Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units

Recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) Working Group on Health Monitoring of Rodent and Rabbit Colonies accepted by the FELASA Board of Management, 9 June 2001


Contents

1 Preamble 20
2 General considerations 21
3 Risk of introducing unwanted microorganisms 22
4 Frequency of monitoring and sample size 23
5 Test methods and samples 26
6 Health monitoring: agents to be monitored 27
7 Reporting test results 28
8 References 29
9 Appendices
   Appendix 1: Some points to consider when monitoring animals from experimental units or various housing systems 31
   Appendix 2: Comments on agents 32
   Appendix 3: Health monitoring reports 38

1 Preamble

These recommendations are primarily intended to standardize health monitoring programmes and reporting. In this way they may also help to standardize the microbiological quality of animals. However, it is not a requirement of these recommendations that animals tested are free from all of the microorganisms listed.

Health monitoring is a complex issue. Therefore, it is recommended that a person with sufficient understanding of the principles of health monitoring (FELASA Category D, Nevalainen et al. 1999) be identified as the individual responsible for devising and maintaining a health monitoring policy for the facility.

It should be noted that health monitoring is not confined to laboratory reporting. There should also be engendered a culture of communication between animal technicians, facility managers, veterinarians and researchers so that observed abnormalities in breeding animals and experimental data can rapidly be evaluated and appropriate action taken.

Animals that are standardized as much as possible are important prerequisites for reproducible animal experiments.
Microbiological standardization aims to produce animals that meet preset requirements of microbiological quality, and to aid in the maintenance of this quality during experiments. Health monitoring is therefore an integrated part of any quality assurance system, e.g. good laboratory practice (GLP), the accreditation programme of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) (www.aaalac.org), or the International Standards Organization (ISO). In addition to infections (Bhatt et al. 1986, Lussier 1988, Nicklas et al. 1999), other exogenous (environmental) and genetic factors and their interactions may influence the suitability of an animal for research.

Outbreaks of infectious diseases in animals occur from time to time and emphasize the need to consider the microbiological quality of the animals concerned. Several groups of microorganisms (viruses, mycoplasmas, bacteria, fungi, and parasites) are responsible for infections in rodents and rabbits. Most infections do not lead to overt clinical symptoms (disease), and may be latent. Thus, an absence of clinical manifestations of infection has only limited diagnostic value. However, these latent infections can have a considerable impact upon the outcome of animal experiments. There are numerous examples of the influences of microorganisms on the physiology of the laboratory animal and hence of the interference of latent infections on the results of animal experiments (behaviour, growth rate, relative organ weight, immune response) (Nicklas et al. 1999). All infections, apparent or inapparent, are likely to increase biological variability and hence result in an increase in animal use. Infection in animals can also lead to contamination of biological materials such as transplantable tumours and other tissues, cell lines and sera (Nicklas et al. 1993) and may also lead to contamination of animals. Some of the microorganisms that may be present in laboratory animals can also infect humans (zoonoses). For all these reasons, it is of vital importance that each institution establishes a laboratory animal health monitoring programme.

This report proposes a scheme for health monitoring of laboratory animal breeding and experimental colonies, with the intention of harmonizing procedures primarily among countries associated with FELASA, but also worldwide. The use of the recommendations will be facilitated by a basic knowledge of microbiological standardization and diseases of laboratory animals, and we therefore recommend the following texts relevant to these subjects (National Research Council 1991, Boot et al. 1993, van Herck et al. 1993, Weisbroth et al. 1998, Percy & Barthold 2001).

The present recommendations replace previous FELASA recommendations for the health monitoring of breeding and experimental colonies of rodents and rabbits (Kraft et al. 1994, Rehbinder et al. 1996).

This document is aimed at all breeders and users of laboratory animals (animal facility managers, veterinarians and scientists using animals for experimental purposes).

These recommendations will be under periodical review and amendments will be published as necessary (www.felasa.org).

2 General considerations

These recommendations constitute a common approach for health monitoring of laboratory animals and the reporting of results. Actual practice may differ from these recommendations in various ways depending on local circumstances, such as research objectives, local prevalence of specific agents, the existence of national monitoring schemes, regulations related to the production of sera and vaccines (e.g. EU Note for Guidance III 1993, ICH Harmonised Tripartite Guideline 1997). Health monitoring schemes must be tailored to individual and local needs. However, quality aims must be clearly defined and an appropriate system of preventive hygienic measures (e.g. barrier systems) developed to meet those aims. Finally, a health monitoring programme should be established in every facility to demonstrate whether the quality aims have been met by monitoring the effectiveness of the preventive measures.
The term ‘unit’ is here understood to describe a self-contained microbiological entity. Space and traffic of personnel and goods essentially separate units. Depending on the actual measures taken and on the professional judgement of the person responsible for the health monitoring programme, a unit might be:

- the total facility;
- various animal rooms within different buildings which are attended by the same group of people (without special preventive measures);
- a classical barrier facility with various rooms (irrespective of how many species or strains are maintained within it);
- an animal room that is protected by preventive measures, such as changing clothing;
- an isolator or isolators between which animals are freely transferred with no special preventive measures, using procedures that are appropriate to the use of isolators;
- an individually ventilated cage (IVC), which is opened only within a laminar flow cabinet using procedures that are appropriate to the use of IVCs.

A breeding unit is here understood as a self-contained microbiological entity in which animals are bred for scientific purposes. This means that only those persons that are involved in housing and breeding animals have access to the unit. On rare occasions animals may be introduced, but only after following strict measures for microbiological security. Only a very few experimental materials (chemicals, drugs, biological materials) are necessary in a breeding unit (e.g. for genetic monitoring).

An experimental unit is here understood as a self-contained microbiological entity in which animals are housed or used for scientific experiments. Usually, introduction of animals from outside sources (commercial breeders, institutional breeding units, experimental units) is necessary. Additional personnel must have access to conduct experiments, and different kinds of experimental materials have to be introduced into an experimental unit. In addition, breeding of laboratory animals might be performed in such a unit.

Preventive measures that reduce the spread of infection between animal rooms, isolators or IVCs may eventually result in splitting a microbiological unit into several units that have to be monitored separately.

Depending on the judgement of the person responsible for health monitoring, the total facility may be considered as multiple units or a single unit. Therefore, different monitoring programmes may be necessary in the same facility.

The cost of preventive measures and health monitoring may seem high, but is very low in relation to the total cost of the research project and is a fully justified means of enhancing the reliability of data generated in animal experiments.

Within the institution, there should be a documented health monitoring policy and a documented policy for the introduction of animals and biological materials (quality system).

Additional investigations may be deemed necessary. Should these indicate the presence of an agent which, although not listed in these recommendations, is suspected of being important, this agent should be mentioned in successive reports and treated as a listed agent.

3 Risk of introducing unwanted microorganisms

The risk of inadvertently introducing microorganisms (viruses, bacteria, fungi and parasites) into breeding units is generally lower than for experimental units. Introduction of unwanted microorganisms is mainly due to one or more of the following factors: animals, biological materials, equipment and staff (Boot et al. 1993, Nicklas 1993).

Animals

Experimental units usually contain various animal species and strains, originating from various sources. It is recommended that animals to be introduced are from sources that follow at least these FELASA health monitoring recommendations. This, however,
may not be possible, for example, in the case of mice of transgenic strains that cannot be obtained from commercial sources. In these cases, **rederivation, quarantine** or other form of risk management of animals from suspect sources should be considered.

**Biological materials**

The use of biological materials such as **cells, sera, ES cells, and sperm derived from animals** may result in the introduction of unwanted agents (Petri 1966, Collins & Parker 1972, Bhatt et al. 1986, Nicklas et al. 1988, Nicklas et al. 1993, Dick et al. 1996, Lipman et al. 2000). It is recommended that biological materials be considered as contaminated and that animal experiments be performed under conditions of strict containment (isolation), unless the biological materials have been tested and found free of contamination.

**Personnel**

The importance of research staff and animal care staff to the microbiological integrity of an animal unit should not be underestimated. Personnel may act as effective carriers of infections from contaminated to non-contaminated units (La Regina et al. 1992, Tietjen 1992). Microorganisms may be carried in the hair, on the hands and on the clothing of personnel who have been in contact with infected animals. It is recommended that facilities establish a quarantine policy for personnel to minimize the risk of them acting as unwitting vectors of infection. Furthermore, it is recommended that a policy for entering animal facilities also be established.

It should be remembered that animals are usually infected and capable of transmitting infection before showing clinical signs and certainly before producing antibodies. Therefore personnel or equipment moving within the unit, i.e. between rooms or other subunits of the whole unit, can act as vectors or the source of an infection before there is any indication of its presence.

Most infections will persist in the unit when susceptible animals are continuously being introduced. The infectious cycle can, however, be interrupted by removing all animals from a unit at the end of experiments and cleaning and disinfecting animal rooms before new animals are admitted (‘all in–all out’ system). If such procedures are applied to short-term experiments (of less than 6 weeks), the risk of spreading the infection is reduced.

4 **Frequency of monitoring and sample size**

Colonies should be monitored at least quarterly. Depending on local circumstances and needs, more frequent monitoring may be carried out for a selection of some frequently occurring agents that have a serious impact on research.

Sick and dead animals should be submitted for necropsy. These animals should be examined in addition to those already scheduled for routine monitoring. The outcome of the necropsy may prompt an increase in the sample size and frequency of monitoring.

As the question of host specificity of infections is not fully understood, in animal (microbiological) units containing more than one animal species, each species must be screened separately, according to the test schedule. Similarly, there may be strain differences in susceptibility to infection and serological response to agents. Therefore, if more than one strain of a species is present, all strains should be screened and each strain should be monitored at least once a year, where possible.

In microbiological units consisting of two or more rooms or subunits, the sample should comprise animals from as many rooms or subunits as possible.

To detect a single infected animal in a population at a defined confidence level, the number of animals examined (the sample size) is inversely proportional to the percentage of uninfected animals (ILAR 1976, Cannon & Roe 1986). To increase the confidence, the sample size needed to detect an infection then increases substantially. The formula is applicable only in populations of at least 100 animals, if the infection is randomly distributed in the unit and if the animals are
randomly sampled (Table 1). The prevalence of an infection may however be dependent on age and sex. Therefore, a sample size of at least 10 animals per microbiological (breeding and experimental) unit is recommended. However, note that infections having a prevalence of less than 30% may not be detected with a 95% confidence level. The detection rate for a given infection depends on the test method employed. Seromonitoring methods often measure higher prevalences than direct methods that detect the presence of (parts of) the microorganism. Using seromonitoring, the level of confidence may therefore be increased by screening the same number of animals.

Due to the higher risk of infection in experimental colonies, smaller numbers of animals are sometimes examined at higher frequency. Theoretically, this procedure will reveal more actual data on the status of a colony and in most cases will help to detect infection earlier, but a decrease in sample size will lead to a decrease in the likelihood of detecting infections with low prevalence (Table 1).

**Sentinel animals**

In some experimental units and colonies of genetically modified or immunodeficient animals, there may be an insufficient number of animals available for health monitoring. It may also be inappropriate to carry out health monitoring in such colonies (for example, serological testing of immunodeficient animals may be misleading). Health monitoring may then be carried out on sentinel animals, which act as surveillance substitutes. However, the use of sentinels may not be covered by the ILAR formula (ILAR 1976) for the sampling of animal colonies.

Sentinel animals must be free from all agents to be monitored; for example when using sentinels to monitor immunodeficient animals, the sentinels must be initially free

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**Table 1 Calculation of the number of animals to be monitored**

Diseases with an infection rate of 50% or more (Sendai, MHV) require far fewer animals to detect their presence than diseases with low infection rates.

**Assumptions**

1. Both sexes are infected at the same rate
2. Population size > 100 animals
3. Random sampling
4. Random distribution of infection

The sample size is calculated from the following formula:

\[
\frac{\log 0.05}{\log \hat{N}} = \text{Sample size}
\]

\[\hat{N} = \text{percentage of non-infected animals}\]

\[0.05 = 95\% \text{ confidence level}\]

**Relation of sample size to prevalence rate**

<table>
<thead>
<tr>
<th>Suspected prevalence rate (%)</th>
<th>95%</th>
<th>99%</th>
<th>99.9%</th>
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<tr>
<td>10</td>
<td>29</td>
<td>44</td>
<td>66</td>
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<td>20</td>
<td>14</td>
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<td>40</td>
<td>6</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
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</table>

Example: 10 animals should be monitored to detect at least one positive animal if the suspected prevalence rate of an infection is 30% (confidence level: 95%)
from *Pneumocystis carinii*. In long-term experiments, sentinels may be housed with the experimental animals from the outset to guarantee that the minimal sample size will be available throughout the whole period of the experiment. Alternatively, sentinels may be introduced periodically to obtain a constant update of current infections. When short-term experiments or experiments in multipurpose units are performed, the unit can be restocked repeatedly. In this case, sentinels removed for monitoring can easily be replaced during restocking with experimental animals from time to time. Sentinel animals, used in an animal room, should be distributed on different cage racks and housed in open cages among the experimental animals for at least 6 weeks. If both stocks are handled similarly, health monitoring data obtained from sentinels will be representative of the microbiological status of all experimental animals of that species held within the unit. Provided that the animals in the general population are in open cages, exposure of sentinels to possible infectious agents might be enhanced by putting them into open cages throughout the unit in locations where possible exposure to infectious agents is known or thought to be maximal. The transmission of infectious agents may be further enhanced by exposing the sentinel animals to soiled bedding, water and feed taken from the cages of the experimental animals, and by exposing sentinel animals directly to experimental animals by placing them in the same cage. Note, however, that some agents, for example Sendai virus (Artwohl et al. 1994) and CAR bacillus (Cundiffe et al. 1995), may not be transmitted successfully using dirty bedding. Immunodeficient strains that are particularly prone to specific infections might be used for detection of some viral, bacterial and protozoal infections. However, immunodeficient animals may not produce an adequate immune response and are therefore unsuitable for serology. It should be noted that animals used in this way may act as enhanced transmitters of infection and may themselves be a hazard to the animals for which they act as sentinels because they may shed pathogenic organisms as a result of their persistent infection.

Preventive measures which reduce the spread of infection between animal rooms within a unit may eventually lead to the creation of different microbiological units that contain so few animals that the ILAR formula (ILAR 1976) is no longer applicable. Similarly, isolators and IVCs may have such small population sizes that sampling according to the ILAR formula (ILAR 1976) is not possible. In such cases, smaller sample sizes (e.g. 3–5 animals per sampling) are recommended if an appropriate sentinel programme is used which leads to an increased probability of agent transmission to sentinel animals. It is difficult to formulate recommendations to cover all of the circumstances in which isolators and IVCs are used. However, some suggestions are given in Appendix 1.

The recommended minimum sampling frequency, age and number of animals to be sampled are summarized in Table 2. It should be noted that animals of other ages might be more appropriate for the detection of specific agents (e.g. < 8 weeks for the detection of *Spiroplasma* sp.). For monitoring of rabbits, samples may be taken that do not involve the killing of animals (e.g. blood or serum samples, swabs from nose, vagina or prepuce, faecal samples) but as this may be less sensitive than testing fresh samples from sacrificed animals, a larger sample size should be chosen.

<table>
<thead>
<tr>
<th>Sampling frequency</th>
<th>Age</th>
<th>No. of animals</th>
<th>Virology</th>
<th>Bacteriology</th>
<th>Parasitology</th>
<th>Pathology</th>
</tr>
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<tbody>
<tr>
<td>Every 3 months</td>
<td>&gt; 8 weeks</td>
<td>10</td>
<td>+</td>
<td>+</td>
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Table 2  Recommended minimum frequency of monitoring and sample size for rodent and rabbit units
5 Test methods and samples

(1) Diagnostic laboratories should follow a quality system which implies, among other requirements, the existence of detailed written procedures. This will be the case if testing is done in compliance with the International Standards Organization (ISO) 9000 series of norms. However, FELASA advocates accreditation of diagnostic laboratories according to ISO 17025 [formerly European Norm (EN) 45001], in which special emphasis is placed on competency of the staff, validation of (in-house) test methods, and participation in inter-laboratory testing programmes (Homberger et al. 1999). Professional competency is of fundamental importance for pre- and post-analytical advice on testing and interpretation of test results. It is therefore recommended that testing be performed under supervision of staff carrying an academic degree in veterinary medicine, medicine, microbiology or equivalent, who have additional experience in laboratory animal diagnostics and laboratory animal science at the level of FELASA category D (Homberger et al. 1999, Nevalainen et al. 1999).

(2) Test methods: the presence of infection in a population can be detected by a variety of direct methods by which the agent or parts of it are detected, and by indirect methods, such as serology in which antibodies to infectious agents are detected. Direct methods are also used in disease diagnostics. The use of a suitable test method does not necessarily imply a reliable test outcome. Experience shows that results obtained from different diagnostic laboratories may vary considerably.

(3) Samples should be taken from randomly selected individual animals or sentinels and not pooled.

(4) Virology: serology is the method of choice for monitoring viral infections in animals, and is also used to test animals that are used in antibody production tests (see Section 6.4). Suitable test methods include the enzyme linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test (IFA) and the haemagglutination inhibition (HI) test. In general, ELISA and IFA are more sensitive than HI and so should be used as primary tests. The specificity of the tests is primarily determined by the antigen chosen and the methods used for antigen preparation (purification etc). ELISA and IFA, for example, measure cross-reacting antibodies to various paroviruses, whereas HI is specific for the virus (e.g. MVM and Toolan’s H-1 virus). The immunoblot technique (Western blot) is not suitable as a test for routine screening. The major drawback is that the technique is labour- and cost-intensive. However, Western blot is highly specific and sensitive and can be used to confirm questionable results. The presence of LDV (the most frequent contaminant of biological material of mouse origin) can be determined by testing mice injected with the material for an increase in the plasma level of lactate dehydrogenase enzyme, or by using a polymerase chain reaction (PCR) test on the material itself.

(5) Bacteriology: bacteria are cultured from samples taken from the upper respiratory tract (nasopharynx, trachea), intestinal tract (cecal contents or faeces) and genitals (prepuce/vagina). As such samples contain numerous non-pathogenic bacteria, selective media should be used in combination with non-selective media whenever possible to facilitate the isolation of the more fastidious bacteria. The culture of some fastidious bacteria requires the use of enriched media. Agar media should be incubated under aerobic conditions. Addition of CO$_2$ or microaerophilic conditions may increase the likelihood of isolating some species. Identification of unwanted bacteria should proceed to the species name, e.g. Corynebacterium kutscheri. In some cases, involvement of specialized reference laboratories should be considered. Commonly used kits for identification of human and veterinary pathogenic bacteria are sometimes not suitable to correctly identify bacterial strains from laboratory animals e.g. Pasteurellaceae and Citrobacter rodentium. Molecular methods (e.g. PCR) may be used for detection and identification.
Cultures techniques are usually used for the detection of most bacterial agents. Serological methods (mainly ELISA and IFA) exist for the detection of antibodies to various bacterial pathogens (Boot 2001) but there is a higher risk of false positive reactions (compared to viruses) due to their complex antigenic structure. Molecular biological methods also exist for the detection of some bacteria.

(6) Parasitology: The pelt should be examined for evidence of ectoparasites. Wet preparations of the large and small intestines and faeces should be examined for evidence of intestinal endoparasites. It should be noted that older animals may be less suitable for microscopic examination because of increased resistance to parasites with age. Identification of parasites should proceed as far as possible to the species name. Serological methods exist for the detection of antibodies to some parasites such as *Encephalitozoon cuniculi*. Serological findings should be confirmed by appropriate alternative test methods.

(7) The choice and preparation of antigen used primarily determines the specificity and the sensitivity of serological tests. The presence of antibodies in animal sera is only an *indicator* of previous or current infection. Positive results should be confirmed by other methods such as culture, PCR, histopathology or another serological method. It is also advised that positive results be confirmed by another laboratory. The results should also be confirmed by repeated testing/sampling from the animal colony. In the case of conflicting results between laboratories, final diagnosis can only be made on the basis of testing by other than serological methods. This is applicable to all groups of agents. Serological tests can differ greatly in sensitivity and specificity. Together with the (sero) prevalence of the infection, both test properties determine the predictive value of a positive and a negative test (Tyler & Cullor 1994).

Further, when a number of sera is subjected to a battery of serological tests, some false positive test results must be expected, even when tests are highly specific e.g. 95\% (Tyler & Cullor 1994, Jacobson & Romatowsky 1996).

(8) Pathology: A full routine necropsy to detect the presence of gross abnormalities should be performed to include examination of: skin, oral cavity, salivary glands (rat only), respiratory system, aorta (rabbit only), heart, liver, spleen, gastrointestinal tract, kidneys, adrenals, urogenital tract (including testes), and lymph nodes. The aetiology of alterations in tissues and organs should be further investigated by histopathology and microbiology, as appropriate. Pathology, including immunohistochemistry and molecular techniques, may be suitable to detect infections.

6 Health monitoring: agents to be monitored

The viruses, bacteria (including mycoplasmas) and parasites to be monitored are listed for each animal species in Appendix 3 (= FELASA Approved Health Monitoring Reports). Rederived and restocked breeding colonies should be monitored at least for the agents listed for the appropriate species. Thereafter, breeding colonies should be tested for the most relevant infections listed at least quarterly. The remaining agents should be monitored at least annually.

A similar monitoring approach is advised for experimental animal colonies in which experiments are continuously performed without application of the so-called ‘all in–all out’ system (at least quarterly).

Monitoring for additional agents and their declaration in a health report is advised under specific circumstances, e.g.

- when associated with lesions;
- when associated with clinical signs of disease;
- when there is evidence of perturbation of physiological parameters or breeding performance;
- when using immunodeficient animals.

Biological material must be evaluated for the presence of relevant agents, including lactate dehydrogenase elevating virus (LDV). This is usually done using mouse, rat or hamster
antibody production tests (MAP, RAP, HAP). Molecular testing may be used as an alternative method. Animals that are to be used in MAP, RAP or HAP tests must be free from all the infections listed in the appendices for which the biological material will be tested. Such tests should be performed under maximal containment conditions (e.g. an isolator) in order to protect other animals in the facility, and to avoid infection of the test animals from other sources.

7 Reporting test results

(1) Health monitoring data should be made available to those researchers using the animals. The data are part of the experimental work and should therefore be evaluated for their influence on the results of experiments, and included in scientific reports and publications as part of the animal specification.

(2) In order to easily compare monitoring reports from different breeders and users, the FELASA approved health monitoring report must be used to present health status information on animals and biological materials. Monitoring reports have been developed for all common species of laboratory rodents and rabbits (Appendix 3).

(3) The health monitoring report of a unit should include the following information:

- Unit designation and description (non-barrier, barrier, IVC, isolator).
- Identification of all species and strains present within the unit for which the report is valid, and the date of issue of the report.
- Positive results of other species held within the same unit should be reported.
- All viruses, mycoplasmas, bacteria, and fungi for which monitoring is recommended (ordered alphabetically) and ecto- and endoparasites identified to the species level.
- Date of latest investigation (per species), method used, designation of antigen used in serology, the name of the testing laboratory.
- Results of latest investigation and 18 months cumulative results of all investigations: number of positive animals/number of animals examined.
- Results of testing not included in the standard health monitoring programme should be added as supplementary information (for example disease diagnoses).
- Results of pathological examinations should be recorded as: Pathological macroscopic lesions were/were not observed in the organs examined.
- Pathological changes should be listed separately for each species and strain.

(4) It should be emphasized that negative results mean only that (antibody activity to) the microorganism monitored has not been demonstrated in the animals screened by the test(s) used. The results are not necessarily a reflection of the status of all the animals in the unit.

(5) An agent must be declared present if it is identified in one or more of the animals screened. Essentially the same is true if antibodies are detected, but positive serological results must have been confirmed (see 5.7).

(6) Agents known to be present need not be monitored at subsequent screens provided that they are declared in the health report. The unit must continue to be reported as positive (at subsequent screens) until the organism has been eradicated, for example by means of rederivation or restocking by animals from another source. Eradication of the infection(s) will be confirmed by subsequent testing according to FELASA recommendations. If the animals have been treated in any way, for example by vaccination, or anthelmintic therapy for pinworm infections, this must be stated on the health monitoring report.

(7) An agent may be considered to be eradicated if all results of monitoring done in accordance with FELASA recommendations (i.e. with appropriate and sensitive methods, representative sampling) during 18 months after the last positive results are negative. This represents at least 6 subsequent screens done quarterly.
8 References

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La Regina M, Woods L, Klender P, Gaertner DJ, Paturzo FX (1992) Transmission of sialodacryoadenitis virus (SDAV) from infected rats to rats and mice through handling, close contact and soiled bedding. Laboratory Animal Science 42, 344–6
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Schauer DB, Zabel BA, Pedraza IF, O’Hara CM, Steigerwalt AG, Brenner DJ (1995) Genetic and biochemical characterization of *Citrobacter rodentium* sp. nov. *Journal of Clinical Microbiology* 33, 2064–8


Tietjen RM (1992) Transmission of minute virus of mice into a rodent colony by a research technician. *Laboratory Animal Science* 42, 422 (abstract)


9 Appendices

Appendix 1 Some points to consider when monitoring animals from experimental units or various housing systems

This appendix is to be used in conjunction with the main document and should not be used as a stand-alone document.

Frequency of monitoring
A similar monitoring programme (frequency of monitoring, sample size) for breeding colonies is advised for experimental animal units if animals are housed in open cages under barrier conditions or conventionally and in units in which animals are introduced only occasionally or where only long-term experiments are performed. More frequent monitoring is necessary if animals or biological materials are frequently introduced into the unit. Infected animals on the site also increase the risk of infection. Monthly or even more frequent monitoring might be advisable in order to obtain reliable information on the actual status. In such cases it is recommended that a minimum of 3–5 animals is a sufficient sample size of animals to be monitored per month. The frequency of monitoring is dependent on the risk of introducing agents (Table 3).

Results of monitoring are presumed to be valid for all animals of the same species within the same unit, independent of the type of experiment.

Sample size
Generally, a sample size of 10 animals per microbiological unit is recommended. In some units of experimental or genetically modified animals (e.g. transgenic breeding), there may be insufficient numbers

Table 3 Some factors that increase the risk of introducing agents into an experimental unit, therefore requiring more frequent monitoring

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<th>High risk:</th>
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<tr>
<td>• Multipurpose units with various kinds of experiments</td>
</tr>
<tr>
<td>• Frequent introduction of animals (&gt; 1 x per month)</td>
</tr>
<tr>
<td>• Frequent entry of research personnel in addition to animal care staff</td>
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<tr>
<td>• Frequent change of personnel working in the unit</td>
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<tr>
<td>• Introduction of animals from different breeding units (from one or several breeders)</td>
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<tr>
<td>• Introduction of biological materials (e.g. sera, tumours, tissues, (ES) cells) originating from the same animal species that are housed in the unit</td>
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<tr>
<td>• Infected animals on the site</td>
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<th>Medium risk:</th>
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<tr>
<td>• Occasional introduction of animals</td>
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<tr>
<td>• One or few types of experiments</td>
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<tr>
<td>• Long-term experiments (only occasional introduction of animals)</td>
</tr>
<tr>
<td>• ‘All in–all out’ system</td>
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<tr>
<td>• Introduction of chemicals only, no biological materials</td>
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of animals available for health monitoring. Serologic monitoring of immunodeficient and many strains of genetically modified animals may yield false-negative results because these animals do not always produce sufficient amounts of antibodies. Often, small populations have to be monitored (e.g. isolator, IVC). In such cases random sampling may not be possible or reasonable (see Table 4). The formula given in the main document (Table 1) is therefore not applicable in many experimental units. In such cases, smaller sample sizes (e.g. 3–5 animals per sampling) together with an increased monitoring frequency are acceptable if an appropriate sentinel programme is used which enhances the probability of agent transmission to sentinel animals.

Animals which show clinical signs unrelated to the experiment should be necropsied and subjected to histopathology and to a microbiologic, parasitologic and serologic examination independent of scheduled testing.

**Monitoring animals from various housing systems**

Conventional or barrier units do not pose a problem for monitoring because a sufficient population size is available. If necessary, sufficient space is usually available for housing sentinels. Space might, however, be limited in filtered cabinets or rooms, isolators or filter top cages (static or individually ventilated cages, IVCs).

If animals housed in filtered cabinets or rooms or in isolators are to be monitored, an efficient sentinel programme (one with appropriate use of soiled bedding and feed) is important for increasing the likeliness of agent transmission to the small number of sentinels. If germ-free or gnotobiotic animals are housed in isolators, monitoring for bacteria (environmental organisms) is more important than monitoring for viruses or parasites due to the higher risk of the former being introduced. Due to space restrictions, only 3–5 animals are usually available for health monitoring of isolator-housed animals.

Reliable information on the infection status in filter top cages or individually ventilated cages (IVCs) is difficult to obtain. If properly handled, every cage represents a microbiological unit, and the system prevents the transmission or spreading of agents between cages. Dirty bedding from as many cages as possible must be placed in a separate ventilated cage in which sentinels are housed. The changing of bedding-donors gives a good insight into the colony status. Other examples of methods for monitoring that may be considered are the use of contact sentinels and the testing of exhaust filters or cage surfaces using PCR.

**Appendix 2 Comments on agents**

These comments have been added because:

- some agents, for which monitoring was recommended earlier, were removed from the list or the frequency of monitoring was changed;
- some new agents have been added.

The information given here should help readers of the recommendations to understand better why monitoring for specific agents is recommended or why changes were made (as compared to previous recommendations). Therefore, very basic information

<table>
<thead>
<tr>
<th>Barriers: breeding</th>
<th>Sufficient No. of animals per unit</th>
<th>Random sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barriers: experiment</td>
<td>Available</td>
<td>Possible</td>
</tr>
<tr>
<td>Isolators</td>
<td>Usually available</td>
<td>Usually not possible</td>
</tr>
<tr>
<td>Filter top cages and IVCs</td>
<td>Usually not available</td>
<td>Usually not possible</td>
</tr>
</tbody>
</table>

IVCs = individually ventilated cages
is given on most agents. A few references are added on recently described agents or agents that are rarely mentioned in the literature.

However, one should realize that much information, for instance, on the impact, the epizootiology, testing, etc. of several agents is controversial. For details, the reader is advised to consult specialists in the field and the scientific literature.

**Bacteria, fungi**

*Bordetella bronchiseptica*: subclinical infection is most frequent in rabbits and occasionally occurs in guineapigs and rats.

*CAR bacillus*: has been implicated in chronic respiratory disease in mice, rats and rabbits, but their role is obscured by frequent simultaneous infection by Mycoplasma and viruses. Cage to cage transmission of the infection is slow, and CAR bacilli are usually not transmitted to sentinel by dirty bedding.

*Chlamydia spp.*: infections are usually persistent and subclinical. *C. psittaci* may cause inclusion conjunctivitis and pneumonia in guineapigs. *C. trachomatis* mouse biotype may under certain circumstances cause pneumonia in mice but the significance is low.

*Citrobacter rodentium*: was formerly known as *‘Citrobacter freundii 4280’*. It has now been characterized, and taxonomic studies showed that it is definitely a separate species (Schauer *et al.* 1995). Presence of the bacterium has been reported to lead to transmissible colonic hyperplasia in mice.

*Clostridium piliforme*: The causative agent of Tyzzer’s disease (formerly *Bacillus piliformis*) does not grow on bacterial culture media. Screening for Tyzzer’s disease by histopathology is insensitive. Positive serological reactions occur frequently without clinical signs of disease and may be indicative of recent active infection. However, the interpretation of serological testing is currently controversial. The suitability of PCR is at present unclear. Immunosuppression of a significant number of the population has been used to demonstrate the presence of this agent in animal colonies.

*Corynebacterium bovis*: a bacterium resembling *C. bovis* is the aetiological agent of ‘scaly skin disease’ or ‘corynebacterial hyperkeratosis’ of nude mice (Clifford *et al.* 1995, Scanziani *et al.* 1997). The clinical disease in nude mice can disappear spontaneously, but high mortality is possible, especially in newborns. *C. bovis* may also cause lesions in mice with fur, e.g. SCID mice (Scanziani *et al.* 1998). While monitoring is not mandatory in immunocompetent mice, they may carry this agent. Monitoring is recommended in immunodeficient mice.

*Corynebacterium kutscheri*: subclinical and symptomatic infection (pneumonia) has mainly been detected in mice and rats.

*Dermatophytes*: *Microsporum* spp. and *Trichophyton* spp. infections (dermatomycoses) occasionally occur in guineapigs and rabbits. Lesions are rare.

*Helicobacter spp.*: various species of this genus have been described since their first isolation from rodents about 10 years ago. At present, there is evidence that some species have the potential to induce clinical disease or may have impact on animal experiments (e.g. *H. hepaticus*, *H. bilis*, *H. typhlonicus*) (Fox & Lee 1997, Franklin *et al.* 1999), whereas no such effects have been described for other species (e.g. *H. rodentium*). Additional species are likely to be described in the near future, and a general recommendation regarding which agents are to be monitored can therefore not be given presently.

*Lawsonia intracellularis*: (Intracellular *Campylobacter*-like organisms) is a likely cause of proliferative enteritis (wet tail) in hamsters. Screening is not recommended, as infection is supposed to lead invariably to clinical disease with characteristic lesions in the intestines.

*Leptospira spp.*: Monitoring for these zoonotic bacteria may be considered if laboratory animals are at increased risk of infection, for instance by contact with wild rodents. Seromonitoring is done by specialized laboratories. Costs are high.

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Laboratory Animals (2002) 36
as monitoring for several serotypes is necessary. Occurrence of *Leptospira* spp. in contemporary colonies is unclear. Leptospirosis has however been found in 'clean conventional' mice (Alexander 1984).

**Mycoplasma spp.**: *M. pulmonis* is at present the most relevant species in mice and rats. Screening is usually done by serology, but antibody response varies greatly between mouse and rat strains. Culture is difficult but may additionally detect various other mycoplasma species. Detection of *Mycoplasma* spp. by PCR is possible.

**Pasteurellaceae**: As in the previous FELASA recommendation for experimental units, monitoring for all Pasteurellaceae is recommended. *Pasteurella pneumotropica* describes a genetically diverse group of organisms. It has been shown repeatedly that different laboratories come to different conclusions on the same strain of rodent Pasteurellaceae, and commercial identification kits do not identify them properly.

**Pneumocystis carinii**: is an important fungal pathogen in immunodeficient animals and may lead to clinical disease or death. Monitoring is recommended for rat and mouse strains with inherited or induced immunodeficiency (e.g. Foxn1nu, Prkdcscid, Rag1tm1Mom).

**Pseudomonas aeruginosa**: the significance is low in immunocompetent animals, but it may cause clinical disease in immunodeficient or immunosuppressed hosts.

**Salmonella spp.**: infrequently found in all animal species. Infected rodents and other hosts, including personnel, may be sources of infection. Such risks are especially great in multipurpose research institutes that house animals of varying pathogen status.

**Staphylococcus aureus**: This bacterial species is ubiquitous in rodent populations where there is direct contact between humans and animals and has the potential to induce clinical signs of disease (e.g. abscesses, wound infections). Exceptionally, other *Staphylococcus* species may also induce clinical signs, at least in immunodeficient animals (Bradfield *et al.* 1993).

**Streptobacillus moniliformis**: infections have been detected during the last decades in colonies of mice, rats and guineapigs. Culture of the bacterium from asymptomatic animals is notoriously difficult. Quarterly monitoring in rats is recommended because this species is the natural host.

**Streptococcus spp.**: (α-haemolytic *S. pneumoniae* and β-haemolytic other species) rarely induce clinical disease and are important primarily in immunodeficient animals but may also lead to clinical signs in immunocompetent individuals.

### Viruses

**Coronaviruses** (MHV in mice, RCV/SDAV in rats): occur frequently and are strongly immunomodulating. Infections are usually self limiting but may be persistent in immunodeficient animals.

**Ectromelia virus**: recent infections came mostly from contaminated biological materials (sera, cells) and contact with wild mice and pets. Susceptibility and antibody response greatly differ among mouse strains.

**Guineapig adenovirus**: This virus has been identified repeatedly as a causative agent of disease or death in guineapigs. The virus cannot be propagated in cell culture, and antigen for serological tests is therefore difficult to obtain. Mouse adenovirus (K87 or FL) is commonly used as an antigen to test guineapig colonies for antibodies to guineapig adenovirus, but there is conflicting information on the degree of cross-reactivity between mouse and guineapig adenoviruses and the validity of these tests (Butz *et al.* 1999).

**Guineapig cytomegalovirus** (GpCMV = Gp herpesvirus type 1): this host specific infection may lead to clinical disease in breeding females. Vertical transmission of the virus is considered common. Seromonitoring results can be confirmed by antigen detection in organs of animals under severe immunosuppression. There is no cross-reactivity with other herpesviruses.
Hamster parvovirus (HPV): weanling and adult hamsters develop clinically silent infections but infection of neonatal Syrian hamsters may result in severe and often lethal disease. Monitoring is recommended as soon as an antigen is available.

Hantaviruses: Wild rodents are natural reservoirs for this group of zoonotic viruses. Laboratory rats and rat material have repeatedly been the source of Seoul serotype Hantavirus infections in research personnel. None of the many other serotypes (e.g. Puumala) has so far been detected in laboratory animal colonies (Meyer & Schmaljohn 2000). Hantavirus infections in rats are inapparent.

K virus: (Mouse pneumonitis virus): previously annual testing was recommended, but infections have not been reported for more than two decades.

Kilham’s rat virus (KRV, RV): see Parvoviruses.

Lactate dehydrogenase elevating virus (LDV): infects mice only and is transmitted within a population vertically or by direct contact (blood). The most important mode of transmission is by experimental procedures (injections, animal-to-animal passages of tumours, microorganisms, parasites, etc.). It is unlikely to be found in breeding units, but it is an important contaminant of biological materials after animal passages. It should be included in monitoring programmes for biological materials and mice if such materials are passaged in mice.

Lymphocytic choriomeningitis virus (LCMV): Only mice and hamsters are known to transmit this zoonotic virus, but other species (e.g. rabbits, guineapig, rats) also seem to be susceptible to experimental infection. Detection of enzootic infection in mice by serology may be difficult (depending on the mode of infection) due to immunotolerance.

Minute virus of mice (MVM): see Parvoviruses.

Mouse adenovirus: It was shown that both strains of mouse adenovirus do not always cross-react in serological tests. Therefore, both strains (FL, K87) should be used as antigens (Lussier et al. 1987). Positive reactions have also been found in rats, and it is recommended that rats are also monitored.

Mouse cytomegalovirus (MCMV): the prevalence of this virus in contemporary laboratory mice is thought to be negligible except in instances in which stocks may have been contaminated by wild mice.

Mouse hepatitis virus (MHV): see Corona viruses.

Mouse parvovirus (MPV): see Parvoviruses.

Mouse polyomavirus: previously annual testing was recommended, but infections have not been reported for more than two decades.

Mouse rotavirus (EDIM): previously annual testing was recommended. The virus has been found in many mouse colonies in recent years. Mouse rotavirus does not infect other species.

Mouse thymic virus (MTV): previously annual testing was recommended, but infections have not been reported for more than two decades.

Parvoviruses: In addition to well-known parvoviruses (MVM, KRV, H-1), additional species have been found during the last decade (mouse parvovirus, MPV; rat parvovirus RPV). Different strains exist for these viruses, and propagation in cell culture is not easily possible. Therefore, antigens are difficult to obtain, and only a few laboratories are able to test for these agents by specific tests (Jacoby et al. 1996).

Pneumonia virus of mice (PVM): infects mice and rats. Previously monitoring of hamsters, guinea pigs and rabbits was recommended, but the virus has not been isolated from any of these species.

Rabbit haemorrhagic disease virus (RHDV): This highly contagious calicivirus causes high mortality in rabbit populations. However, apathogenic caliciviruses exist which interfere with serological tests (Capucci et al. 1996, Chasey 1997). Positive serological reactions for RHDV may therefore be caused by cross-reaction with such virus strains. Positive reactions should be interpreted with care.

Rabbit enteric coronavirus: infections seem to occur frequently in rabbitries, but the
Rabbi virus has not been isolated (hence monitoring is not possible).

*Rabbit parvovirus*: infections seem to occur frequently in rabbitries. Monitoring is recommended as soon as an antigen is available.

*Rabbit pox virus* (myxomatosis): monitoring was recommended earlier, but as the natural mode of transmission is by insects, the infection is not likely to be found in well managed laboratory colonies. Diagnosis can be easily made by clinical signs and by post mortem examination.

*Rabbit rotavirus*: infection is non persistent. Seromonitoring must be carried out using a serogroup A antigen (as in mice).

*Rat parvovirus* (RPV): see Parvoviruses.

*Rat respiratory virus* (RRV): this yet unclassified virus induces mild to moderate lung lesions (interstitial lymphohistiocytic pneumonia, increased bronchus-associated lymphoid tissue) in all strains of rats, usually at an age of 8–10 weeks. Clinical signs have not been reported. Diagnosis is presently based on histopathology. Antigens and serological tests are at present not available, and monitoring on a broad basis is therefore not possible (Elwell et al. 1997, Riley et al. 1997, Slaoui et al. 1998).

*Reovirus type 3*: Besides mice and rats, antibodies have been found also in asymptomatic hamsters, guineapigs (for which monitoring was recommended earlier) and in rabbits, but the virus has not been isolated from any of these species.

*Sendai virus*: rodents (mice, rats) are the natural host for this virus. Seropositives among other species (including man) are likely to be due to closely related, serologically cross-reacting viruses (e.g. other paramyxoviruses). Since transmission via dirty bedding is not reliable, the use of cage contact sentinels is recommended.

*Sialodacryoadenitis virus* (SDAV)/

*Rat coronavirus* (RCV): see Coronaviruses.

*Simian virus 5* (SV5): was earlier recommended for guineapigs, but no documented infections are known.

*Theiler’s murine encephalomyelitis virus* (TMEV): Positive reactions have been reported in rats which might be due to a yet uncharacterized virus (‘rat cardiovirus’) (Ohsawa et al. 1998). Positive findings have also been reported in guineapigs suffering from lameness.

**Toolan’s H-1 virus**: see Paroviruses.

**Parasites**

*Amoebae* (*Entamoeba sp.*): are commensal protozoans found in the large intestine. Infections are subclinical, and no examples of interference with research have been reported. They might, however, be an indicator of hygiene failures or contact with wild or infected animals.

*Cestodes*: most species require an intermediate host and are therefore unlikely to be found in well-managed animal facilities. Some, however, may have a direct life cycle (e.g. *Hymenolepis nana*) by ingestion of eggs and have been detected in rodent colonies.

*Coccidia*: these host-specific protozoans are common pathogens in rabbits and guineapigs and may cause enteritis and death, primarily in young animals. Coccidia infections may also occur in mice and rats but are uncommon.

*Ectoparasites*: colonies of laboratory animals may severely suffer from ectoparasites (mites, fleas, lice, mallophages).

*Encephalitozoon cuniculi*: this microsporidian parasite can occur in all species, mostly in rabbits and guineapigs. It causes multifocal nephritis and encephalitis (mostly subclinical). Infectious spores are excreted in urine.

*Giardia muris*: causes subclinical infection in laboratory rodents.

*Klossiella sp.*: members of this genus are coccidia and are found in kidney tubules or endothelial cells of blood vessels in mouse (*K. muris*) and guineapigs (*K. cobayae*). The infection is clinically occult but lesions in the kidneys are usually visible macroscopically.

*Nematodes*: several species have been reported from most species of laboratory animals. They may colonize different parts of the intestinal tract (e.g. stomach, liver, caecum, colon) and even the urinary
bladder of rats (*Trichosomoides crassicauda*). Due to differences in their life cycles and different predilection sites in their hosts, several detection techniques (e.g. perianal examination with cellophane tape, flotation, wet mount of caecum contents) may be necessary to detect or exclude parasitic stages of pinworms in mice and rats (*Syphacia sp.*, *Aspiculuris*) with sufficient certainty.

*Spironucleus sp.*: insufficient information is available on transmission of these flagellates between different rodent species (mouse, rat, hamster). They may induce clinical signs and have impact on various types of experiments.

*Toxoplasma gondii*: monitoring was earlier recommended, but as infectious forms are excreted by Felidae only, spread of the infection within rodent and rabbit colonies does not occur.

*Trichomonads*: at present no evidence exists that these obviously apathogenic flagellates have any impact on the physiologic parameters of their host. They are, however, likely to be species-specific and thus might be an indicator of a leak in the barrier system or of direct or indirect contact with wild rodents.
## Appendix 3 Health monitoring reports

### Health Monitoring in Accordance with FELASA recommendations

**Date of issue:**

**Location:**

**Housing:** (Barrier/Non-Barrier/IVC/Isolator):

**Species:** Mouse

**Strain:** (Strain)

Species and strains present within the unit:

<table>
<thead>
<tr>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (≤ 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
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<td></td>
</tr>
<tr>
<td>Mouse hepatitis virus</td>
<td>3 months</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse rotavirus (EDIM)</td>
<td>3 months</td>
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<td></td>
<td></td>
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<tr>
<td>Paroviruses</td>
<td></td>
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</tr>
<tr>
<td>Minute virus of mice</td>
<td>3 months</td>
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<tr>
<td>Mouse parvovirus</td>
<td>3 months</td>
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<tr>
<td>Pneumonia virus of mice</td>
<td>3 months</td>
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<tr>
<td>Sendai virus</td>
<td>3 months</td>
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<tr>
<td>Theiler’s murine encephalomyelitis virus</td>
<td>3 months</td>
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<tr>
<td>Ectromelia virus</td>
<td>Annually</td>
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<tr>
<td>Lymphocytic</td>
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</tr>
<tr>
<td>Choriomeningitis virus</td>
<td>Annually</td>
<td></td>
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<tr>
<td>Mouse adenovirus type 1 (FL)</td>
<td>Annually</td>
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<tr>
<td>Mouse adenovirus type 2 (K87)</td>
<td>Annually</td>
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<tr>
<td>Mouse cytomegalovirus</td>
<td>Annually</td>
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<tr>
<td>Reovirus type 3</td>
<td>Annually</td>
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<tr>
<td><strong>Additional organisms tested:</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteria, mycoplasma and fungi</strong></td>
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<tr>
<td>Citrobacter rodentium</td>
<td>3 months</td>
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<tr>
<td>Clostridium piliforme</td>
<td>3 months</td>
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<td></td>
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<tr>
<td>(Tyzzer's disease)</td>
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<tr>
<td>Corynebacterium kutscheri</td>
<td>3 months</td>
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<tr>
<td>Mycoplasma spp.</td>
<td>3 months</td>
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<tr>
<td>Pasteurellaceae</td>
<td>3 months</td>
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<tr>
<td>Salmonella spp.</td>
<td>3 months</td>
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<tr>
<td>Streptococci</td>
<td>3 months</td>
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<tr>
<td>β-haemolytic (not group D)</td>
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<tr>
<td>Streptococcus pneumoniae</td>
<td>3 months</td>
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<tr>
<td>Helicobacter spp.</td>
<td>Annually</td>
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<tr>
<td>Streptobacillus moniliformis</td>
<td>Annually</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Additional organisms tested:</strong></td>
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</tr>
<tr>
<td><strong>Parasites</strong></td>
<td></td>
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</tr>
<tr>
<td>Ectoparasites:</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species designation</td>
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<td>Endoparasites:</td>
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<td>Species designation</td>
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<tr>
<td><strong>Pathological lesions observed</strong></td>
<td>3 months</td>
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</tbody>
</table>

Data are expressed as number positive/number tested

Positive findings in other species in the same unit:

Abbreviations used in this report:

- ELISA = enzyme linked immunosorbent assay
- MICR = microscopy
- IFA = immunofluorescence assay
- CULT = culture
- PATH = gross pathology
- PCR = polymerase chain reaction
- HIST = histopathology
- NT = not tested
# Health Monitoring in Accordance with FELASA recommendations

**Date of issue:**

**Location:**

**Housing:** (Barrier/Non-Barrier/IVC/Isolator):

**Species:** Rat

**Strain:** (Strain)

## Species and strains present within the unit:

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (≤ 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvoviruses</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Kilham rat virus</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Rat parvovirus</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Toolan’s H-1 virus</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Pneumonia virus of mice</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Sendai virus</td>
<td>3 months</td>
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<tr>
<td>Sialodacyroadenitis/Rat coronavirus</td>
<td>3 months</td>
<td></td>
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</tr>
<tr>
<td>Hantaviruses</td>
<td>Annually</td>
<td></td>
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<td></td>
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<tr>
<td>Mouse adenovirus type 1 (FL)</td>
<td>Annually</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse adenovirus type 2 (K87)</td>
<td>Annually</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Reovirus type 3</td>
<td>Annually</td>
<td></td>
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</tr>
</tbody>
</table>

### Additional organisms tested:

- **Bacteria, mycoplasma and fungi**
  - *Bordetella bronchiseptica* 3 months
  - *Clostridium piliforme* 3 months
  - (Tyzzer’s disease)
  - *Corynebacterium kutscheri* 3 months
  - *Mycoplasma spp.* 3 months
  - *Pasteurellaceae* 3 months
  - *Salmonella spp.* 3 months
  - *Streptobacillus moniliformis* 3 months
  - *Streptococci* 3 months
    - β-haemolytic (not group D)
  - *Streptococcus pneumoniae* 3 months
  - *Helicobacter spp.* Annually

### Additional organisms tested:

- **Parasites**
  - Ectoparasites: 3 months
  - Species designation
  - Endoparasites: 3 months
  - Species designation

### Pathological lesions observed

3 months

Data are expressed as number positive/number tested

### Positive findings in other species in the same unit:

**Abbreviations used in this report:**

- ELISA = enzyme linked immunosorbent assay
- MICR = microscopy
- IFA = immunofluorescence assay
- CULT = culture
- PATH = gross pathology
- PCR = polymerase chain reaction
- HIST = histopathology
- NT = not tested
Health Monitoring in Accordance with FELASA recommendations

Date of issue:
Location: 
Species: Hamster
Housing: (Barrier/Non-Barrier/IVC/Isolator):
Species and strains present within the unit:

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (≤ 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytic choriomeningitis virus</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sendai virus</td>
<td>3 months</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Bacteria, mycoplasma and fungi</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Clostridium piliforme (Tyzzer’s disease)</td>
<td>3 months</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pasteurellaceae</td>
<td>3 months</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Salmonella spp.</td>
<td>3 months</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Corynebacterium kutscheri</td>
<td>Annually</td>
<td></td>
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</tr>
<tr>
<td>Helicobacter spp.</td>
<td>Annually</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Parasites</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ectoparasites:</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species designation</td>
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</tr>
<tr>
<td>Endoparasites:</td>
<td>3 months</td>
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<tr>
<td>Species designation</td>
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</tr>
<tr>
<td>Encephalitozoon cuniculi</td>
<td>Annually</td>
<td></td>
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</tr>
</tbody>
</table>

Pathological lesions observed

3 months

Data are expressed as number positive/number tested

Positive findings in other species in the same unit:

Abbreviations used in this report:
ELISA = enzyme linked immunosorbent assay, MICR = microscopy, IFA = immunofluorescence assay, CULT = culture, PATH = gross pathology, PCR = polymerase chain reaction, HIST = histopathology, NT = not tested
Health Monitoring in Accordance with FELASA recommendations

Date of issue:

Location: 
Species: Guineapig

Species and strains present within the unit:

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (≤ 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guineapig adenovirus*</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sendai virus</td>
<td>3 months</td>
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</tr>
<tr>
<td>Guineapig cytomegalovirus</td>
<td>Annually</td>
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</tr>
</tbody>
</table>

Bacteria, mycoplasma and fungi

<table>
<thead>
<tr>
<th>Bacteria, mycoplasma and fungi</th>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (≤ 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella bronchiseptica</td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydia psittaci</td>
<td>3 months</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Corynebacterium kutscheri</td>
<td>3 months</td>
<td></td>
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<td></td>
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<tr>
<td>Dermatophytes</td>
<td>3 months</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pasteurellaceae</td>
<td>3 months</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Streptobacillus moniliformis</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Streptococci β-haemolytic (not group D)</td>
<td>3 months</td>
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<tr>
<td>Streptococcus pneumoniae</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Yersinia pseudotuberculosis</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Clostridium piliforme (Tyzzer's disease)</td>
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</tr>
</tbody>
</table>

Additional organisms tested:

- Additional organisms tested:

- Parasites
  - Ectoparasites: 3 months
  - Species designation
  - Endoparasites: 3 months
  - Species designation
  - Encephalitozoon cuniculi 3 months

Pathological lesions observed

- Pathological lesions observed 3 months

Data are expressed as number positive/number tested. *Indicate antigen(s) used in serological testing

Positive findings in other species in the same unit:

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Health Monitoring in Accordance with FELASA recommendations

Date of issue: 
Location: 
Housing: (Barrier/Non-Barrier/IVC/Isolator): 
Species: Rabbit 
Strain: (Strain)

Species and strains present within the unit:

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Test frequency</th>
<th>Latest test date</th>
<th>Latest results</th>
<th>Testing laboratory</th>
<th>Test method</th>
<th>Historical results (&lt; 18 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit haemorrhagic disease virus</td>
<td>3 months</td>
<td></td>
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<tr>
<td>Rabbit rotavirus</td>
<td>3 months</td>
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<tr>
<td>Additional organisms tested:</td>
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<table>
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<td>Dermatophytes</td>
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<tr>
<td>Pasteurella multocida</td>
<td>3 months</td>
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<tr>
<td>Other Pasteurellaceae</td>
<td>3 months</td>
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<tr>
<td>Salmonella spp.</td>
<td>3 months</td>
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<td>Additional organisms tested:</td>
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Pathological lesions observed | 3 months |

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