Society for Laboratory Animal Science

GV-SOLAS

Working group on Hygiene
Hygienic monitoring of mice and rats in various housing systems

General

According to FELASA recommendations at least 10 animals of each hygienic unit should be submitted to necropsy and serological, bacteriological and parasitological routine tests in at least quarterly intervals (1). This applies to barrier and conventional environments of populations exceeding 100 animals if certain additional presuppositions are given (2). Very often by using compartments intensively (e.g. filter lid cages, IVC [individual ventilated cages], isolators) the number of animals per hygienic unit is much lower. This recommendation is intended to assist in the hygienic monitoring of such animal populations.

Definition: Hygienic unit

One hygienic unit includes the area of a laboratory animal facility that is cared for by the same personnel (animal care personnel, research scientists).

Examples could be:

- a barrier unit with several rooms, accessible through locks (shower, changing of clothes, disinfecting of the hands)
- several conventional animal rooms in one building
- several animal rooms on different floors or in different buildings, which are entered by the same persons without any preventive measures
- an isolator
- a microisolator cage (filter lid cage, IVC). Is the isolator cage only opened in ventilated workstations by using an appropriate sterilisation agent for the hands (gloves), it may act as an efficient barrier (see “Handling of microisolator cages”, GV-SOLAS).

Sample size

The necessary sample size depends on the size of the animal stock, the prevalence rate of an eventual infection and the examination interval. With an inventory of more than 100 animals, at random sampling by routine examination of 10 animals results in a 95% chance of detecting an infection if at least 30% of a population is infected. Theoretically, with 4 examinations per year, an infection with a prevalence of 10% can be detected with nearly 99% certainty (3). If the risk of introducing agents into an experimental unit is very high (e.g. introduction of animals more than one time per month, introduction of animals from different breeders, frequent entry of research personnel in addition to animal care staff) more frequent monitoring is necessary. In such cases it is recommended that a minimum of 3-5 animals is a sufficient sample size of animals to be monitored at least per month.

Choice of animals

The choice of the animals used for routine examination depends on several factors. Most suitable are animals directly from the animal facility, which were born there or have spent a long time at this place. If such animals are not available, sentinels (see below) should be used. Preferably old breeding animals (retired breeders) should be used, as these, due to their long presence in the facility and their decreasing immune competence, very often harbour the broadest spectrum of agents. Except for serological and some parasitological examinations (helminths), also young animals with at least 10 weeks of age are very suitable. The animals are preferably taken from different locations and cages of one hygienic unit.

Sick animals

Valuable additional information is delivered through sick or dead animals. Generally, all sick and dead animals should be submitted to examination in addition to scheduled testing so that occurring infections can be detected at a very early stage. The research personnel should be instructed to deliver all animals to microbiological examination which show any sign of sickness or appear moribund or dead.

Sentinel animals

Definition and utilisation

Sentinel animals are introduced into hygienic units in order to detect existing infectious agents. These animals are kept with a high infection risk, intentionally, so they get infected easily by the agent occurring in this area. The sentinel animals are not chosen by chance. They are used, when no animal from the hygienic unit to be examined can be submitted for the microbiological examination (e.g. animals involved in an experiment, transgenic animals, small populations) and in case examining the specific strain does not make sense as e.g. serological testing of immunodeficient animals is not reliable.

In general, one can distinguish between various options of using sentinel animals:

1. **Contact sentinels** = Keeping sentinels in the same cage as the animals to be examined.
2. **Bedding sentinels =** Keeping sentinels on used bedding from several cages of the animals to be examined (e.g. in IVCs; however, not all pathogens can be transferred this way (see: "Probability to transfer specific infections by applying sentinel methods")
3. **Exhaust air sentinels =** Keeping sentinels in cages filled with the exhaust air of the cages of animals to be examined (e.g. in IVCs; however, not all pathogens can be transferred this way (see: "Probability to transfer specific infections by applying sentinel methods")

Selection criteria for sentinel animals

If sentinel animals are not bred within the colony to be examined they have to be taken from a colony with well known and regularly checked hygienic status.

- Animals should be free from all agents to be monitored (incl. parasites) and free from antibodies to these agents.
- The animals should be of the same animal species like the one of the population to be examined.
- It should be young adult animals, which show a good immune reaction (serology).

There are several opinions concerning the genetics of the sentinel animals to be used:

- Outbred animals are relatively cheap, usually quite robust and very fertile. In general, they are susceptible to a wide range of pathogens; however, they show less often clinical symptoms of disease.

- Inbred strains (e. g. DBA/2, BALB/c) often are more expensive as outbred animals. The susceptibility to specific infectious agents and to clinical diseases differs between strains. A/J mice, e.g., usually do not show any clinical symptoms with an MHV infection, whereas there may be a high morbidity and mortality rate in other strains, e.g. C57BL/6. In contrast, an ectromelia virus infection in C57BL/6 mice and other resistant strains like C57BL/10 is often inapparent, but this infection shows a high morbidity and mortality rate concerning 80-90% of the colony in more susceptible strains like A, DBA/2, BALB/c and C3H.

- Immunodeficient animals usually are quite expensive. They are more susceptible to clinical diseases than immunocompetent animals and have to be kept away from other potentially infecting agents. Often they do not show seroconversion. In this case infections can not be evaluated by serological tests and they are not suitable as sentinel animals for these agents. Often, however, immunodeficient animals remain infected persistently and allow an isolation of the infectious agent, whereas infections in immunocompetent animals usually are self-limiting. The assumption immunodeficient animals are good sentinels for the verification of parasitic infections is not approved for *Spironucleaus muris* (28) and for *Syphacia obvelata* it is only approved that immunodeficient animals excreted worm ova for a prolonged period of up to 19 weeks; whereas the excretion of worm ova of immunocompetent mice nearly stopped after 13 weeks (27).

As a general rule, one should use outbred animals as sentinels. The use of inbred animals or mutants as sentinels would be a good option if special agents for which these strains are known to be sensitive shall be detected or excluded, respectively.

The use of immunodeficient animals is only recommended in rare cases (e.g. detection or exclusion of opportunistic agents like *Pneumocystis* spp. or proving internally inconsistent results concerning *Clostridium piliforme*) since other methods to detect even inapparent infections are available nowadays.

In experimental animal units sentinels used are routineously animals of the same species as the animals which are kept in this area. If possible they should derive from the same breeder (out of the same breeding area) as the experimental animals and should be ordered at the same time. It is of decisive importance that by thorough choice of the breeding areas an

introduction of agents via added sentinel animals will be excluded, i.e. they should be free from all infectious agents to be monitored and free from antibodies to these agents. This is of special importance for populations of immunodeficient animals.

Acquisition of sentinels

In hygienic areas where re-occupation occurs (short-term experiments, multipurpose units), at least (10)-20 animals should always be available at short notice. It is recommended to order the demand of sentinels for a longer period, e.g. for 6 months. Thus, an adequate long exposition period in the area to be examined is ensured.

In units where no re-occupation occurs (long-term experiments, only one type of experiment, all-in all-out practices), the number of sentinels is based upon the estimated time of the experiment. If possible, an adequate number of sentinels should be available during the complete time period of the experiment. Thus, the risk of agent introduction by added sentinels can be eliminated.

With immunodeficient animals (e. g. thymusaplastic *Foxn1*^{nu}-mice or *Whn*^{rnu}-rats, B-and T-cell deficient *Prkdc*^{scid} or *Rag*^{tm1Mom} mice) immunocompetent animals will be kept as sentinels in the same unit (it is best to use heterozygous litter siblings with nude mice). It is important that the sentinel animals are free of opportunistic agents (e.g. *Staphylococcus aureus, Pneumocystis spp.*) that can cause diseases in immunodeficient animals.

Animal husbandry of sentinel animals

- Same husbandry conditions as for the rest of the stock
- In order to avail a transmission of an infection, the sentinel animals should receive used bedding from as many (or all) cages as possible. They are also to be fed from the used feeding devices and drinking bottles.
- Exposition period in the area to be examined: 6 weeks at least (8-10 weeks or even longer would be best)
- Cages preferably well beneath on the rack, evenly distributed in the room
- Cages with sentinel animals should be handled and changed at last

With the above mentioned form of keeping animals diagnostically conclusive results can be achieved even with a small number of tests (animals). When reusing bedding usually 50% clean and 50% used bedding should be applied, thus, keeping on partially used bedding is not as stressful for the sentinel animals as applying 100% used bedding. For reducing dust exposure, however, the sentinel animals can also be put into a used cage during cage changing procedure. Each time the sentinel animals then have to be put into another cage to make sure they got in contact with each cage in the IVC rack.
Within small animal colonies (e. g. IVCs, isolators) there is a high chance of transferring pathogens by using contact sentinels who are to be kept in the same cage as the animals to be examined (7).

Specifics of various housing systems

1. **Health monitoring in conventional or barrier units**

(see also “sample size”)
The FELASA recommendations should be used with the mostly practised conventional or barrier animal husbandry of rodents (in open cages).

2. Health monitoring in isolators

Mostly, very small populations are kept in isolators and there is not enough space in order to keep the number of animals for health monitoring as recommended for open husbandry. Appropriate monitoring is therefore only possible by keeping a realistic number of animals in an isolator consequently on used bedding (and with soiled feed and drinking bottles) from preferably many (all) cages. Use of contact sentinels may also be appropriate. According to the size of the isolator one or more cages with sentinel animals should be scheduled and for infection controls approximately 3-5 animals are recommended per sampling together with an increased monitoring frequency (1). (For keeping aseptic animals please see „Mikrobiologische Untersuchung von keimfreien Tieren“, GV-SOLAS).

3. Health monitoring in filtered cabinets

Filter cabinets represent a hygienic unit, wherein, by proper handling smaller animal populations can be isolated from other animal populations, which are kept outside a cabinet or in other cabinets. Within a cabinet it is possible – in case of animal husbandry in open cages – that pathogens spread between cages or will be transmitted through the air. Due to the small number of animals kept in such cabinets and the risk or extent of a transfer of pathogens from cage to cage (which are both difficult to estimate), a sentinel programme similar to animal husbandry in isolators or microisolators is reasonable.

4. Health monitoring in microisolator cages (IVCs, filter lid cages)

(Also see “Handling of microisolator cages”, GV-SOLAS)

When microisolator cages are used, the hygienic unit is not the room but every single cage. This principle inhibits very efficiently the transfer of infections among the cages if proper husbandry techniques are used. At the same time it hampers the use of sentinel animals for hygiene monitoring, as a homogeneous spread of a pathogen within the population will be reduced or even prevented. This refers to static microisolator cages (i.e. filter lid cages without forced air supply) as well as to individually ventilated microisolator cages (IVCs) on special racks. It is now generally accepted that filter lid cages are not recommended for regular husbandry because ammonia and CO_2 concentrations reach harmful levels rapidly and temperature and relative air humidity are elevated. As for practical reasons not from all cages a sample for health monitoring can be taken, one has to make a compromise. The following procedure enables a relatively reliable monitoring without breaching the microisolator principle:

- Sentinel animals are also kept in filter lid cages/IVCs.
- After exchanging all cages in the IVC rack (except from the sentinel animals´ cages) under the sterile workbench bedding and food samples should be taken from as many used cages as possible. These samples are to be mixed with 50% of clean bedding and put into the sentinel animals´ cages (learn more about other methods in „Animal husbandry of sentinel animals“). Furthermore, the sentinel animals should get a used drinking bottle. Then the sentinel animals are to be kept in this cage.
- Weekly changing of the bedding, food and water bottle donors results in a good profile through the colony.

- The sentinel animals will be treated with used bedding, food and water bottles for 10-12 weeks. The final hygienic examination should take place 4-6 weeks after the last contaminated bedding change. The most reasonable method is keeping a large group of animals for a maximum period of 6 months with a progressive removal of sentinel animals.

Probability to transfer specific infections by applying sentinel methods

Every known infectious agent in mice and rats (except from retroviruses) can be transmitted by long term exposition to contact sentinels. To detect agents primarily or exclusively spreading from animal to animal through direct contact (e. g. *CAR* (cilia-associated respiratory)-*Bacillus*, *Pasteurella pneumotropica*, LDV), using other sentinel types (exhaust air sentinels or bedding sentinels) does not make sense (10). In this case only an examination of animals out of the colony or direct contact of infected animals with sentinel animals will bring reliable results.
An exposure of sentinel animals to exhaust air (some manufacturers of IVCs offer the opportunity of leading exhaust air from the other cages through a sentinel cage) can be useful for detecting pathogens also spreading through the air (e. g. mouse hepatitis virus, Sendai virus) (6, 7).
However, other pathogens like mouse rotavirus, mouse parvovirus or *Helicobacter* spp. usually do not spread throughout the air but can remain infectious in used bedding (4-25). Even mites (*Myobia musculi*) did infect other animals by using soiled bedding but effectively (i.e. 75% of the cages) not until after 5 months (26). Keeping sentinel animals on used bedding and additionally supplying them with exhaust air of other cages at the same time increases the efficiency of infection control in IVCs (8).
Transmission of infections by used bedding was experimentally verified for the following mostly faecally excreted pathogens: ectromelia virus (11), mouse hepatitis virus (4, 9, 26), rotavirus (6), sialodacryoadenitis virus (SDAV) (12), a variety of mouse and rat parvoviruses (also Kilham rat virus) (9, 13, 14, 15, 25), Theiler´s murine encephalomyelitis virus (TMEV) (16), mouse norovirus (23), *Clostridium piliforme* (17, 18), *Helicobacter hepaticus* and *H. bilis* (19, 22) and for Sendai virus (20), which, however, was not contagious in another examination – maybe because of its low stability (4). A transmission of fairly resistant pathogens like the mouse adenovirus seems also possible (21). Concerning endoparasite transmission there are only a few reliable sources. With *Spironucleus muris* an inconsistent transmission of the infection to mice by used bedding was described possibly caused by the fairly fast dehydration of the excreted cysts (most of them lost their infectiousness after 2h). Furthermore, *Spironucleus muris* infectiousness was very depending on the time frame the sentinel was exposed to the used bedding. In this study it took at least 6 (up to 16) weeks. (28). Up to now the infectiousness of worms (*Syphacia obvelata*) was only described by using contact sentinels; in this study immunodeficient animals showed an extended excretion time, whereas in immunocompetent animals excretion stopped after 13 weeks (27). With other pathogens – especially infectious pathogens of the airways – transmission through used bedding was not proven (*CAR-Bacillus*) (10) or not yet tested (mouse pneumonia virus, *Mycoplasma pulmonis*) (21). *Pneumocystis carinii*, however, also an infectious pathogen of the airways, was transferred from chronically infected animals to sentinel animals ($Prkdc^{scid}$ mice) after 12 weeks on used bedding. (22). This infection, however, took a long time and was less efficient compared to experiments with contact sentinels. Contradictory results were found for *Pasteurella pneumotropica*. One working group achieved infecting bedding sentinels, however not in every case, with *Pasteurella pneumotropica* after 12 weeks (22) while another working group did not manage to achieve an infection at all (24).

The efficiency of a transmission of infectious pathogens by used bedding especially depends on stability and dose of the pathogen. Thus, an experimentally verified infectiousness of used bedding (with a high pathogen rate from the acute infection) is not completely comparable to the infectiousness of used bedding in case of chronic infections where often only small amounts of pathogens are excreted (21, 24). The approach of transmitting mouse parvovirus (MPV) and mouse hepatitis virus (MHV) by used bedding revealed that high infection rates can only be achieved at the climax of virus excretion (i.e. after 1 week p.i. with MPV and after 3 days p.i. with MHV) (9). In another experimental investigation high infection rates (90-100%) with MPV-1 could 2-3 weeks p.i. only produced with high virus doses. Whereas, with lower doses infection rates of only 20- 60% were achieved within 2-6 weeks, depending on the dose given. (25). The approach of transmitting MHV from a chronically infected population with bedding sentinels was successful for 50% of the animals after 4 months and completed after 5 months (100%) (26). The approach of transmitting the mouse norovirus with bedding sentinels resulted in an infection rate of 80% after 12 weeks (23).
In addition to bedding sentinels the use of exhaust air filters or the impression method applied on the cage surface for infection control by PCR („Environmental Monitoring“) is possible since some viral and bacterial nucleic acids are detectable for weeks/months (6, 7, 25).

In summary it can be said, therefore, that none of these methods alone shows reliable results concerning the detection of all pathogens and gaining a complete overview on hygienics. Especially in case of compartmental animal husbandry (e.g. IVCs) this complete overview of the hygienic situation is hard to gain. Therefore, a complex course of action is necessary in which the pathogens specifics and the different structures of the animal husbandry facility are to be determined on an individual basis. This is why planning an efficient hygiene programme for animal husbandry in IVC-racks is quite challenging.

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Authors: Felix R. Homberger, Yale University; Werner Nicklas, DKFZ Heidelberg
Revised by Bettina Kränzlin, Heidelberg University.

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