Journal of the American Association for Laboratory Animal Science
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 53, No 6
November 2014
Pages 633–640

Position Statement

AALAS/FELASA Working Group on Health Monitoring of Rodents for Animal Transfer

Kathleen R. Pritchett-Corning (convenor),[1, 2, 3] Jan-Bas Prins (convenor),[4] Ricardo Feinstein,[5] Jeffrey Goodwin,[6] Werner Nicklas,[7] and Lela Riley[8]

The rise of the genetically modified rodent has seen the corresponding rise of shipments of animals around the world. Although there are fewer restrictions on shipment of cryopreserved rodent germplasm, not all institutions have the capacity to either easily generate germplasm from desired mice or to reconstitute germplasm into live animals. Thus, live rodents are still moved internationally and this has necessitated close attention to animal health and animal health reporting. In some cases, regional organizations have suggested minimally acceptable health monitoring (HM) programs as well as reporting formats [7]. The reporting of institutional health monitoring results remains wildly disparate, however, experience from FELASA countries shows that recommendations on a common reporting format may lead to more uniform reporting. Disparity in reporting not only creates uncertainty, but increases the time necessary to critically review results as well as increasing the overall number of communications between institutions as part of the shipping process, both of which slow the acquisition process. In addition, the lack of a reporting standard can lead to critical information being missed, which may result in the introduction of an unwanted agent to a facility. Recently, the FELASA health monitoring recommendations have been revised and are now freely available.[4] In the meantime, a joint working group established by of FELASA and AALAS has evaluated the potential for a common health report to be used for international transfer. This proposed health report is more detailed than that suggested by the FELASA Working Group on Health Monitoring, but is inclusive of the changes that group made.

Recipients of laboratory rodents routinely ask for health information to get an impression of the microbiological status of the shipping institution and thus of the risk of introducing agents into their facility. While animals from commercial breeders are usually of high health quality, animals from universities or research institutions are more frequently colonized or infected by unwanted agents[5; 6; 8-10]. To considerably reduce the risk of agent introduction with these animals it is important that sufficient information on the health status is provided for the colony of origin and that this information is carefully read and critically interpreted. This information should be provided in the form of a health monitoring report, not a collection of laboratory reports. Health monitoring reports should be produced by a person (or office) in charge of the HM program with sufficient understanding and insight into operational procedures in the animal facility, and ideally at least reviewed by the designated veterinarian. They should be made available to interested parties within an institution and when animals are shared between institutions. Data reflecting the health status of animals used in an experiment 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.[3]

It should also be clearly stated that all health monitoring programs have limitations. One of the most basic is time; animals are rarely sampled at exactly the point at which an agent enters a facility and begins to infect animals. This time lag means that the agent often spreads before it is detected. Some other limitations are also known, such as the fact that many agents are inefficiently transmitted by dirty bedding sentinels or that different strains of mice have different susceptibilities to agents. Another common limitation is that using the "ILAR formula" to determine the number of animals chosen for testing, unless very specific conditions apply, may underpower the HM program [1; 2]. Some of this uncertainty may be eliminated with the use of direct animal monitoring via non-terminal PCR samples, which allow samples to be easily taken from valuable research animals. Limitations of direct sampling and testing via PCR include the fact that PCR only detects nucleic acid from the agents, not whether or not the organism is present in an infectious form or dose. For screening, another limitation compared to serology is that in short lasting infections (i.e., MHV) the PCR detects the virus during the period of infection if the correct tissues are examined, but an animal that seroconverts remains seropositive for a long period of time, even after the causative agent has been eliminated from the body. Serological tests, therefore, may find more positive animals than PCR despite the high sensitivity of PCR. Environmental sampling of soiled surfaces, such as IVC rack plenums and filters, which see air from every cage, may be a valuable supplement to sentinel or colony animal testing and may supplant the testing of live animals entirely in the future. Limitations of these newer HM programs have yet to be determined.

Most laboratory animal facilities are compartmentalized which prevents agents becoming a risk for all animals housed in a facility. A site is a clearly defined area within which may be located several facilities with interactions of people and animals. At the site level, common services and procedures may exist that can be used by, or be applicable to several facilities situated on that site. A facility describes a physically separated entity consisting of one or more microbiological units. The term microbiological unit is here understood to describe a self-contained microbiological entity. Space and traffic of animals, personnel, and materiel essentially separate units. The HM program description and HM reports may be customized to

[1]*Harvard University Faculty of Arts and Sciences, Cambridge, MA USA;* [2]*Charles River, Wilmington, MA USA;* [3]*University of Washington, Seattle, WA, USA;* [4]*Leiden University Medical Center, Leiden, Netherlands;* [5]*National Veterinary Institute, Uppsala, Sweden;* [6]*University of California, Irvine, CA, USA;* [7]*German Cancer Research Center, Heidelberg, Germany;* [8]*IDEXX RADIL, Columbia, MO, USA*

the microbiological unit or address the entire facility or site. In practice, definition of each microbiologic unit in facilities having many units can be extremely difficult, e.g., if animals are housed in IVCs. For colonies housed in IVC cages, strict isolation of animals, although it can be achieved, is often impossible with the requirements of breeding and animal management. In this case, the "microbiological unit" is frequently defined at the IVC rack level (sentinels are usually assigned per rack or rack side) or the room level. Regardless, a proper understanding of the local definition of the microbiological units of a facility is essential when evaluating its health monitoring reports.

The list of agents provided on this health report format is a suggestion, based on input from experts associated with both FELASA and AALAS. Although it is impossible to define a complete global and final list of agents which fits all situations and all institutions, this Working Group has provided a list of agents in order to create a standardized health report. The presence or absence of an agent on this list should not imply that facilities should or should not test for that agent. Each institution must define which agents are acceptable or not under certain conditions or for specific experiments or animals, but standardizing the reporting of agents will help both receiving and shipping institutions better understand the microbiological status of animals being shipped. As an example, agents such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* may be considered important for immunodeficient animals as well as for selected groups of immunocompetent animals, but not of significance for certain other animals or experiments. This means that information on the presence or absence of specific agents may not be found on a health report because an agent is not considered sufficiently important. However, the agent should remain on the list, so that receiving institutions can easily evaluate the testing status of a particular agent. In general, testing should be performed for all viruses, bacterial agents and parasites that have a significant pathogenic potential for animals or humans or which can affect physiological parameters and thus influence results of scientific experiments, and for those that are still prevalent in contemporary colonies of laboratory rodents.

However, a health monitoring program must also be flexible and test for agents and include them in a health report if they are associated with lesions or with clinical signs of disease (e.g., *Staphylococcus aureus* in abscesses in immunocompetent mice, or other opportunistic pathogens in immunodeficient animals) or which are otherwise suspected to be important, and for agents that were newly detected or shown to be of general significance (e.g., *Pneumocystis* spp.). The list of agents must therefore be adapted to newer knowledge when appropriate. Exotic agents with expected importance (e.g., *Bordetella hinzii*) should also be mentioned if found. A health report should give information on both agents that were found and on agents for which tests were performed but which were not found.

An agent must be declared on the health report if it is identified and confirmed in one or more of the animals screened. Confirmatory testing is usually initially performed by the testing laboratory and should involve both confirmation of the positive sample by a different method and ideally demonstration of the agent in a second animal/sample, although this is not always possible. Confirmatory testing may also be performed on the same sample or other samples by a second laboratory. Confirmatory testing is usually performed on positive samples which may result in overlooking false negative results. Agents known to be present in a microbiological unit do not need to be monitored at subsequent screens if they are declared in the health report. Once a unit has reported a positive test result, the unit must continue to be reported as positive on subsequent health reports until the organism has been eradicated. Subsequent testing should be used to confirm eradication of the infectious agent(s). For the purposes of the HM report, a unit may be reported as negative for an infectious agent if it is not found during regular testing after the 18 month historical reporting period.

Positive results on a health report do not mean that the animals are not fit for use. Agents that are reported as not tested or positive should be evaluated in the context of the future use of the animals and the overall health status of the microbiological unit for which they are destined. Negative results on a health report may also be misleading in that they indicate the health status of the microbiological unit at the time of sampling, which may have been weeks or months previously.

Methods appropriate for the detection of a specific agent or infection must be applied for health surveillance (serology, bacterial culture, parasitology, molecular methods, histopathology). Methods used may differ in their sensitivity and specificity. High specificity is an advantage, but it may be a relative limitation in PCR or serology for viruses prone to mutate or occurring in many strains. This may, however, be less significant if appropriate confirmatory methods are used in addition to primary tests. It must also be considered that other factors related to a health monitoring program (e.g., sentinel testing vs. testing of colony animals vs. testing of environmental surfaces) may have an effect on the detection of many agents. This can be very important as many agents (e.g., ectoparasites, some respiratory agents) are not easily transmitted to sentinels. Reporting of test methods used for each reported agent is essential for a health report.

Pathogens causing lesions and disease have been successively eradicated from many modern rodent facilities and most agents colonizing laboratory rodents in many countries at the present time do not cause disease or lesions. Therefore, the absence of clinical manifestation or lack of lesions at necropsy has no or very limited diagnostic value. Necropsy results may be reported, however, if this is a normal part of an institution's health monitoring program. Histopathology may detect lesions caused by known agents, but also unexpected infections caused by agents not included in the regular screening lists, or by previously unidentified microorganisms. The results of diagnostic examinations performed on ill animals from the primary colony (i.e., necropsy, histopathology, microbiology, etc.) are therefore an essential complement to regular health monitoring.

Ideally, the number of animals necessary to detect an infection depends on the expected prevalence rate of an infection. Agents with high infectivity (e.g., MHV) spread rapidly and infect more animals and are more likely to be detected than agents spreading slowly (e.g., MPV). In this case more animals need to be tested than in the case of highly infectious agents. Pathogens occurring at a low prevalence in the microbiological unit may give sporadic positive or erratic results (i.e., positive followed by negative at subsequent testing). The true prevalence rate cannot be estimated from the results of health monitoring, and depends on properties of the agents, housing systems (IVC vs. open cages), animal genetics (susceptible vs. resistant host), immune status (immunocompetent vs. immunodeficient), and husbandry procedures. Furthermore, the availability of animals suitable for testing and financial constraints also may limit the number of animals submitted for testing. As a result of these constraining factors, animal numbers usually submitted for testing are too small to allow detection of agents with low prevalence. It is therefore recommended that results from repeated testing are combined into a health report which provides all significant information about the health status obtained during a certain period of time.

Health Monitoring Report for Mice

(This form contains a list of microbial and parasitic agents that may infect mice. Please provide results for agents for which you test. Facilities may not test for all agents. Inclusion of an agent on the list does not imply that it is recommended that testing be performed. Veterinarians at the receiving institution make the determination regarding accepting or rejecting individual shipments. A positive for any agent does not necessarily mean the animals are not fit for use.)

Institution:

Date:

Microbiological Entity/Unit *(choose level suitable for your facility)*

- Building
- Room
- Rack

Entity/Unit type *(mark the correct type)* **:**

- Conventional
- Barrier
- Isolator
- Other

Contact person *(name, e-mail, phone)*

Testing Results:				**Most recent testing**		**Historical results** Collected over ___ months	
	Testing laboratory	**Testing method**	**Tested subject** *(sentinel, colony animal, environmental, etc.)*	**Date**	**No. positives/ No. tested**	**No. positives/ No. tested**	**Testing frequency**
Viruses: all virus names in accordance with ICTV taxonomy 2013 release							
Ectromelia virus[1]							
Hantaviruses							
Lactate dehydrogenase-elevating virus							
Lymphocytic choriomeningitis virus[1]							
Mammalian orthoreovirus type 3[1]							
Mouse norovirus[1]							
Murid herpesvirus 1 (Murine cytomegalovirus)							
Murid herpesvirus 3 (Mouse thymic virus)							
Murine adenovirus (type A and B)[1] (Mouse adenovirus type 1 and 2)							
Murine coronavirus[1] (Mouse hepatitis virus)							
Murine pneumonia virus[1] (Pneumonia virus of mice)							
Murine polyomavirus							
Rodent protoparvovirus 1[1] (Parvoviruses)							
- NS1							
- Mouse parvovirus 1[1]							
- Minute virus of mice[1]							
Rotavirus A[1] (Mouse rotavirus)							
Sendai virus[1]							
Theilovirus[1] (Theiler's murine encephalomyelitis virus)							
Bacteria & fungi:							
β-hemolytic *Streptococcus* spp. (not group D)[1]							
Bordetella bronchiseptica							
Bordetella hinzii							
Cilia-associated respiratory bacillus							
Citrobacter rodentium[1]							
Clostridium piliforme[1]							
Corynebacterium kutscheri[1]							
Helicobacter spp.[1]							
Klebsiella oxytoca							

1 The 2014 FELASA recommendations include this agent

Figure 1. The health report form for mice.

Testing Results (continued):				Most recent testing		Historical results	
						Collected over ___ months	
	Testing laboratory	Testing method	Tested subject (sentinel, colony animal, environmental, etc.)	Date	No. positives/ No. tested	No. positives/ No. tested	Testing frequency
Bacteria (continued)							
Klebsiella pneumoniae							
Mycoplasma pulmonis [1]							
Pasteurella pneumotropica [1]							
Pneumocystis murina							
Pseudomonas aeruginosa							
Salmonella spp.[1]							
Staphylococcus aureus							
Streptobacillus moniliformis [1]							
Streptococcus pneumoniae [1]							
Parasites[1]:							
Mites							
Myobia musculi							
Myocoptes musculinus							
Radfordia affinis							
Pinworms							
Aspiculuris tetraptera							
Syphacia spp.							
Protozoa							
Entamoeba muris							
Giardia muris							
Spironucleus muris							
Tritrichomonas muris							
Other parasites							

Additional agents for which testing has been done:

Pathology:

Additional comments:

(This section may be used to provide additional details regarding diagnostic testing including a brief description of corrective actions taken related to any positive results, dates of treatment, dates of retesting and results and, if appropriate, information on positive test results for other units and/or other species within the facility.)

1 The 2014 FELASA recommendations include this agent

Figure 1. The health report form for mice (cont.).

Health Monitoring Report for Rats

(This form contains a list of microbial and parasitic agents that may infect rats. Please provide results for agents for which you test. Facilities may not test for all agents. Inclusion of an agent on the list does not imply that it is recommended that testing be performed. Veterinarians at the receiving institution make the determination regarding accepting or rejecting individual shipments. A positive for any agent does not necessarily mean the animals are not fit for use.)

Institution:

Date:

Microbiological Entity/Unit *(choose level suitable for your facility)*

- Building
- Room
- Rack

Entity/Unit type *(mark the correct type)*

- Conventional
- Barrier
- Isolator
- Other

Contact person *(name, e-mail, phone)*

Testing Results:				**Most recent testing**		**Historical results**	
			Tested subject			Collected over ___ months	
	Testing laboratory	**Testing method**	*(sentinel, colony animal, environmental, etc.)*	**Date**	**No. positives/ No. tested**	**No. positives/ No. tested**	**Testing frequency**
Viruses: all virus names in accordance with ICTV taxonomy 2013 release							
Hantaviruses[1]							
Lymphocytic choriomeningitis virus							
Mammalian orthoreovirus type 3[1]							
Murine adenovirus (type A and B)[1] (Mouse adenovirus type 1 and 2)							
Murine coronavirus[1] (Rat coronavirus, SDAV)							
Murine pneumonia virus[1] (Pneumonia virus of mice)							
Rodent protoparvovirus 1[1] (Parvoviruses)							
- H1[1]							
- Kilham rat virus[1]							
- Rat minute virus[1]							
- Rat parvovirus[1]							
Sendai virus[1]							
Theilovirus[1] (Rat theilovirus)							
Bacteria and fungi:							
β-hemolytic Streptococcus spp. (not group D)[1]							
Bordetella bronchiseptica							
Bordetella hinzii							
Cilia-associated respiratory bacillus[1]							
Clostridium piliforme[1]							
Corynebacterium kutscheri							
Helicobacter spp.[1]							
Klebsiella oxytoca							
Klebsiella pneumoniae							
Mycoplasma pulmonis[1]							
Pasteurella pneumotropica[1]							
Pneumocystis spp.[1]							
Pseudomonas aeruginosa							
Salmonella spp.[1]							
Staphylococcus aureus							
Streptobacillus moniliformis[1]							
Streptococcus pneumoniae[1]							

1 The 2014 FELASA recommendations include this agent.

Figure 2. The health report form for rats.

Testing Results:	Testing laboratory	Testing method	Tested subject (sentinel, colony animal, environmental, etc.)	Most recent testing: Date	Most recent testing: No. positives/ No. tested	Historical results: Collected over ___ months: No. positives/ No. tested	Historical results: Collected over ___ months: Testing frequency
Parasites[1]:							
Mites							
Myobia musculi							
Myocoptes musculinus							
Radfordia ensifera							
Pinworms							
Aspiculuris tetraptera							
Syphacia spp.							
Protozoa							
Entamoeba muris							
Giardia muris							
Spironucleus muris							
Tritrichomonas muris							
Other parasites							

Additional agents for which testing has been done:

Pathology:

Additional comments:
(This section may be used to provide additional details regarding diagnostic testing including a brief description of corrective actions taken related to any positive results, dates of treatment, dates of retesting and results and, if appropriate, information on positive test results for other units and/or other species within the facility.)

1 The 2014 FELASA recommendations include this agent.

Figure 2. The health report form for rats (cont.).

Health Monitoring Report for Mice

Institution: Hudson University

Date: In the future

Microbiological Entity/Unit

Building: Eastern Laboratory

Room(s): Area 27 (rooms 27 A, B, C)

Entity/Unit type

Conventional

[Barrier]

Isolator

Other

Contact person *(name, e-mail, phone):* W. R. Veterinarian, DVM, docvet@hudson, +1 212 555 1212

Testing Results:

	Testing Laboratory	Testing method	Tested subject (sentinel, colony animal, environmental, etc.)	Most recent tests: Date	Most recent tests: No. positives/ No. tested	Historical results (Collected over 18 months): No. positives/ No. tested	Historical results (Collected over 18 months): Testing frequency
Viruses: all virus names in accordance with ICTV taxonomy 2013 release							
Ectromelia virus[1]				NOT TESTED			
Hantaviruses	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Lactate dehydrogenase-elevating virus	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Lymphocytic choriomeningitis virus[1]				NOT TESTED			
Mammalian orthoreovirus type 3[1]	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Mouse norovirus[1]	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Murid herpesvirus 1 (Murine cytomegalovirus)	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Mouse cytomegalovirus	WRL	ELISA	SENTINEL	2-May-14	0/30	0/30	Yearly
Mouse hepatitis virus[1]	In-house	ELISA	SENTINEL	2-May-14	0/30	0/180	Quarterly
Mouse norovirus[1]				PRESENT IN FACILITY			

Figure 3. A partial mouse health report, with example data supplied. This example illustrates how the health report might be customized for use without changing the list of agents supplied.

The higher the risk of introducing agents into a population the more frequently animals should be tested. Risk factors may include frequent introduction of animals, introduction of unscreened biological materials, or frequent access of research personnel, to name a few. This means that the risk of introducing agents into a closed breeding unit is likely to be far lower than into a multipurpose experimental unit and that less frequent testing may be acceptable. It is common practice that testing is performed at least quarterly. It is reasonable that populations are not tested or tested at a lower frequency (e.g., annually) for agents that have not been found in laboratory rodents for longer periods of time. The recently published FELASA Health Monitoring Recommendations for Rabbits and Rodents [4] reflect these changes in prevalent infectious agents, as does the document proposed by this group. Whatever the frequency of testing chosen, this should be reported on the health report for each agent tested.

Given the statistical limitations of the results, the health report should not be the sole basis of the decision to allow entry of imported animals to a facility. The health history of the facility of origin and its HM program, as well as the risks of possible contamination, should also be taken into account. In addition, the health status of animals may change during transport depending on the type of transport used and the way the animals are packed for transport. Facilities should evaluate the risks inherent in the introduction of animals and develop an appropriate plan (e.g. quarantine/rederivation and testing process). It is convenient to keep in mind that in breeding facilities such as those of a commercial vendor animals live "undisturbed" in a protected environment, whereas animals used in experiments may have been operated, irradiated, or subjected to other procedures or treatments that make them more susceptible to infections. Therefore, in experimental colonies it may be necessary to consider additional agents, perhaps including agents that were insignificant at the colony of origin.

The health report format developed by this group attempts to address these concerns and the basic tenets of health monitoring, while still being simple for the end user. We have created a customizable spreadsheet (Figures 1 and 2), which may be downloaded in Mac or PC format from here (https://www.aalas.org/about-aalas/position-papers/health-reporting-for-international-transfer-of-rodents). Although the form is fully editable, we encourage users not to alter the list of agents. Altering the list of agents defeats the purpose of a standardized reporting format. If the list is altered, it becomes harder to discern which agent is missing from a shipping institution's health monitoring to compare it to the receiving institution's health status. Rather, if an agent is not reported or not monitored by your facility, type NOT TESTED into that agent's row. If an agent is known to be endemic in your facility, number of positives and numbers tested may be reported, or PRESENT IN FACILITY may be typed into that agent's row. Opportunistic bacteria not included in the lists may be important in selected animals or animal models. These organisms could be added under the section labeled "Additional Agents": (i.e., *Enterococcus* spp., *Proteus* spp., etc.) See Figure 3 for an example partial health

monitoring report from a non-existent university, populated with fictional data.

A useful health report does not only give results from testing but should also be accompanied by additional information about housing and maintenance procedures, any treatment provided, and a detailed description of the health monitoring program. It is also important to provide contact data for a competent person who is responsible for the HM program in the animal facility in case additional information is requested. These data are not easily included in a health report, and ideally institutions should also provide a 1-2 page health monitoring program description with the HM results [4]

Both FELASA and AALAS hope that this proposed Health Monitoring Report format will make a significant contribution to the harmonization of reporting on the health status of mouse and rat colonies worldwide. We encourage its use by our colleagues all over the world.

References

1. **Dubin S, Zeitz S**. 1991. Sample size for animal health surveillance. Lab Anim (NY) **20**:29-33.
2. **ILAR**. 1976. Long-term holding of laboratory rodents. ILAR News **19**:L1-L25.
3. **Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG**. 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol **8**:e1000412.
4. **Mähler M, Berard M, Feinstein R, Gallagher A, Illgen-Wilcke B, Pritchett-Corning K, Raspa M**. 2014. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim.
5. **Mähler M, Kohl W**. 2009. A serological survey to evaluate contemporary prevalence of viral agents and Mycoplasma pulmonis in laboratory mice and rats in western Europe. Lab Anim (NY) **38**:161-165.
6. **McInnes EF, Rasmussen L, Fung P, Auld AM, Alvarez L, Lawrence DA, Quinn ME, Utteridge TD, del Fierro GM, Vassallo BA, Stevenson R**. 2011. Prevalence of viral, bacterial and parasitological diseases in rats and mice used in research environments in Australasia over a 5-y period. Lab Anim (NY) **40**:341-350.
7. **Nicklas W**. 2008. International harmonization of health monitoring. ILAR J **49**:338-346.
8. **Pritchett-Corning KR, Cosentino J, Clifford CB**. 2009. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim **43**:165-173.
9. **Schoondermark-van de Ven EM, Philipse-Bergmann IM, van der Logt JT**. 2006. Prevalence of naturally occurring viral infections, *Mycoplasma pulmonis* and *Clostridium piliforme* in laboratory rodents in Western Europe screened from 2000 to 2003. Lab Anim **40**:137-143.
10. **Won YS, Jeong ES, Park HJ, Lee CH, Nam KH, Kim HC, Hyun BH, Lee SK, Choi YK**. 2006. Microbiological contamination of laboratory mice and rats in Korea from 1999 to 2003. Exp Anim **55**:11-16.