Diagnostic Testing of Mouse and Rat Colonies for Infectious Agents

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Rodent health monitoring programs make an essential contribution to biomedical research by identifying the presence of infectious agents that might confound animal research. The authors discuss the types of diagnostic tests available, which agents deserve monitoring, and the appropriate frequency for such interventions.

Infectious diseases of mice and rats are important to the scientific community because they can introduce unwanted variables that can alter experimental outcomes. The goal of laboratory animal facilities is to maintain disease-free animals so as to eliminate these unwanted variables, and the goal of rodent health monitoring programs is to determine the presence or absence of pathogenic microbes (viruses, bacteria, endoparasites, ectoparasites, protozoa, and fungi) within colonies of laboratory rodents. Because most of the pathogens of laboratory animals do not cause overt clinical disease, identification of these important pathogens depends on a variety of specialized diagnostic tests. Characteristics central to the success of monitoring mouse and rat colonies for infectious agents include knowledge of the pathogenesis, epizootiology, and prevalence of the infectious agents; also necessary are familiarity with available diagnostic tests and access to specific information about the husbandry conditions of individual animal groups. This information will ultimately dictate which agents to survey, how frequently testing is needed, and what method of testing to employ. This article focuses on available testing methodologies, and general issues to consider when choosing the agents to be monitored and the frequency of testing. In addition, the reader will obtain current information on the prevalence of infectious agents as an indicator of the potential risk of these infectious agents invading a research mouse or rat colony.

Testing Methodology Options

The tests available for evaluating mice and rats for infectious agents are diverse, and variables such as cost, sample requirements, and the potential for the test to detect the presence of or exposure to the agent will determine the type of test selected for each infectious agent. Common primary testing methods and available confirmatory testing methodologies for the various infectious agents of mice and rats appear in Table 1.

Serology

Serology continues to be the primary means of testing rodent colonies for exposure to all viruses and a few bacteria, largely because serological tests are sensitive and specific, are relatively inexpensive, and allow screening for a multitude of infectious agents with one serum sample. Standard serological assays used for rodent health monitoring detect antibodies against infectious agents. Of the many available serological formats, enzyme-linked immunosorbent assays (ELISA) are the most commonly used primary tests because they are easily automated for high-throughput testing and the results obtained provide an objective measure of the immunoreactivity of the sample. Although indirect fluorescent antibody (IFA) assays are the most commonly used secondary serological tests because of their sensitivity, they are more labor-intensive than ELISAs, and interpretation of the results is subjective.

Development of pathogen-specific serum antibodies requires the generation of an immune response that usually occurs 5–10 days after exposure to a pathogen. Circulating antibody titers typically persist for months after infection. In practice, testing animals less than two weeks after exposure to a pathogen often will result in negative results because the animals have not had sufficient time to develop detectable titers of pathogen-specific antibodies. Thus, serology is a valuable tool that provides an indirect measure of past exposure to an infectious agent, but can fail to identify an...
The need to detect viral, bacterial, fungal, and protozoal infections in laboratory mice and rats has led to the development of polymerase chain reaction (PCR) assays. These assays directly test for the presence of nucleic acids from the infectious agent. PCR will detect DNA, and reverse transcriptase PCR (RT-PCR) will detect RNA. In this discussion, the term PCR will refer to both PCR

**TABLE 1. Commonly used testing methodologies for mouse and rat pathogens**

| Agent (species) | Species* | Primary testing methodology (sample tested) | Confirmatory testing methodology |
|----------------|----------|---------------------------------------------|---------------------------------|
| **Viruses**    |          |                                             |                                 |
| Ectromelia     | M        | Serology (serum)                            | PCR, Histology                  |
| Hantaan (HTN)  | R        | Serology (serum)                            | PCR                             |
| K virus        | M        | Serology (serum)                            | PCR                             |
| Lymphocytic choriomeningitis virus (LCMV) | M, R | Serology (serum) | PCR |
| Lactate dehydrogenase-elevating virus (LDEV) | M | Bioassay (serum) | PCR |
| Mouse adenovirus 1 (MAD 1) | M, R | Serology (serum) | PCR |
| Mouse adenovirus 2 (MAD 2) | M | Serology (serum) | PCR, Histology |
| Mouse cytomegalovirus (MCMV) | M | Serology (serum) | PCR, Histology |
| Mouse hepatitis virus (MHV) | M | Serology (serum) | PCR, Histology |
| Mice minute virus (MMV) | M | Serology (serum) | PCR |
| Mouse parvovirus (MPV) | M | Serology (serum) | PCR |
| Mouse rotavirus (MRV) | M | Serology (serum) | PCR, Histology |
| Mouse thymic virus (MTV) | M | Serology (serum) | PCR |
| Pneumonia virus of mice (PVM) | M, R | Serology (serum) | PCR |
| Polyoma virus (polyoma) | M | Serology (serum) | PCR |
| Rat coronavirus (RCV) | R | Serology (serum) | PCR, Histology |
| Rat paroviruses (rat parvos) | R | Serology (serum) | PCR |
| Reoivirus type 3 (REO 3) | M, R | Serology (serum) | PCR |
| Sendai virus (Sendai) | M, R | Serology (serum) | PCR |
| Thielier’s murine encephalomyelitis virus (TMEV) | M, R | Serology (serum) | PCR |
| **Bacteria**   |          |                                             |                                 |
| Cilia-associated respiratory (CARI) bacillus | M, R | Serology (serum)/PCR (trachea) | Histology |
| Citrobacter rodentium | M | Culture (cecal contents) | PCR, Histology |
| Corynebacterium kutscheri | M, R | Culture (NP)* | PCR |
| Helicobacter spp. (any) | M, R | PCR (feces) | Culture |
| Helicobacter hepaticus | M, R | PCR (feces) | Culture |
| Helicobacter bilis | M, R | PCR (feces) | Culture |
| Helicobacter typhlonius | M, R | PCR (feces) | Culture |
| Helicobacter rodentium | M, R | PCR (feces) | Culture |
| Helicobacter sp. unidentified | M, R | PCR (feces) | Culture |
| Mycoplasma pulmonis | M, R | Serology (serum)/PCR (NP) | Culture |
| Pasteurella pneumotropica | M, R | Culture (NP) | PCR |
| Proteus mirabilis | M | Culture (cecal contents) | PCR |
| Pseudomonas aeruginosa | M | Culture (cecal contents) | PCR |
| Salmonella spp. | M, R | Culture (cecal contents) | PCR |
| Clostridium piliforme | M, R | ELISA (serum) | PCR, Histology |
| **Parasites**  |          |                                             |                                 |
| Aspiculuris tetraperta | M | Direct exam (colon contents) | Direct exam (fecal floatation) |
| Myobia musculi | M | Direct exam (pelage) | Direct exam (pelage) |
| Myocoptes musculinus | M | Direct exam (pelage) | Direct exam (pelage) |
| Radfordia affinis | M, R | Direct exam (pelage) | Direct exam (pelage) |
| Radfordia ensifera | M | Direct exam (pelage) | Direct exam (pelage) |
| Rodentepetes nana | M, R | Direct exam (small intestine) | Direct exam (perianal tape test) |
| Syphacia obvelata | M | Direct exam (cecal contents) | Direct exam (perianal tape test) |
| Syphacia muris | R | Direct exam (cecal contents) | Direct exam (perianal tape test) |
| **Fungus**     |          |                                             |                                 |
| Pneumocystis carinii | M, R | PCR (lung) | Histology |
| **Protozoan**  |          |                                             |                                 |
| Encephalitozoon cuniculi | M, R | ELISA (serum) | PCR, Histology |

*aM, Mouse; R, rat.

*bNP, nasopharynx.

*cMonitored only in immunodeficient mice and rats.
and RT-PCR assays. Thus, PCR provides information about the current infection status of the animal. For agents that produce chronic infections such as helicobacters, cilia-associated respiratory (CAR) bacillus, Pneumocystis carinii, and Mycoplasma pulmonis, PCR is an excellent primary test to monitor for infection because of the large amount of time available to detect the pathogen. In contrast, for agents that produce only acute infections in immunocompetent mice, such as ectromelia virus and mouse hepatitis virus (MHV), PCR is not as valuable in defining the infection status of a colony because of the limited time period during which PCR can detect these agents in a given animal. However, if the goal of testing is to determine if an individual animal is actively infected, PCR is an excellent tool for many agents, provided that samples are available from appropriate animals and tissues for testing. It is important to consider this difference, because PCR and serology evaluation of a given animal may yield conflicting results. For example, in rats infected with rat coronavirus (RCV), virus is present in the Harderian, parotid, and exorbital glands 7 days after infection, but absent by 14 days, which is about the time that detectable RCV-specific serum antibody titers are beginning to appear. It is important to assure, when using PCR tests, that the tissue sample required for testing is specific to the pathogen of interest. For example, the mesenteric lymph nodes are the target tissue for mouse parvovirus (MPV) PCR, feces is the proper sample for helicobacter PCR, and the trachea or tracheal swab samples are the appropriate samples to select for M. pulmonis PCR.

Generally, PCR assays are extremely sensitive and specific, but they are labor-intensive, relatively expensive, and susceptible to cross-contamination due to high test sensitivity.

Direct Examination

Direct microscopic examination is the primary means of detecting parasites of laboratory animals. Examination of intestinal contents, fecal floatations, or perianal tape tests are common procedures for the detection of endoparasites, such as pinworms. Examination of the pelage allows possible detection of ectoparasites, such as fur mites. Whereas these tests provide a definitive diagnosis and are relatively inexpensive to conduct, they offer only moderate sensitivity. Thus, false-negative results may occur when evaluating animals harboring low numbers of organisms.

Culture

Microbiological culture of the nasopharynx and cecum is a routine method of evaluating mice and rats for most bacterial pathogens. Standard bacteriology relies on cultivation of the organism on a variety of cell-free media; bacterial identification depends on bacterial colony morphology, Gram stain characteristics, and biochemical profiles of the isolate. Standard bacteriology can lack sensitivity in the detection of fastidious bacteria or bacteria present in low numbers. Also, bacteriology can lack specificity in that not all bacteria of the same species have the same biochemical profile and closely related bacterial species can have the same morphological characteristics and biochemical profiles. For these reasons, scientists are developing and increasingly using alternative tests such as PCR that can provide greater test sensitivity and specificity than culture, to detect murine bacterial pathogens. Diagnostic laboratories do not routinely use cultures to detect viral pathogens of mice and rats largely because the process is laborious and expensive and, for routine testing purposes, does not offer advantages over other, less expensive, and higher throughput assays such as serology and PCR.

Histopathology

Histopathological examination of selected target tissues of mice and rats is an excel-
chronic disease in these animals that is detectable by histopathological examination. In addition, histopathology has been central to the identification of novel murine pathogens. For example, histopathological identification of hepatitis in A/JCr mice was the first clue leading to the identification of *Helicobacter hepaticus* as a pathogen of mice\(^9\). More recently, histopathological examination of the lungs of rats revealed a novel interstitial pneumonia\(^7,8\), for which a viral pathogen, rat respiratory virus (RRV), is the suspected cause\(^6\).

**Agents to Monitor**

The decision as to which agents to monitor, often termed creating an agent exclusion list, will differ among rodent populations and is largely affected by the potential negative influence of a particular infectious agent on ongoing research or, for zoonotic agents, the potential risk the agent may pose to the health of animal care personnel and researchers. Inclusion of an agent on an exclusion list depends on the nature of the pathogen, the species of animals in the colony, the genotype of the animals in the colony (i.e., immunodeficient vs. immunocompetent), and the type of research being conducted. Another practical consideration is the potential ability to keep the pathogen out of the animal colony in light of the uncertain health status of animals entering the colony or surrounding colonies as well as the ability and availability of diagnostic tests to detect the pathogen. This is currently an issue with the novel agent RRV, which causes interstitial pneumonia in laboratory rats and for which no sensitive commercial diagnostic assays exist\(^6\). An addition-al issue often overlooked when developing an agent exclusion list is the proposed plan for dealing with the agent if it is detected. One can argue that if no action is to be taken once a pathogen is detected in a colony of animals, then there may be no justification for testing for it. However, defining the infection status of a group of animals from which the agent need not be excluded is justifiable if these animals are a potential source of contamination to other animal groups from which the agent must be excluded.

**Frequency of Monitoring**

Once an agent exclusion list exists, the next question regards the frequency of testing. Although the potential for a pathogen to affect ongoing research negatively or to pose a threat to the health of personnel influences the frequency of testing, the potential risk of the infection being introduced into a colony of animals is also a consideration. A way to evaluate this risk is to examine the current and recent historical prevalence of these infectious agents not only in individual animals, groups, and rooms within the research facility, but also in the local surrounding research campus and among research mouse and rat populations in general. This information, along with animal husbandry conditions and the movement of animals and people among groups of animals, can give an indication of the risk that a particular infectious agent will gain entry into a population of animals. It follows that the greater the potential for an infectious agent to gain entry into an animal population, the more frequently should a facility monitor the population.

Information about local prevalence data will vary between research institutions, which must collect and analyze these data on a case-by-case basis. In this discussion, we will provide current information on the prevalence of microbes in research mice and rats in general as one indicator of the risk that an infectious agent will invade a naive (clean) rodent population. Figures 1 and 2 provide a summary of the microbiological reporting data from the University of Missouri Research Animal Diagnostic Laboratory (MU RADIL) from 1 November
TABLE 2. General mouse pathogen testing recommendations

| Pathogens to monitor frequently | Pathogens to monitor less frequently | Pathogens to monitor infrequently |
|---------------------------------|-------------------------------------|----------------------------------|
| **Viruses**                     |                                     |                                  |
| Mouse hepatitis virus           | Ectromelia                          | Lactate dehydrogenase-elevating virus |
| Mice minute virus               | Lymphocytic choriomeningitis virus  | Mouse cytomegalovirus            |
| Mouse parvovirus                | Mouse adenovirus 1                  | Mouse thymic virus               |
| Mouse rotavirus                 | Mouse adenovirus 2                  |                                  |
| Sendai virus                    | Pneumonia virus of mice             |                                  |
| Theiler’s murine encephalomyelitis virus | Polyoma virus                   |                                  |
|                                 | Reovirus type 3                     |                                  |
| **Bacteria**                    |                                     |                                  |
| Helicobacter spp.               | Pasteurella pneumotropica           | Cilia-associated respiratory bacillus |
| Mycoplasma pulmonis             | Pseudomonas aeruginosa              | Citrobacter rodentium           |
|                                 | Salmonella spp.                     | Clostridium piliforme           |
|                                 |                                     | Corynebacterium kutscheri       |
|                                 |                                     | Proteus mirabilis               |
| **Parasites**                   |                                     |                                  |
| Pinworms                        | Fur mites                           |                                  |
|                                 |                                     |                                  |
| **Fungi**                       |                                     |                                  |
| Pneumocystis carinii*           | Encephalitozoon cuniculi            |                                  |

*Monitored only in immunodeficient mice and rats.

2001 to 31 October 2002, and include primary test results for viral, bacterial, fungal, protozoal, and parasitic infectious agents of laboratory mice or rats, respectively. It is noteworthy that these data represent a biased sample population in that animals tested and tests requested were selected by the clients of the RADIL and do not represent a randomly selected population. However, the MU RADIL carries out testing for many academic and private research institutions in geographically varied regions across North America, so these data likely provide a valid relative measure of pathogen prevalence in research mouse and rat colonies in the United States and Canada.

Figures 1 and 2 show that most of the murine pathogens that are subject to routine monitoring were detected in research rodent colonies during the past year. In mice, mouse parvovirus (MPV), mouse hepatitis virus (MHV), and mouse rotavirus (MRV) were the most prevalent viruses, helicobacters were the most prevalent bacteria, and pinworms were the most commonly detected parasites. In rats, the most frequently detected viruses were the parvoviruses, pneumonia virus of mice (PVM), and Theiler’s murine encephalomyelitis virus (TMEV), with Sendai virus and rat coronavirus (RCV) being detected less frequently. It should be noted that seroreactivity to TMEV in rats has not been linked to infection with the TMEV viruses of mice and likely represents infection with other viruses in the Picornaviridae family of unknown significance. As in mice, helicobacters were the most common bacteria identified and pinworms were the most common parasites detected. These data are consistent with the 1998 study by Jacoby and Lindsey that used surveys to investigate the prevalence of infectious agents in North American research institutions. Interestingly, in our prevalence study, some agents in research mice and rats were not detected. These agents were Hantaan virus, K virus, mouse thymic virus, Citrobacter rodentium, Corynebacterium kutscheri, and Encephalitozoon cuniculi for mice, and Hantaan virus, lymphocytic choriomeningitis virus (LCMV), mouse adenovirus 1, Salmonella spp., Rodentolepis nana, and Encephalitozoon cuniculi for rats.

If one uses these prevalence data to aid in developing a list of agents for which to test and to help determine the frequency of testing, a cost-conscious and justifiable approach would be to test frequently for the most prevalent agents and less frequently for the rarest agents, while considering the impact that each of these agents would have on the ongoing research or the research staff if detected in the rodent population. Using mouse pathogens as an example, it seems reasonable to test frequently for MHV, MPV, and MRV, which are highly prevalent agents that can alter the physiology of infected mice. LCMV and ectromelia are not very prevalent agents, but because LCMV poses a human health risk and ectromelia is a highly contagious virus that causes devastating disease in mice, one could argue that these agents should be monitored, but perhaps less frequently. In contrast, while agents such as E. cuniculi and the CAR bacillus can cause severe disease, they are not highly contagious and are not prevalent among research mice and therefore could be subject to infrequent testing. The definitions of “frequently” and “infrequently” will also vary substantially among research facilities and will depend on several of the variables mentioned earlier; generally, though, frequently may mean monthly or quarterly and infrequently may mean semiannually, annually, or only on a case-by-case basis. Respectively, Tables 2 and 3 provide general recommendations regarding which pathogens of mice and rats to monitor and the frequency to test for these agents. Readers should recall that these are only general guidelines that can aid in developing specific testing needs and frequencies at each research institution for the rodent subpopulations of interest. In particular, if colonies of mice or rats are at an increased risk of exposure to a specific agent because of a previous or current outbreak, it would be prudent to monitor more frequently for this agent in vulnerable populations.

An Example of a Testing Program

After considering the budget available for testing, one must prioritize the list of agents for monitoring in terms of prevalence and potential animal or human health impact, then determine the frequency and type of testing compatible with the budget and available diagnostic tests. For example, a logical course of action on a quarterly basis might be...
to collect serum to monitor for antibodies to the most prevalent and serious viral and bacterial pathogens, collect feces for Helicobacter spp. testing by PCR, and concomitantly check for evidence of pinworm infection by conducting perianal tape tests to detect Syphacia spp. and fecal floatations to identify Aspiculuris tetraptera in mice. On an annual basis, institutions should consider a more comprehensive monitoring approach in which whole animals are submitted to a diagnostic laboratory for a more exhaustive serological testing battery, bacterial culture for enteric and respiratory pathogens, a more detailed parasitological examination that includes both ecto- and endoparasites, and possibly histopathological screening of a battery of tissues for evidence of pathogens or disease processes. It should be stressed that this is a general recommendation, and individual testing needs and frequencies will differ between rodent subpopulations.

**Specific Testing Methodologies for Highly Prevalent Mouse Pathogens**

Helicobacters. From the data cited earlier, 32% of all mouse fecal samples tested were positive for a helicobacter, making helicobacters the most prevalent pathogen in mice used for biomedical research. This is not surprising, given that the first reports of pathogenic intestinal helicobacters were in 1994 (refs. 5, 20), and testing and control measures to exclude or eliminate these pathogens from research mouse colonies have not been extensively used until recently. The pathogenic potential of the different Helicobacter spp. varies, but in general, they all can cause or have been associated with intestinal or liver inflammation, depending on the genotypic infection of the infected mouse.²⁻²⁵. Conducting PCR on DNA extracted from feces is the primary method used to test mice for helicobacter infection. The usual approach is to do a helicobacter PCR assay that will detect all bacteria of the Helicobacter genus²⁶,²⁷. If positive samples are detected, then one can do species-specific PCR assays to define which Helicobacter species are present.

Livingston *et al.* showed that Helicobacter hepaticus was transmitted from colony mice to sentinel mice rather quickly²⁸. However, in another study, sentinel mice failed to become infected by *H. hepaticus* from some known positive groups of mice²⁹. These researchers also reported delayed and inconsistent transmission of *Helicobacter bilis* to sentinel mice²⁸,²⁹ and we have seen failed transmission of *H. bilis* and *H. rodentium* to sentinel mice exposed to contaminated bedding from infected colonies. Recognizing the potential for false-negative results with sentinels and recognizing that fecal PCR assays are antemortem tests, we recommend screening of colony animals for helicobacter infections. To help minimize the cost associated with helicobacter PCR testing, one can pool sentinel groups of mice²⁹. These researchers have slightly greater sensitivity in detecting infected mice²⁸, because PCR of mesenteric lymph nodes can also detect MPV-infected mice³³, because MPV persists in lymphatic tissue of infected mice³⁴. Investigators usually reserve PCR for confirming serological test results because of its expense and because serology appears to have slightly greater sensitivity in detecting infected mice³³ (B. Bauer, unpublished data). For example, in an endemically infected colony, all of ten 7.5-month-old Semar mice tested positive for MPV by serology, but only a few of ten mice were positive for MPV by PCR (B. Bauer, unpublished data). Laboratories also use MPV PCR to test for environmental contamination, to aid in detecting MPV-contaminated equipment, and to assess effectiveness of decontamination efforts after an MPV outbreak.

**Mouse parvovirus.** MPV is among the most prevalent mouse viral pathogens in research mice and can adversely affect research data obtained from infected mice¹²,¹³. Serology is the preferred primary testing method for detecting MPV-infected mice. Recently, several investigators have developed ELISA assays that use viral capsid proteins as antigens to detect anti-MPV antibodies in infected mice with high sensitivity and specificity¹¹,¹², providing highly accurate, low-cost, high-throughput assays for the detection of MPV-infected mice. Subsequent IFA assays are in common use as secondary serological tests for samples positive for MPV by ELISA. These IFA tests are sensitive, but lack specificity and are more labor-intensive than ELISA. Hemagglutination-inhibition (HAI) assays also serve as secondary serological confirmation for MPV. Although MPV HAI tests are specific, they lack sensitivity, are labor-intensive, and must be subjectively interpreted. PCR of mesenteric lymph nodes can also detect MPV-infected mice, because MPV persists in lymphatic tissue of infected mice.³⁴. Investigators usually reserve PCR for confirming serological test results because of its expense and because serology appears to have slightly greater sensitivity in detecting infected mice³³ (B. Bauer, unpublished data).

| Table 3: General rat pathogen testing recommendations |
|------------------------------------------------------|
| **Pathogens to monitor frequently** | **Pathogens to monitor less frequently** | **Pathogens to monitor infrequently** |
| **Viruses** | **Viruses** | **Viruses** |
| Rat paroviruses | Lymphocytic choriomeningitis virus | Hantaan |
| Pneumonia virus of mice | Reovirus type 3 | Mouse adenovirus 1 |
| Sendai virus | | |
| Theiler’s murine encephalomyelitis virus | | |
| **Bacteria** | **Bacteria** | **Bacteria** |
| Helicobacter spp. | Pasteurella pneumotropica | Cilia-associated respiratory bacillus |
| Mycoplasma pneumoniae | Salmonella spp. | Clostridium piliforme |
| | Streptococcus pneumoniae | Corynebacterium kutscheri |
| **Parasites** | **Parasites** | **Parasites** |
| Pinworms | Fungus | Fur mites |
| Pneumocystis carinii²⁹ | | |
| aMonitored only in immunodeficient mice and rats. | | |
prevalent among research mouse colonies and continues to have a severe impact on biomedical research using mice. Serology continues to be the best method for screening immunocompetent mice for MHV infection. Several investigators have also described PCR assays for MHV\(^{35–37}\); they can be used to confirm infection. In immunocompetent mice, PCR assays can detect MHV in the feces\(^6\) or mesenteric lymph nodes\(^{38}\) for as long as four weeks after infection. However, immunocompromised and genetically engineered mice have shown persistent infection and shedding of MHV\(^{37,38}\). Researchers have used serological and fecal PCR monitoring for MHV as a means to facilitate the re-establishment of MHV-free mice from infected colonies\(^37\). In this approach, a laboratory re-establishes the colony by selecting as breeders mice that were seropositive for MHV (i.e., indicating history of infection with MHV) but consistently negative for viral shedding as determined by fecal PCR (i.e., indicating clearance of infection).

**Testing of Biological Materials**

Experimental protocols involving mice or rats make use of a variety of transplantable tumors, cell lines, antibodies and other biological materials that become potential sources of infectious agents for research mouse and rat colonies. A complete rodent disease prevention program should include screening these materials for the presence of pathogens to prevent the possible introduction of infectious agents into research mice and rats. Traditionally, one examines such biological materials for the presence of infectious agents with antibody production tests, such as the mouse or rat antibody production (MAP or RAP) test, in which a portion of the biological material is inoculated into naive animals and then, four to six weeks later, the animal is tested for antibodies against various mouse or rat pathogens. Recently developed PCR assays permit direct testing of biological materials for the presence of these pathogens and provide an alternative to antibody production tests\(^{39}\). Advantages of these PCR-based tests over antibody production tests are that they provide equal or greater sensitivity in detecting the pathogens, results can be obtained more rapidly, and they do not require the use of animals.

**Confirmatory Testing**

Diagnosticians continually strive to develop new and more accurate tests. However, it is essential to emphasize that, in practice, no diagnostic test is 100% sensitive or 100% specific, so there is always a chance of obtaining a false-negative or false-positive test result. Therefore, confirmation of unexpected or unusual test results should always precede their use in making major colony management decisions. The best way to investigate unexpected or unusual results is to gather enough additional information to substantiate or refute the initial finding. Options for confirmatory testing include repeating the same test or doing a suitable alternative test on the same sample or a new sample derived from the same animal, or conducting the same or different tests on samples collected from cohort animals. In practice, all of these approaches are often necessary. For example, if a serum sample tests positive for an agent by ELISA, one should attempt to confirm the result by retesting the sample using an alternative serological assay, such as an IFA test. Testing additional animals from the colony by serology for the same agent is also prudent. In addition, if a suitable alternative test such as PCR, culture, or histopathology exists for the pathogen, it is wise to test animals from the same colony using these techniques. However, suitable alternative testing methods are not available for all pathogens. Readers should recall that when multiple diagnostic approaches are used to test for the same agent, it is possible to obtain conflicting results. This is most common when using PCR assays or histopathology as confirmatory tests for serologically detected agents that cause acute infections. It is essential to remember that serology provides an indirect measure of exposure to an agent, whereas PCR directly detects the presence of the agent and histopathology detects pathological changes induced by the microbe. Therefore, it is quite possible for an animal to have been exposed to an agent, to have developed a detectable antibody response, and to test positive serologically, but to have since cleared the infection and thus test negative for the agent by PCR or histopathology. Conversely, it is possible for an animal to be acutely infected with a pathogen, to lack a detectable antibody response, and to test negative for the agent by serology, but still test positive for the presence of the agent by PCR or show acute lesions suggestive of infection. Understanding the epizootiology of infectious agents within a given animal population and having knowledge of the advantages and limitations of each diagnostic methodology will help sort out these potential differences, and can help in deciding on appropriate methods and samples to use for confirmatory testing. In addition, experts at diagnostic laboratories can provide insight on test performance, help with data interpretation, and aid in developing a plan for health monitoring and follow-up testing.

**Conclusions**

Although there is no single diagnostic testing plan that can be applied to all mouse and rat colonies, one can develop individual testing programs by assessing the risk posed by the various infectious agents (i.e., risk to research, risk to personnel, and risk of exposure of the colony to the infectious agents) and by having a thorough knowledge of available diagnostic tests and testing methodologies. One can obtain this information by reading reference material\(^{1,10,40–44}\) and scientific literature, as well as by consulting with laboratory animal experts and laboratory animal diagnosticians.

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