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# Specialist information

## from the Committee (GV-SOLAS) for Hygiene

# Validity of health reports: Critical remarks on the use of sentinels for the determination of the infectious status in laboratory animal facilities

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Frequently, colony animals are not available for health monitoring of laboratory animal populations. This may be the case when all animals housed in a unit are used for experiments or in case of valuable genetically modified animals so that animals cannot be submitted for whole body examination. In specific situations it may not be reasonable to use such animals for testing, e.g., when animals are immunodeficient or immunovague and when it is not definitely known whether they produce antibodies that are detectable in serological tests.

Therefore, animals from a population with well-known microbiological status are commonly introduced to monitor the colony. These animals are considered representative for the animal population and are submitted to testing after a certain period of exposure to the colony animals. Such animals are commonly called “sentinels” and are exposed to agents potentially present in a population either by direct or by indirect contact. The term “sentinel” is now generally used for animals that are considered representative for a population and which are therefore tested to gain insight into the microbiological status of a population.

It is of crucial importance that sentinels - prior to their introduction into a population - are free from all agents for which the relevant population is to be tested or which are unwanted in this colony. Otherwise it may happen that agents are introduced e.g. into a colony of immunodeficient animals by immunocompetent sentinels which may be pathogenic for the immunodeficient population (e.g., *Staphylococcus aureus*, *Pneumocystis* spp.). Furthermore, the introduction of viruses into animal populations by sentinel animals has been described (Pullium et al. 2004).

During traditional housing in open cages, transfer of agents to sentinel animals is permanently possible by aerogenic transmission or by fomites. In addition, handling of animals housed in open cages may support agent transfer as change of gloves or hand disinfection is usually not implemented when animals are handled or transferred to clean cages. Regardless, long exposure periods of 10 – 12 weeks or more are necessary to detect infections caused by the majority of agents with sufficient certainty.

These possibilities of agent transmission are not given when animals are housed in individually ventilated cages (IVC) and properly handled. It is therefore important to assess sentinel-based health information together with the type of housing. The validity of such information also depends on the type of the sentinel programme (e.g., “contact sentinels”, “dirty bedding sentinels”) and the duration of exposure. It is therefore necessary that such information is given in a health report.

Information on the health status of a population must always be evaluated critically. This information is of crucial importance for the assessment of the infectious status of a population and the risk of introducing agents by sentinels. Unfortunately, health reports are frequently prepared and interpreted by persons who do not have sufficient understanding of the principles of health monitoring or do not have sufficient insight into details of an animal population.

There are different types of health information:

- A **test report** is prepared by the diagnostic laboratory and is valid only for the animals tested. Frequently, the testing laboratory does not get detailed information on the origin of the animals (“hygienic unit”), the health monitoring programme, genetic background, immunodeficiency, etc., so that these important data are usually not included in a test

report. A reliable evaluation of the risk of agent introduction is therefore, solely based on test reports, usually not possible.

- A **health report** summarises data from regular and repeated testing. The information given is therefore based on higher animal numbers. Additional information (e.g., clinical observations, housing conditions, monitoring programme, testing of colony animals vs. sentinels) is part of a meaningful health report so that, for example, sample size, frequency of testing etc. are documented.

Meaningful **health reports** [see also (Nicklas et al. 2002, Mähler et al. 2014) should provide information on the following items:

- Exact designation of the origin of animals („microbiological unit“), e.g., barrier unit, room number, etc
- Housing conditions (e.g., barrier housing, open cages, IVC, isolator)
- Animal species and strains
- Name(s) of the testing laboratory(ies)
- Date of restocking or last rederivation
- Date of latest testing, number of animals tested and results from testing
- Cumulative results of all tests performed during a defined period (e.g., 12 or 18 months) or since restocking
- Test methods (clinical, microscopy, serology, culture, histopathology, PCR, etc.)
- Name(s) of agent(s) tested
- Name(s) of agent(s) detected
- Name(s) of agent(s) not detected
- Treatment, vaccination
- Contact person

More details are given by the Working Group on Hygiene (Harmonisation of Health Monitoring Reports (http://www.gv-solas.de/fileadmin/user_upload/pdf_stellungnahme/2018stell_hyg-Harmonisation.pdf, Nicklas and Seidel 2018).

Additional information is necessary for the interpretation of a health report and for the assessment of the risk of agent introduction. Details should be provided regarding the health monitoring programme (testing of colony animals, sentinels, sentinel programme), clinical observations, and additional characteristics and factors leading to an increased risk of agent introduction (e.g., access of experimenters, introduction and use of biological materials, introduction of animals from outside and their origin). Frequently, details on the use of personal protective equipment are requested. However, this information does not really allow to assess the quality of the animals.

Evaluation of information from health monitoring is particularly difficult when animals are housed in IVC. Direct testing of colony animals may be possible under these conditions, however, due to the partitioning into many small units it would be necessary to test large animal numbers. Such populations are commonly tested by use of sentinels. Usually, pooled used bedding (sometimes also food, used drinking bottles, etc.; there are numerous variations and modifications) is collected from several cages and put into one or several sentinel cages. Sentinel animals are subsequently tested after a certain exposure period. Using this approach, few sentinels may represent a rack or parts of a colony, and health monitoring can be performed inexpensively. It is important to consider that detection of an infection depends on

the transmission of agents to sentinel animals. This means that sentinels must be exposed to an **infectious dose** of an agent.

It must be expected that, due to the high degree of partitioning in IVC housing systems and isolation of animals on the cage level, infections can spread only very slowly so that an infection may be limited to a few cages. Only restricted amounts of soiled bedding can be transferred into sentinel cages which means that during pooling of bedding from several cages the number of agents may be diluted to an extent that the number of agents may be too low to lead to seroconversion.

Several agents such as pinworms, mouse hepatitis virus (MHV) (Thigpen et al. 1989, Dillehay et al. 1990, Brielmeier et al. 2006, Compton et al. 2004, Smith et al. 2007), rat coronaviruses (RCV/SDAV) (La Regina et al. 1992) or *Clostridium piliforme* (Gibson et al. 1987, Waggie et al. 1984, Motzel and Riley 1992) are usually sufficiently transmitted with dirty bedding and are detectable with an appropriate sentinel programme. In addition, transmission of parvoviruses (Smith et al. 1993), murine norovirus (MNV) and *Helicobacter* species to sentinels has been described (Whary et al. 2000, Manuel et al. 2008).

It is known from other agents that they are insufficiently transmitted to sentinels and that the use of sentinels can be problematic for their detection (Shek 2008, Weisbroth et al. 1998, Mähler and Nicklas 2012). Among them are many respiratory agents like Sendai virus (Artwohl et al. 1994, Dillehay et al. 1990), *Filobacterium rodentium* (formerly known as CAR bacillus) (Cundiff et al. 1995), *Pasteurellaceae* (Scharmann and Heller 2001) or mycoplasms and also agents with zoonotic potential such as the lymphocytic choriomeningitis virus (LCMV) (Ike et al. 2007). Some agents are highly adapted to their host so that they survive outside their host only for short periods of time (Scharmann and Heller 2001) (e.g. *Pasteurellaceae*, mycoplasms, *Streptobacillus moniliformis*). Several publications report that mites are also not easily transmitted to sentinels by used bedding and may therefore not be detected (Lindstrom et al. 2011, Thigpen et al. 1989). This is also true for intestinal flagellates such as *Spironucleus muris* (Perdue et al. 2008).

It may happen that animals develop resistance to certain agents with increasing age. This has been published e.g. for mouse parvovirus (MPV) (Besselsen et al. 2000) and mouse rotavirus (EDIM) (Riepenhoff-Talty et al. 1985). However, reports exist also in which an age-dependent sensitivity to specific agents was not detected (Grove et al. 2012).

Strain-specific sensitivity to various agents has also been described. BALB/c mice proved to be resistant to an experimental infection with *Streptobacillus moniliformis*, while massive clinical signs and death were observed in C57BL/6 mice within few days (Wullenweber et al. 1990). On the other hand, C57BL/6 mice are less susceptible to an infection by MPV so that, compared to other mouse strains (including outbred stocks), a higher viral dose is necessary for an infection and for seroconversion (Besselsen et al. 2000). Vice versa, it was shown by experimental infection with minute virus of mice (MVM) that C57BL/6 mice can easier be infected compared to outbred mice (Janus et al. 2008). These examples demonstrate that the selection of strains used as sentinels can be crucial for the detectability of infections caused by certain agents. Other agents like *Pneumocystis* sp. or *Corynebacterium bovis* are best detected by traditional methods when certain immunodeficient strains are used as sentinels. Finally, it must be considered that seroconversion to several agents may occur very late. Seroconversion to most viral infections is usually detectable within two weeks post infection.

In contrast, agents colonising mucous membranes such as mycoplasms, *Pasteurellaceae* or *Streptobacillus moniliformis* frequently result in seroconversion after 10 weeks or later so that such infections may remain undetected despite long exposure periods of sentinel animals.

The reasons given above show that it is advisable to apply additional measures to increase the reliability of agent detection. In addition to dirty bedding sentinels colony animals should be tested from time to time in order to verify the suitability of a health monitoring programme. Frequently, “hygienic units” can be defined when animals are housed in IVC (e.g., certain experiments or lines). Especially sick animals may give good insight into the infectious situation of a population and should therefore be tested preferentially. Specific IVC systems have been existing in which the exhaust air of all cages was directed through a sentinel cage but this approach did also not lead to the transmission of all agents to sentinels (Compton et al. 2004). Several agents (e.g., *Corynebacterium bovis*) can be directly detected by PCR testing of swabs sampled from inner surfaces of cages. It has been shown for various agents that detection is possible by PCR or similar approaches in dust sampled from exhaust air. Furthermore, ectoparasites can be detected by testing swabs from exhaust air pipes (Jensen et al. 2013). Contact sentinels may be appropriate to test small animal populations, e.g., when genetically modified lines are exchanged between institutions (Lipman and Homberger 2003, Myers et al. 2003). This can be achieved by housing sentinels (usually females to avoid fighting) for 2-3 weeks in the same cage together with the animals to be tested and subsequent housing in a separate cage (eventually use of dirty bedding after the separation). Sentinels can be submitted to laboratory testing after sufficient long exposure time (at least 8-10 weeks after the first contact). It can be expected that this approach leads to transmission of all agents to susceptible sentinels. Alternatively, methods have been developed to detect a large variety of agents in faecal and hair samples and in oral swabs, for example during quarantine (Henderson et al. 2013).

In conclusion, it becomes obvious that sentinel-based health information should be handled with care. This is especially the case for animals housed in IVC’s. It is to be assumed that the relatively high prevalence rate of ecto- and some endoparasites reported in research colonies (Carty 2008, Marx et al. 2017) is a consequence of poor transmissibility to sentinel animals. Their presence in single cages may not be detected so that animals infested with parasites may be shipped. It is therefore advisable to check the microbiological quality of animal shipments upon arrival by appropriate tests whenever there are any doubts regarding the reliability of health information.

A high risk of agent introduction emanates from the frequent exchange of genetically modified animals as these usually originate from experimental units in which unwanted agents are more prevalent compared to breeding populations. It has been shown many times that the health information provided when exchanging animals between research institutions does not reliably allow assessment of their microbiological quality. In addition to problems regarding the sentinel issue, additional factors such as insufficient testing frequency or sample sizes may hamper a reliable quality assessment. Frequently, other information may be insufficiently provided such as details on the health monitoring or sentinel programme. To minimize the risk of agent introduction it is of crucial importance that appropriate quarantine and individual testing of animals are possible. As the health information provided is occasionally not sufficiently meaningful, a quarantine unit must be able to safely house animals for a limited period irrespective of the health information provided so that agent transmission to other animals is impossible.

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