservation for storage and quarantine pending results of any diagnostic procedures. The processing laboratory may be part of a specifically designed collection and processing unit, or a suitably adapted part of an existing building. It may be on the premises where the donor animals are kept but in this case should be physically separated from animals.

Additionally:

1. The processing laboratory should be under the supervision of the institute veterinarian and be inspected by an Official Veterinarian.
2. While embryos for export are being handled prior to their storage in ampoules, vials or straws, no embryos of lesser health status should be processed.
3. The processing laboratory should be constructed with materials which permit its effective cleansing and disinfection. This should be done frequently, and always before and after each occasion on which embryos for export are processed.

Article 4.10.2tris.

Risk management

With regard to disease transmission, transfer of *in vivo* derived embryos is a very low risk method for moving the genetic material of laboratory animals. Irrespective of animal species, there are three phases in the embryo transfer process that determine the final level of risk:

1. The first phase comprises the risk potential for embryo contamination and depends on:
 - a) the disease situation in the exporting country and/or zone;
 - b) the microbial status of the colony (i.e. defined, conventional or undefined) and the donors from which the embryos are collected;
 - c) the pathogenic characteristics of the specified disease agents that are of concern to the Veterinary Authority of the importing country.
2. The second phase covers risk mitigation by use of internationally accepted procedures for processing of embryos which are set out in the IETS Manual². These include the following:
 - a) Depending on microbial status of the colony, the embryos should be washed up to ten times with at least 100-fold dilutions between each wash, with a fresh pipette being used for transferring the embryos through each wash.
 - b) Only embryos from the same donor should be washed together, and no more than ten embryos should be washed at any one time.
 - c) Sometimes, for example when removal of certain viruses (e.g. herpesviruses) is required, the standard washing procedure should be modified to include additional washes with the enzyme trypsin, as described in the IETS Manual².
 - d) The zona pellucida of each embryo, after washing, should be examined over its entire surface area at not less than 50X magnification to ensure that it is intact and (apart from the mucin layer in the case of rabbit embryos) free of adherent material.
3. The third phase, which is applicable to diseases of concern to the Veterinary Authority of the importing country, encompasses risk mitigation resulting from:

- a) post-collection surveillance of the microbial status of the donor colony based on the recognized incubation periods of the diseases of concern to determine retrospectively the health status of the colony whilst the embryos are stored (in species where effective storage by cryopreservation is possible) in the exporting country;
- b) post-mortem testing of the donor(s) or other samples such as blood, embryo-collection (flushing) fluids and non-viable embryos, in a laboratory for presence of specified disease agents.

Article 4.10.3.

Conditions applicable to the embryo team/institute veterinarian

1. ~~The veterinarian, certified in laboratory animal care or laboratory animal accredited, must ensure that the required colony health profiling procedures are implemented, and the results are reviewed and properly recorded before shipment of embryos. He/she is also responsible for confirming that proper animal management/sanitation conditions have been maintained~~ It is the responsibility of the institute veterinarian to ensure that required health testing procedures are implemented to demonstrate microbial status of the colony (i.e. defined, conventional or undefined). Colony microbial status should be reviewed by the institute veterinarian before shipment of the embryos.
2. The veterinarian is responsible for certifying that the embryo handling procedures and laboratory conditions were maintained in accordance with ~~the IETS Manual² Articles 4.10.2. and 4.10.2bis.~~
3. ~~The veterinarian must supervise all quarantine practices to protect against unwanted contamination and spread of disease, and to ensure that valid results are generated~~ is responsible for the risk management procedures outlined in Article 4.10.2tris.
4. ~~The veterinarian must~~ should authorise all embryo shipments, ensuring that the correct embryo collection records and veterinary certification documents ~~and embryo collection records are~~ have been completed and are included in the shipments.

~~Article 4.10.4.~~

Test programmes for donor animals

~~Sentinel animals in each donor colony should be subjected to routine monthly microbial screening. Testing for specific pathogens is species dependent and will undoubtedly also be influenced by geographic location. Recommendations regarding specific microbial agents to be tested for in mice, rats, cotton rats, hamsters, guinea pigs, gerbils and rabbits have been published elsewhere³.~~

Article 4.10.5.

Conditions applicable to ~~the embryo/animal handling~~ donors from animal colonies of different microbial status

It should be noted that the conditions applicable to donor animals vary according to the microbial status of the colony from which they originate, i.e. defined, conventional or undefined.

Sentinel animals in each donor colony of defined and conventional status should be subjected to routine microbial screening, preferably monthly, but at least quarterly. Testing for specific pathogens depends on the animal species and may be influenced by geographical location. Recommendations regarding specific microbial agents to be tested for in different laboratory animal species have been published elsewhere.

1. Defined microbial conditions status

- a) ~~Germfree and m~~Microbiologically defined colonies (Article 4.10.1.), ~~barrier maintained animals~~ represent the cleanest sources of gametes, and the embryos recovered from these animals can be regarded as pathogen free.
- b) Since the ~~animals themselves~~ male and female donors are pathogen free ~~or possess defined flora~~ (usually based on random, monthly testing of sentinel animals), dissection of the female reproductive tract and embryo ~~isolation~~ collection procedures ~~can~~ should be performed under aseptic laboratory conditions, ~~and do not require the use of~~ using a biological safety cabinet if appropriate.
- c) ~~Strict aseptic procedures should nevertheless be followed and, while embryo washing is not essential to safeguard against any possible air-borne contamination in the laboratory, it is recommended that embryos undergo at least a 3-step washing procedure. In each wash, embryos should be gently agitated in the medium, and the wash volume must constitute at least a one hundred fold dilution of the volume in which the embryos are transferred~~ Embryo washed as described in point 2 of Article 4.10.2tris is not necessary but it is recommended that embryos are washed 2 or 3 times. In each wash, embryos should be gently agitated in the medium.
- d) ~~Microbial testing of flush or washing media is not required.~~
- ed) ~~Cryopreserved embryos should be designated, in the appropriate records,~~ The embryos should be recorded as coming from a germfree or microbiologically defined, barrier maintained colony, thus indicating that ~~additional safeguards~~ special risk management procedures (Article 4.10.2tris.) for pathogen removal are not necessary. ~~Isolation and health status monitoring of~~ The need to quarantine the embryo recipients ~~should be considered but the need to quarantine them is a decision~~ is a matter for the importing laboratory institute.

2. Conventional conditions

- a) ~~Animals maintained under these conditions generally represent closed colonies whose~~ Colonies of conventional microbial status are usually closed and their health status is routinely profiled monitored (Article 4.10.1.). ~~They~~ The animals may have been exposed to various pathogens, resulting in infection ~~the isolation of infectious agents,~~ with positive antibody titres or even active clinical disease. ~~However, prior to embryo collection there should be familiarity with~~ but the pathogen(s) of ~~particular~~ concern in each individual ~~the colony should be well known.~~
- b) Reproductive tracts (uteri, oviducts and/or ovaries) should be removed at a separate site and then taken into the embryo processing laboratory. These procedures should be performed by ~~separate~~ different technicians or, at the ~~very~~ minimum, their protective clothing should be changed between locations. If the animals ~~are to~~ should be handled in the laboratory, the tracts should be dissected out within a biological safety cabinet. This will help protect against the possible shedding of pathogens into the laboratory itself.
- c) Once the reproductive tracts have been removed, embryo recovery should be performed under aseptic conditions. ~~Embryos must be inspected (>100x) for the presence of cracks in the zona pellucida and only zona-intact embryos should be kept. They must then be washed using the standard 10-step procedure, described~~ Depending on which, if any, pathogens are known to occur in the colony, embryos should be processed according to the risk management procedures, including

washing, as described in Article 4.10.2tris, and in the IETS Manual². This recommendation could be waived in the future if sufficient research evidence from embryo pathogen interaction studies warranted it.

- d) Embryos derived from animals that have positive antibody titres or other evidence of specific pathogens should only be transferred into a new colony via a *quarantine* system, using microbiologically defined recipient females. As an additional safeguard, *Quarantine* may also be appropriate if there is any uncertainty about the donor or disease status of the embryos, quarantining of recipients should be applied the microbial status of the donor colony or the donors. In certain situations where the embryos might could have been exposed to bacterial infection (e.g. *mycoplasma*), they should be cultured in a medium containing an appropriate antibiotic for 24 h pre-freezing, or post-thawing and prior to transfer before cryopreservation, or in the interval between thawing and transfer into recipients.
- e) ~~If the embryos were not handled in the recommended manner, this must be indicated on the shipment records, and mandatory quarantining of the recipient dam and offspring should be imposed by the recipient institution until their health status is confirmed. The recipient dam should then be tested post-weaning for pathogens, and introduction of the progeny into the colony should only take place if test results are satisfactory.~~ If the recipient institution does decide to *quarantine* the recipient dam and offspring until their health status is confirmed, the recipients should be tested post-weaning for pathogens of concern, and introduction of offspring into the colony should only take place if the test results are satisfactory.

3. Undefined microbial conditions

- a) ~~These animals are derived from either the wild~~ Embryos from free ranging animals or from colonies of unknown health status ~~and embryos from them require maximum precautions~~ the full range of risk management procedures that are described in Article 4.10.2tris and in the IETS Manual². The health status of breeder males and donor females should be determined The procedures resemble those used for embryos of livestock as recommended in Chapter 4.7. and Chapter 4.8. of this *Terrestrial Code*. Ideally, the breeder males and donor females should be separated from other animals and tested 15 days before and on the day of breeding (for males) or at embryo collection (for females). Alternatively, the animals could be incorporated into a conventional colony, where, over time, a health history can be documented to reduce the strict monitoring and embryo handling requirements.
- b) ~~A~~ Biological safety cabinet should be used for all animal, tissue and embryo handling donors and reproductive tissues, and for processing embryos.
- c) Post-mortem testing of the donor females for *diseases* or pathogens of concern to the *importing country* may be appropriate after the embryos/oocytes have been collected. Alternatively if embryos are collected surgically ~~A~~ an aliquot of flush fluid from each donor, or a pooled sample, should be tested for the presence of specific pathogens of concern to the *importing country* and laboratory.
- d) Embryos must be washed at least 10 times in accordance with the protocols in the IETS Manual² ~~(i.e. the 10-step wash, possibly including trypsin treatment in the case of certain herpesviruses) and an aliquot of media from the last four (pooled) washes should be tested for pathogens~~ trypsin treatment should be used if presence of certain pathogenic herpesviruses is of concern.

- e) Cryopreserved embryos must be stored in the exporting laboratory until such time as the necessary disease screening of colonies, tissues and or fluids is completed and the certification supporting documents for certification completed and signed by the institute veterinarian.
- f) On arrival in the importing country the All embryos from these animals must should be transferred into a colony via recipients in a quarantine system, as discussed above. Recipients should be tested at intervals appropriate to recognized incubation periods of the diseases of concern. In addition to testing the recipients dam after transfer, all the offspring should be tested at 12 weeks of age and/or individuals from successive generations should be tested before their introduction into breeding colonies outside the quarantine facility.

Article 4.10.5.bis.

Conditions applicable to the storage and transport of embryos

1. Embryos for export should be frozen in fresh liquid nitrogen and then stored in fresh liquid nitrogen in cleaned and disinfected tanks or containers.
2. The embryos should be stored in sealed sterile ampoules, vials or straws under strict hygienic conditions at a storage place approved by the Veterinary Authority of the exporting country. Only embryos from the same donor should be stored together in the same ampoule, vial or straw.
3. Ampoules, vials or straws should be sealed at the time of freezing (or prior to export where cryopreservation is not possible) and they should be clearly identified according to or similar to the system recommended in the IETS Manual². Identification should include details of the species/genotype of the donors, microbial status (e.g. defined, conventional or undefined), collection/cryopreservation date, number and developmental stage of the embryos, container number and details of any specialized procedure such as *in vitro* fertilization, micromanipulation.
4. Liquid nitrogen storage containers should be sealed under the supervision of the Official Veterinarian prior to shipment from the exporting country.
5. Embryos should not be exported until the appropriate veterinary certificates are completed.

Article 4.10.6.

Special experimental circumstances Procedures for *in vitro* fertilization and micromanipulation

If embryos are to be cryopreserved following specialised produced by *in vitro* fertilization of oocytes, it is advised that the washed sperm should be used so as to minimize the risk of possible pathogen exposure. If embryos are to undergo micromanipulation procedures that involve penetration of the zona pellucida, they must undergo the required washing steps (depending on colony status) before treatment. In the case of *in vitro* fertilisation, to minimise possible pathogen exposure, it is also advised that only washed sperm should be used. Embryos should be washed again before cryopreservation any required risk management steps (including washing) should be carried out first, as described in Chapter 4.9.