Terrestrial Animal Health Standards Commission Report

October 2008

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) microbes[1] 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).

Article 4.10.2.

Conditions applicable to the embryo production team/laboratory

1. The embryo production team ~~must~~ should be composed of competent technicians supervised by an experienced ~~embryologist~~ professional ~~holding a graduate academic degree (e.g. M.S., Ph.D., D.V.M.)~~.

2. Team personnel should be trained in the principles of *disease* control and the use of aseptic techniques in embryo handling. Laboratory sanitary procedures must conform with requirements in the IETS Manual[2].

3.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. Restrictions should be established to prevent free access of personnel into the embryo handling laboratory after their exposure to other animal facilities.

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 sheets[2] 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.

5. It is the responsibility of the ~~chief embryologist (i.e.~~ laboratory supervisor~~)~~ to ensure that the embryos are properly stored in sterile, sealed containers (e.g. ampules or straws). 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).

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.

2. The veterinarian is responsible for certifying that the embryo handling procedures and laboratory conditions were maintained in accordance with the IETS Manual[2].

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.

4. The veterinarian must authorise all embryo shipments, ensuring that the correct veterinary certification documents and embryo collection records are completed and 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 elsewhere[3].

Article 4.10.5.

Conditions applicable to the embryo/animal handling

1. Defined microbial conditions

   a) Germfree and microbiologically defined, barrier maintained animals represent the cleanest sources of gametes, and the embryos recovered from these can be regarded as pathogen free.

   b) Since the animals themselves are pathogen free or possess defined flora (usually based on random, monthly testing of sentinel animals), dissection of the reproductive tract and embryo isolation procedures can be performed under aseptic laboratory conditions, and do not require the use of a biological safety cabinet.

   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.

   d) Microbial testing of flush or washing media is not required.

   e) Cryopreserved embryos should be designated, in the appropriate records, as coming from a germfree or microbiologically defined, barrier maintained colony, thus indicating that additional safeguards for pathogen removal are not necessary. Isolation and health status monitoring of the embryo recipients should be considered but the need to quarantine them is a decision for the importing laboratory.

2. Conventional conditions

   a) Animals maintained under these conditions generally represent closed colonies whose health status is routinely ~~profiled~~ monitored. They may have been exposed to various pathogens, resulting in the isolation of infectious agents, positive antibody titres or even active clinical *disease*. However, prior to embryo collection there should be familiarity with the pathogen(s) of particular concern in the colony.

   b) Reproductive tracts (uteri, oviducts and/or ovaries) should be removed at a separate site and then taken into the embryo laboratory. These procedures should be performed by separate technicians or, at the very minimum, their protective clothing should be changed between locations. If the animals are to 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 in the IETS Manual[2]. ~~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, if there is any uncertainty about the donor or disease status of the embryos, quarantining of recipients should be applied. In certain situations where embryos might 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.

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.

3. Undefined microbial conditions

a) These animals are derived from either the wild or from colonies of unknown health status and embryos from them require maximum precautions. The health status of breeder males and donor females should be determined 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.

c) 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 in accordance with the protocols in the IETS Manual[2] (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.

e) Cryopreserved embryos must be stored in the exporting laboratory until such time as the necessary *disease* screening of tissues and fluids is completed. All embryos from these animals must be transferred into a colony via a quarantine system, as discussed above. In addition to testing the recipient dam, all 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 4.10.6.

Special experimental circumstances

If embryos are to be cryopreserved following specialised 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.

1 **Recommendations for the health monitoring of mouse, rat, hamster, guineapig and rabbit breeding colonies**.- Report of the Federation of European Laboratory Animal Science Associations (FELASA), Working Group on Animal Health accepted by the FELASA Board of Management, November 1992.

2 Manual of the International Embryo Transfer Society ~~(1998)~~.

3 Schiewe M.C., Hollifield V.M., Kasbohm L.A. & Schmidt P.M. (1995) - Embryo importation and cryobanking strategies for laboratory animals and wildlife species. *Theriogenology*, **43**, 97-104.