

An Overview of Typical Infections of Research Mice: Health Monitoring and Prevention of Infection

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There are many reasons to keep research mice healthy and free from infections. The two most important of these are to protect the health and welfare of research mice and to prevent infections from negatively impacting research. Just as the genetic integrity of a mouse strain will influence the reproducibility and validity of research data, so too will the microbiologic integrity of the animals. This has been repeatedly demonstrated in the literature of laboratory animal sciences wherein the direct impact of infections on physiologic parameters under study have been described. Therefore, it is of great importance that scientists pay close attention to the health status of their research animal colonies and maintain good communications with the animal facility personnel at their institution about mouse health issues. This overview provides information about animal health monitoring (HM) in research mouse colonies including commonly monitored agents, diagnostic methods, HM program, risk assessment, and animal facility biosecurity. Lastly, matters of communication with laboratory animal professionals at research institutions are also addressed. © 2015 by John Wiley & Sons, Inc.

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INTRODUCTION

The quality of animals used in research has a direct impact on the value of that research. In the context of research mice, this means using animals that are genetically appropriate for the specific investigation and free of the variability induced by infections. From a genetic perspective research scientists address animal quality issues by testing their mice for consistency of genetic background, and in the case of genetically engineered mutant mice (GEM), for direct evidence that specific genes of interest are present (or absent in the case of knockouts). It is prudent to do this testing prior to embarking on an expensive research project (Fahey et al., 2013). Similarly, it is prudent to verify the health status of research mouse colonies to exclude mice compromised

by infections. Even in the absence of clinical signs of disease, infections in mice are well known as a source of variability that can have adverse effects on research or may present a health hazard to laboratory staff (i.e., zoonotic infections of mice) and thus should be avoided when doing research.

Prevalence surveys of murine infectious agents taken at research institutions in North America (Carty, 2008), Europe (Mähler and Köhl, 2009), Japan (Hayashimoto et al., 2013), Australasia (McInnes et al., 2011), and Taiwan (Liang et al., 2009) over the past decade have demonstrated that infectious agents are still quite prevalent in research mouse colonies. The species and prevalence of the agents (viruses, bacteria, parasites and fungi) established in these surveys were dependent on



the geographic areas and institutions under study. Nonetheless, these data point out that despite efforts taken at many research institutions worldwide to eliminate infectious agents and elevate the health status of their animal facilities, infectious agents of mice still persist. Moreover, novel infectious agents of mice are being discovered more frequently now as genome sequencing methods become faster and less expensive. Murine astrovirus (MuAstV) is such an example. The virus was first identified in 1985 by electron microscopy following an outbreak of diarrhea in a colony of nude mice and not further characterized until 2013 by use of viral metagenomics (Kjeldsberg and Hem, 1985; Ng et al., 2013). The availability of sophisticated molecular diagnostic techniques, the increased use of immunodeficient, transgenic, and humanized mice, and the increasing exchange of research animals and animal products among laboratories nationally and internationally guarantee that this trend of emerging/re-emerging infectious agents will continue.

To circumvent problems generated by infections in research mice, mouse colonies should be regularly tested for infectious agents and measures taken to prevent, control and eliminate infections. A survey of animal health professionals at research institutions across the U.S. (Carty, 2008) reported that routine HM programs afford the optimal means of detecting disease outbreaks in animal facilities. The Federation of European Laboratory Science Associations (FELASA) regularly emphasizes this fact by meticulously outlining rodent HM guidelines for research animal facilities (Mähler et al., 2014). HM programs are generally based on long-established standardized lists of target organisms (Tables 1 and 2) as well as recently discovered microorganisms. Yet, it is common for institutional laboratory animal veterinarians to tailor HM programs to meet their facilities' specific needs in a cost-effective manner, since the prevalence of murine pathogens varies by institution. Understanding HM programs is essential to the well-being of one's animals as well as the preservation of data integrity. For this reason, it is important, especially for graduate students, postdoctoral fellows, new investigators and others with limited experience in dealing with infections in mouse colonies that they become familiar with the HM programs at their own institutional animal facilities. In addition to discussions with animal health professionals at your research institution, further resources on this subject are available at a

number of laboratory animal associations (see Internet Resources below) and at professional mouse vendors to assist scientists in understanding the vagaries of health surveillance.

GENERAL REVIEW OF MOUSE HM PROGRAMS

When performed regularly, animal HM provides animal facility veterinarians a continuous flow of test data enabling them to assess and adjust preventative medicine programs designed to reduce and eliminate pathogenic organisms. For example, in the 1960's and 1970's, early in the development of HM programs in animal facilities, murine pathogens were very prevalent and the goal of HM was primarily to identify the disease agents causing illness and deaths in mouse facilities. Today, many of these disease agents have been completely eliminated or severely reduced in mouse colonies and monitoring of mice is aimed at exclusion of unwanted infectious agents.

A HM program should contain the selection of infectious agents, diagnostic methods, preventive measures and response plans for dealing with biosecurity breaches and disease outbreaks. There are basic guidelines for establishment of standard HM programs (Mähler et al., 2014). However, designing such programs requires a risk assessment of the potential exposure of mice to infectious agents relevant to each particular institution and therefore must be adaptable and somewhat flexible. A variety of factors must be taken into consideration: the types of studies being conducted, caging systems, numbers and types of mouse strains being housed, local prevalence of specific infectious agents, animal care practices, health history, facility infrastructure, presence of non-murine animal models, and available funding. Typically, as part of the HM planning process, the facility laboratory animal veterinarians engage in dialog with research investigators to acquire detailed information regarding researchers' individual needs. As such, productive collaborations between research scientists and veterinarians are essential for developing and implementing a cost-effective HM program.

With the rise of worldwide exchange of genetically engineered mutant mice (GEM) and biological materials, international harmonization of HM standards has been proposed along with promoting the 3Rs (i.e., replacement, reduction, and refinement) in animal use (Nicklas, 2008; National Centre for the

Table 1 Viral Infectious Agents of Mice

Viral agents	Virion	Risk level ^a /prevalence	Recommended tissues for PCR testing
ssDNA virus			
<i>Parvovirus</i>			
Minute virus of mice	Non-enveloped	B/low	Mesenteric lymph node, spleen, intestine
Mouse parvovirus	Non-enveloped	B/high	Mesenteric lymph node, spleen, intestine
dsDNA virus			
Ectromelia virus	Enveloped	A/low	Spleen, skin lesion, feces
<i>Adenovirus</i>			
Murine adenovirus-1	Non-enveloped	A/low	Lung
Murine adenovirus-2	Non-enveloped	B/low	Feces, intestine
<i>Herpesvirus</i>			
Murine cytomegalovirus	Enveloped	B/low	Salivary gland, spleen
Mouse thymic virus	Enveloped	B/low	Salivary gland
<i>Polyomavirus</i>			
Mouse polyomavirus	Non-enveloped	C/low	Mammary gland, skin
ss(+)RNA virus			
<i>Coronavirus</i>			
Mouse hepatitis virus (polytropic)	Enveloped	A/high	Mesenteric lymph node, feces, lung
Mouse hepatitis virus (enterotropic)	Enveloped	B/high	Mesenteric lymph node, feces
Murine norovirus	Non-enveloped	D/high	Feces, intestine
Murine astrovirus	Non-enveloped	D/high	Feces, intestine
Theiler's murine encephalomyelitis virus	Non-enveloped	B/low	Feces, intestine
Lactate dehydrogenase-elevating virus	Enveloped	B/low	Spleen
ss(-)RNA virus			
Lymphocytic choriomeningitis virus	Enveloped	A/low	Kidney, urine, blood
Sendai virus	Enveloped	A/low	Trachea, lung
Hantavirus (ambisense ss RNA ±)	Enveloped	A/low	Trachea, lung
Pneumonia virus of mice	Enveloped	C/low	Trachea, lung
dsRNA virus			
Mouse rotavirus (EDIM)	Non-enveloped	B/high	Feces, intestine
Reovirus	Non-enveloped	C/low	Feces, liver, lung

^aRisk level group definitions: A: risk to most research or is a human zoonotic risk; B: risk to research but not zoonotic; C: minimal risk to research and uncommon; D: recently discovered viruses with little or no known risk to research.

Table 2 Common Bacterial, Mycoplasmal, Fungal, and Parasitic Infectious Agents of Mice

Agent	Sample	Methods of diagnosis
Bacteria		
<i>Bordetella spp.</i>	Respiratory swab, wash fluid	Microbiologic culture
Cilia-associated respiratory bacillus (CAR bacillus)	Serum/respiratory swab/respiratory wash fluid	MFI ^a , ELISA ^b , IFA ^c , PCR ^d
<i>Citrobacter rodentium</i>	Colon or cecum/feces	Microbiologic culture
<i>Clostridium piliforme</i>	Serum	MFI ^a , ELISA ^b , IFA ^c
<i>Corynebacterium bovis</i>	Skin scrape/swab, oropharyngeal swab	Microbiologic culture; PCR ^d
<i>Corynebacterium kutscheri</i>	Oropharyngeal swab	Microbiologic culture; PCR ^d
<i>Helicobacter spp.</i>	Colon or cecum/ feces	PCR ^d
<i>Klebsiella pneumoniae</i> <i>Klebsiella oxytoca</i>	Oropharyngeal swab/colon or cecum/feces	Microbiologic culture; PCR ^d
<i>Mycoplasma pulmonis</i>	Serum/oropharyngeal or nasal swab/lung wash	MFI ^a ; PCR ^d ; microbiologic culture
<i>Pasteurella pneumotropica</i>	Oropharyngeal swab/colon or cecum/feces	Microbiologic culture; PCR ^d
<i>Proteus mirabilis</i>	Oropharyngeal swab, colon or cecum/feces	Microbiologic culture; PCR ^d
<i>Pseudomonas spp.</i>	Colon or cecum/feces	Microbiologic culture; PCR ^d
<i>Salmonella spp.</i>	Colon or cecum/feces	Microbiologic culture
<i>Staphylococcus aureus</i>	Oropharyngeal swab	Microbiologic culture; PCR ^d
<i>Streptobacillus moniliformis</i>	Oropharyngeal swab	Microbiologic culture
<i>Streptococcus spp.</i>	Oropharyngeal swab	Microbiologic culture; PCR ^d
Fungi		
<i>Encephalitozoon cuniculi</i>	Serum/urine/kidney/brain	MFI ^a ; PCR ^d
<i>Pneumocystis murina</i>	Lung	PCR ^d
Parasites		
A. Endoparasites		
<i>Spironucleus muris</i>	Cecum/colon	Microscopy; PCR ^d
<i>Trichomonas spp./flagellates</i>	Cecum/colon	Microscopy; PCR ^d
<i>Aspicularis tetraptera</i>	Cecum/colon	Microscopy; PCR ^d
<i>Syphacia obvelata</i>	Cecum/colon/anal tape sample	Microscopy; PCR ^d
<i>Rodentolepis nana</i>	Intestine	Microscopy; PCR ^d
B. Ectoparasites		
<i>Myobia musculi</i>	Fur	Direct microscopy
<i>Mycopetes musculinus</i>	Fur	Direct microscopy
<i>Radfordia affinis</i>	Fur	Direct microscopy

^aMFI: multiplex fluorescent immunoassay.

^bELISA: enzyme-linked immunosorbent assay.

^cIFA: indirect immunofluorescence assay.

^dPCR: polymerase chain reaction.

Replacement, Refinement & Reduction of Animals in Research, 2014). Pharmaceutical companies and commercial vendors have made efforts toward such standards based on recommendations by FELASA and other international institutions. However, HM programs in research institutions and universities are more complex than commercial vendors, given the many different factors to be considered as mentioned above. Universal standards will thus not be applicable or feasible.

INFECTIOUS AGENTS OF RESEARCH MICE

Viral Infections of Mice

Table 1 summarizes viruses that are commonly tested for in-mouse HM programs. Viral infections of mice are the greatest concern to laboratory animal veterinarians and animal facility personnel because of their potential for spread across a facility, the diseases they can cause and their impact on research. There are seventeen viral species in Table 1 many of which have substrains or additional serotypes that infect mice. However, the prevalence of these viruses varies greatly, with some no longer found in research animal facilities. Some viral infections, such as murine cytomegalovirus (MCMV), mouse thymic virus (MTV), murine polyomavirus (MPyV), ectromelia virus (ECTA), reovirus (REO) have declined significantly since the 1990's and have become rare or nonexistent in mouse colonies. Based on serological or polymerase chain reaction (PCR) data from the past fifteen years (Weisbroth, 1999; Prichett-Corning et al., 2009; Mähler et al., 2014), the most prevalent viral agents in contemporary mouse facilities are mouse norovirus (MNV), mouse hepatitis virus (MHV), mouse parvovirus (MPV), and mouse rotavirus (MRV, known as epizootic diarrhea of infant mice, EDIM). Agents such as MNV and MPV are shed in mouse feces for as long as 2 months, even by immunocompetent mice. Additionally, small non-enveloped viruses (i.e., MPV, MNV, MRV) are very resistant to chemical or environmental inactivation. In contrast, although MHV does not remain active for long periods in the environment, it is highly contagious and thus continues to be a major infectious agent in mouse facilities, especially in conventionally housed mice. It is likely that some agents may be prevalent simply because they are newly recognized (i.e., MNV, MuAstV; Kjeldsberg and Hem, 1985; Karst et al., 2003; Ng et al., 2013). A recently

discovered murine virus, murine astrovirus (MuAstV; Ng et al., 2013) has not yet been the subject of widespread surveillance so the prevalence of this virus in research animal facilities is unknown at this time.

The significance of these viral infections to scientists is their potential impact on research, which varies depending on route of infection (i.e., natural versus experimental infection), host factors (i.e., mouse strain, age, immune competency), and virus factors (i.e., virulent versus avirulent strain; Fox et al., 2006; Percy and Barthold, 2007; Besselsen et al., 2008; Mähler et al., 2014). Laboratory animal veterinarians often classify murine viruses into risk groups according to the significance of interference with research, infectivity and potential for spread and difficulties with detection and elimination. In Table 1, a simple classification scheme of A to D is based on these criteria. Viruses in groups A and B are more likely to adversely affect research and for group A, present the risk of human infection. Viruses in group C have minimal impact on research and are not common whereas those in group D are recently discovered viruses whose impact on research is not well known. For example, lymphocytic choriomeningitis virus (LCMV) and hantavirus are at risk level A because of their zoonotic potential, that is, their ability to infect humans as well as mice. A few other viral agents, including murine adenovirus-1 (MAdV-1), ECTA, MHV polytropic strains, and Sendai virus (SV), are also classified as risk level A because they are capable of causing clinical illness in both immunocompetent and immunodeficient adult mice. Viruses at risk level B may interfere with specific experiments involving the host immune system (parvovirus, MCMV, MTV, lactate dehydrogenase-elevating virus [LDEV], MHV enterotropic strains), central nervous system (Theiler's murine encephalomyelitis virus, TMEV), or gastrointestinal systems (MAdV-2, MRV).

Natural transmission of murine viruses occurs most commonly through the fecal-oral route or fomites (e.g., MAdV, ECTA, MNV, MuAstV, EDIM, TMEV, MHV, REO). Other transmission routes include direct contact (e.g., parvovirus, MTV, MCMV, LCMV), respiratory (e.g., MPyV, pneumonia virus of mice [PVM], SV, MHV), and vertical transmission (e.g., LCMV, LDEV; Fox et al., 2006; Percy and Barthold, 2007).

Contaminated biological materials are a common source for inadvertent viral infection during experimentation. For example murine

tumors, hybridomas, ascites fluid, embryonic stem cells, in vitro fertilized embryos, gametes, cell lines, and even viral stocks introduced into mice can cause infections unless the materials are screened for infectious agents first (Nicklas et al., 2010). This is particularly important for banked biologicals that may have been frozen before an infection was detected in the mouse from which the materials were derived, or before sufficiently sensitive tests were available for detection of the specific infectious agent.

Bacterial, Fungal and Parasitic Infections of Mice

Table 2 demonstrates species of bacteria, fungi and parasites for which research mice are commonly tested. Regarding bacterial infections, the actual species of bacteria isolated from infected mice vary to a great extent depending on the genetics and phenotype, age, and immune status of the mice, as well as environmental factors such as heating, ventilation, and air conditioning (HVAC), human traffic flow, husbandry practices (e.g., bedding type, caging system), preparation of animal supplies (sterile versus non-sterile), and human contact with mice. For example, virtually all mouse strains are susceptible to infection with opportunistic bacteria such as *Staphylococcus aureus*, *Klebsiella oxytoca*, *Pasteurella pneumotropica*, *Proteus mirabilis* and *Helicobacter spp.* and these species are commonly found in many mouse facilities (Prichett-Corning et al., 2009; Treuting et al., 2012). Yet, taking certain precautions in mouse-housing facilities can substantially reduce the prevalence of opportunistic bacterial infections. This topic will be discussed below under disease prevention. The preponderance of bacterial infections of mice is non-lethal, subclinical infections. Asymptomatic mice may only be identified at the time of health monitoring when microbial cultures or PCR yield positive results. In a number of animals, however, infections with bacterial opportunists can present as clinical cases, such as bite-wound and retrobulbar abscesses, conjunctivitis, otitis, rectal prolapse and other overt signs of infection (Treuting et al., 2012).

Fungal infections of mice are less common than bacterial infections. The two principle fungal infections of mice are caused by *Encephalitozoon cuniculi* and *Pneumocystis murina*. These are two unusual organisms previously considered to be protozoan parasites, but recently reclassified as fungi based on genomic sequencing data (Katinka et al., 2001; Chabe et al., 2011). Generally, *Encephalito-*

zoon infections are more of a concern in rabbits; however, mice can be susceptible to infection. *Pneumocystis* infections are of greatest concern in immunodeficient mouse strains as described below in the section Monitoring Immunodeficient and GEM Strains.

Both endo- and ecto-parasite infections of mice are still prevalent in research mouse facilities, but their rate of prevalence is far below the more common bacterial and viral infectious agents discussed above (Prichett-Corning, et al., 2009). The existence of parasitic infections in research mouse facilities may be the result of unidentified or untreated enzootic infections of existing mouse colonies; most mice infested with endo- and ecto-parasites are asymptomatic and may harbor a low parasite burden that is difficult to detect via standard HM methods. Other factors that may contribute to the continued presence of parasites in some research colonies may include unregulated movement of mice between institutional animal facilities and a lack of proper quarantine procedures that allow for ample time for testing or treatment of imported mice. Additionally, pinworm infections may be particularly problematic due to treatment failure, persistence of eggs in the environment and diagnostic inefficiencies. Although parasitic infections do not typically cause clinical signs in either immunocompetent or immunodeficient mice, there is evidence that these infections do cause physiologic changes that may significantly impact research studies (Beattie et al., 1980; Bugarski et al., 2006; Michels et al., 2006).

Principal Diagnostic Methods for Infectious Agents of Mice

The standard practice at most research institutions that house mice is to submit HM samples, or mice, to commercial diagnostic laboratories (see Internet Resources for commercial diagnostic laboratories). Some research institutions have in-house laboratory animal diagnostic laboratories, however, these are generally small laboratories that lack the scientific capabilities of the commercial labs. Nonetheless, in-house laboratories can provide quick answers to pressing questions about infections in mice by providing microbiologic culture, or other diagnostic procedures that do not require an intensive investment in scientific instrumentation as is done at commercial laboratories. The information below provides insight into the choice of diagnostic methods used in commercial diagnostic laboratories and the rationale

for their use in determining whether or not an infection is present in a mouse colony.

Serology

Serology is the primary laboratory method used for monitoring viral infections in mouse colonies. Several non-viral infectious agents of mice can also be detected by serology because these infectious agents also reliably provoke an antibody response in immunocompetent mice (e.g., *Mycoplasma pulmonis*, Cilia-associated respiratory [CAR] bacillus, *Clostridium piliforme* and *E. cuniculi*; Table 2). The benefits of serologic testing are that it is relatively inexpensive, yet provides information about current and previous infections that have occurred in a mouse colony because serum antibodies generally persist in mice for months after resolution of an infection. Additionally, current serologic test platforms such as the enzyme-linked immunosorbent assay (ELISA) and multiplexed fluorometric immunoassay (MFI, Luminex) enable the detection of multiple infectious agents from a single serum sample. Both assay platforms can also be used as high throughput systems and are the most cost-effective diagnostic systems for large-scale surveillance. Another serology test, the indirect immunofluorescence antibody (IFA) assay is often used to confirm equivocal results of ELISA or MFI because of its sensitivity and specificity. However, performing IFAs is labor intensive and highly dependent on the technical expertise of the observer, so it is not used as frequently as ELISA or MFI. Commercial diagnostic laboratories will provide the user information on how to prepare blood samples for serology testing as well as providing shipping forms and packaging. Furthermore, commercial diagnostic laboratories will provide information on choosing the best serologic test for a given circumstance.

Molecular diagnostics

PCR is the most common molecular assay for detection of DNA agents (DNA viruses, bacteria, parasites) and reverse transcription (RT)-PCR for RNA viruses used in laboratory animal diagnostic laboratories. These assays detect a specific region of genomic nucleic acid (DNA or RNA) of infectious agents and can be used at any time during active infection. Confirmatory tests for positive PCR results include direct sequencing of PCR-amplified DNA fragments, an alternative PCR assay with the same DNA/RNA, or the same PCR assay with different specimens. PCR has been increasingly used as a part of laboratory animal

HM programs because of its significant advantages over serology or microbiologic culture (Compton and Riley, 2001). PCR is also valuable as a noninvasive antemortem test that has proved both practical and useful for detection of certain infectious agents shed in fecal samples (Tables 1 and 2).

The major limitation of PCR as a diagnostic tool is that for most agents, PCR can only detect active infections. Therefore, PCR is best used as part of routine HM to detect the presence of unknown infections in samples regularly submitted to a diagnostic laboratory. PCR is, however, especially effective in detecting infections in immunodeficient, GEM, and aged mice since infections often persist in these mice and the probability of detecting a pathogen is increased. Since PCR is used to detect the infectious agent itself, tissue selection for PCR testing requires that the individual making this selection has an understanding of the pathogenesis of the specific infectious agent so that tissue tropism and duration of infection are taken into account when deciding on the most appropriate sample to collect (Tables 1 and 2). Another limitation of PCR is its dependence on proper sample handling during collection and processing to avoid cross-contamination or sample degradation, which may result in false-positive or false-negative results.

Microbiologic culture

Microbiologic culture is utilized for detection of bacteria and fungi in samples from mice. Samples are cultured in nutrient broth and then subcultured on nutrient agar plates or selective media in agar when there is increased growth (seen as turbidity) in broth cultures. The presence of bacteria or fungi is assessed by the development of colonies on agar plates. Bacteria of interest can be further identified to species level using instruments such as the VITEK identification card system (BioMérieux), biochemical tests, or simply by colony characteristics (e.g., morphology, color, smell). Sample source and properties of bacteria being monitored determine types of medium (selective or differential) and incubation conditions (aerobic or anaerobic; Fox et al., 2006; Percy and Barthold, 2007). Microbiologic culture and identification of bacteria and fungi is labor, equipment and expertise intensive.

Parasitology

Direct microscopic examination of fresh samples is frequently used as the primary

detection method for identification of endo- and ecto-parasites. Endoparasites such as *Trichomonas* can be identified in cecum and colon tissues minced in saline. The same is true for other protozoan parasites. Identification of pinworms and pinworm eggs can also be done microscopically. To enhance this process the eggs can be concentrated by pre-treatment of gut content samples with zinc or sodium sulfate whose specific gravities enable the eggs to “float” on top of the medium after centrifugation. The eggs are then adhered to a glass cover slide, attached to a microscope slide and observed under a microscope. Ectoparasites such as fur mites are also identified by direct microscopic evaluation of mice or of plucked fur samples from their head and neck. In all cases, expertise in microscopic identification of parasites is required to reliably confirm the presence of parasites. Currently, the trend in parasite diagnostics is leaning towards the use of PCR as the preferred method of detection.

Monitoring Immunodeficient and GEM Strains

Immunodeficient and GEM strains have greater susceptibility to infectious agents than their immunocompetent counterparts and this must be taken into consideration when testing them for infectious agents. For example, *P. murina* is a ubiquitous opportunistic pathogen that in immunocompetent mice produces a clinically silent infection controlled by a T-cell mediated immune response. However in many immunodeficient mouse strains, *Pneumocystis* produces lethal pulmonary infections (Weisbroth, 2006). Since many GEM strains are “immunovague” (Treuting et al., 2012), that is, have an unknown or variable capacity to mount an effective acquired immune response, these strains should be considered potentially susceptible to lethal *Pneumocystis* infections. Currently, the best method of identifying *Pneumocystis* in mice is via PCR on a sample of lung tissue from potentially affected animals. This requires that the mice be humanely euthanized and a piece of lung submitted to a diagnostic laboratory. Ideally, for routine health surveillance of immunodeficient mouse colonies, mice taken directly from immunodeficient stocks or immunodeficient sentinel mice should be tested regularly for the presence of *Pneumocystis* in a mouse room.

Immunodeficient mice are also generally more susceptible to infections with opportunistic bacteria than are their immunocompetent counterparts. Abscesses, bite-wound infections, otitis media, and unthrifty appear-

ance due to internal infections can result from exposure of immunodeficient mouse strains to opportunistic bacteria that might otherwise be non-pathogenic in immunocompetent mice. *Helicobacter spp.*, *K. oxytoca*, *Klebsiella pneumoniae*, *S. aureus*, some species of coagulase negative *Staphylococcus*, *Pasteurella pneumotropica* and *Corynebacterium bovis* are among the more commonly identified opportunistic bacteria in infected immunodeficient mice. Sources of these bacteria (see Table 2) include the local environment, human caretakers and research personnel, unsterile materials (e.g., feed, caging, bedding) that have not been sterilized.

Infectious viral agents usually cause acute infection in immunocompetent mice, followed by complete recovery without clinical signs. In contrast, infections in immunodeficient mice may be asymptomatic and persistent. The mice become chronic carriers of virus and serve as a source of infection for other colonies, especially if the virus is shed in feces.

Prevention of Infections

Measures designed to prevent infections of research mice ensure the health and welfare of these animals that are valuable investments both experimentally and financially. Biosecurity measures, that is, the sum of risk management practices, employ barrier systems to minimize the risk of introduction (bio-exclusion) and spread (bio-containment) of infectious agents within or between laboratory animal units and can be implemented at all levels of a research animal facility from building design to personnel activity in a mouse room. Examples of biosecurity applied at the building level are high-efficiency particulate air (HEPA) filtration of room air, room access controls for personnel so that only approved individuals can enter a mouse room, detailed procedures for movement of personnel and mice from room to room, use of construction materials that can withstand repeated chemical disinfection, and physical separation of areas where clean, sterilized caging materials are prepared and handled away from mouse waste and dirty cages. Generally speaking, research investigators, postdoctoral fellows and graduate students are not involved in the oversight or use of biosecurity measures at this level. These are overseen by the facility management staff. Nonetheless, especially for the novice mouse user, you can discuss the building level biosecurity measures at your institution with the facility

management staff to gain an understanding of the current measures in place.

Mouse room level biosecurity is very critical and presents an opportunity for mouse users to participate in the protection of their animals from exposure to infectious agents. Mice are generally housed in either conventional cages with or without a filter top and placed on racks in an open room, or in individually ventilated cages (IVC), or rack and cage systems in which the cage (with or without filter) is securely attached to a specially designed rack that provides HEPA-filtered input and exhaust air with no exposure to room air. IVC caging systems are considered very secure with regard to exposure of mice to adventitious infectious agents. Though IVC caging offers greater protection of mice to inadvertent exposure to infectious agents, for both cage types, IVC and conventional, the real risks are associated with handling of mice during cage changes, experimental use, and other handling that present opportunities for exposure of mice to infectious agents. Thus, applying strict biosecurity steps at these critical junctures pays off by reducing the risk of an outbreak.

What steps can help? Direct human contact with mice is inadvisable as it increases the risk of transmission of human opportunistic bacteria to the mice by exposure to human skin, breath, nasal or oral secretions. Forceps that have been treated with a disinfectant, or disinfected gloved hands should be used to pick up and handle mice. The specific combination of personal protective equipment (PPE) varies with the institution and is in most cases chosen by the animal facility and veterinary staff; however, in general, personnel that routinely work with mice should always wear gloves and a clean lab coat or scrubs. Mask, bonnet, and shoe covers or dedicated mouse room shoes should be worn in higher health status rooms. Immunodeficient and GEM mice housed in high level barrier rooms generally require more stringent PPE, such as sterilized PPE that may also include face shields or powered air-purifying respirators (PAPRs) to further prevent inadvertent exposure of the mice to infectious agents. Additionally, sterilization of all of the materials to which mice in high barrier rooms are exposed (cages, bedding, feed and water) is performed to eliminate infectious agents that may be present in these materials. The specific PPE you use in preparation for handling mice should be discussed with your animal facility staff.

Following institutional biosecurity practices is prudent, as each of the PPE items mentioned serves to reduce exposure of mice to human microflora and mouse pathogens, as humans may also serve as unintentional fomites for agents that were acquired in the environment outside of the mouse facility.

Further biosecurity steps should be taken during experimental manipulation of mice and movement of animals back and forth to a laboratory, or during movement between mouse rooms. Research procedures ideally should be done in a mouse-room-associated procedure area to avoid transporting the mice out of the housing room. The procedure room and equipment should be routinely disinfected by the users to prevent the transmission of infectious agents between mice from different projects or, in the case of shared procedure rooms, between mice from different mouse rooms. In those cases in which mice are taken from a mouse room to a research laboratory for experimental manipulation, the same precautions for handling the mice in a mouse room apply to handling the mice in a laboratory. Additionally, if possible, the mice should be returned to a mouse room dedicated for the purpose of housing mice that have been in a laboratory or otherwise out of the mouse room of origin. Check with the animal facility staff at your institution to determine if such facilities are available.

It is also important to be aware of institutional procedures pertaining to the movement of mice between different housing rooms. While it would be ideal for all research mouse colonies to be free of all infectious agents and to maintain them using stringent biosecurity measures, this is often not feasible in many institutions due to the high cost of maintaining such operations on a daily basis. Therefore, physical separation of mice based on health status and regulation of mouse movement and human traffic is another biosecurity measure that is employed at many institutions. For this reason, movement of mice between mouse rooms is inadvisable unless it is a controlled move sanctioned by the veterinary staff. Additionally, each mouse room may have its own environmental microflora as well as that present in the mice. For this reason, moving mice around to different rooms has the potential of altering that microflora, which is a change that could impact research results.

It is not in the purview of this article to provide an exhaustive list or discussion of biosecurity procedures. However, it is

important to note that testing all biologicals used in mice (e.g., cells, antibodies, proteins, other biological molecules) for the presence of pathogens is critical for the prevention of inadvertent transmission of infectious agents. Furthermore, quarantine of newly imported mice and testing them for infections prior to release into the general population will prevent the transmission of infections potentially carried by these mice. We strongly advise scientific personnel to contact their animal care staff for information on the biosecurity procedures at their institution.

Communication between Researchers and Animal Care Staff

Although modern detection methods and HM programs have facilitated the eradication of many infectious agents that were common in research colonies decades ago, contemporary research colonies require collaborative efforts in order to prevent unexpected outbreaks.

Generally speaking, animal care personnel consists of veterinarians, facility managers, and animal caretakers, who all share the common goal of ensuring research integrity through the proper care of research mice. To achieve this goal, the animal care staff must be familiar with each investigators' research animal models and objectives. Communication between research personnel and members of the animal care staff plays a critical role in maintaining appropriate HM programs, biosecurity practices, and quarantine procedures that serve to prevent infectious outbreaks. Effective communication between research and animal care personnel ensures that the HM program and biosecurity measures employed are suitable for the types of research mice within a given mouse room and that health testing is properly performed, test results are correctly interpreted, and unforeseen events within the animal facility are promptly dealt with. When unexpected events occur, such as discovery of ill or dead animals or issues with the mouse housing room (e.g., variations in temperature, light, humidity), it is recommended that research personnel discuss the issues with the facility staff immediately to get advice and assistance in dealing with the situation.

The animal care staff can also assist new members of the scientific community in becoming familiar with the use of mice in their research. For example, research institutions have required training programs for new animal users. These programs present an opportunity for scientists to find out pertinent infor-

mation about the overall facility, the animal health programs and the legal and institutional requirements for using mice. We urge you to communicate regularly with the animal care staff at your institution to maintain an open dialog about your research needs and to get updated information about the health status of the facility.

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INTERNET RESOURCES

- <http://www.felasa.eu/recommendations/recommendation/recommendations-for-health-monitoring-of-rodent-and-rabbit-colonies/>
- <http://dels.nas.edu/ilar>
- <http://www.aclam.org/>
- [https://www.aalas.org/Guidelines for HM Programs/Laboratory Animal Health](https://www.aalas.org/Guidelines%20for%20HM%20Programs/Laboratory%20Animal%20Health).
- <http://www.criver.com/products-services/basic-research/health-monitoring-diagnostic-services>
- http://www.idexxbioresearch.com/radil/Health_Monitoring/Health_Monitoring_Overview/
- Commercial Laboratory Animal Diagnostic Laboratories.*
- <http://jaxmice.jax.org/genetichealth/index.html>
- <http://www.taconic.com/breed-your-model/health-testing>
- <http://www.criver.com/products-services/basic-research/health-reports>
- http://www.harlan.com/about_harlan_laboratories/quality_programs
- Mouse Vendor Health Reports.*