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Chapter 11

Health Delivery and Quality Assurance Programs for Mice

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I. INTRODUCTION

This chapter covers health care delivery for individual mice, quality assurance (QA) programs for research colonies of mice, and infectious outbreak sources and management. Comprehensive health care must be provided in all of these areas to be effective. For example, a single sick mouse might represent the index case in what could become an epidemic within a colony, causing widespread morbidity and mortality if not dealt with quickly. Conversely, an unwanted infectious agent that does not cause clinically evident signs could still have a major adverse impact on research results if allowed to disseminate. As a result, the program for providing health care must include detection and care of individual mice that are ill, routine colony surveillance to guard against the undetected entry and spread of previously excluded infectious agents, and a response plan if such agents are detected. Such programs will result in humane care of animals and the best protection of experimental repeatability and validity.
II. HEALTH CARE PROVISION FOR EXPERIMENTAL MICE

A. Detection of Sick Mice

1. Signs of Illness in Mice

As a prey species, mice tend to camouflage signs of illness by retaining normal behaviors as a protective mechanism. When illness is significant enough to override this stoicism, generalized signs of distress predominate. As a result, illness in mice often presents with nonspecific signs such as ruffled fur, hunched posture, and inactivity. Signs related to specific body systems, although not always evident, may be present and may suggest specific potential causes, as listed in Table 11-1. It should be noted that in this era of genetically engineered mutant mice, nearly all abnormal physical findings have the possibility of being due to genetic causes, either as part of the phenotype or a complication related to the phenotype. The expected phenotype of any mutant mouse strain, or the possibility of an unexpected phenotype, must be considered to avoid improper diagnosis.

2. Observations by Animal Care Staff

With larger species such as rabbits or primates, a clinical veterinarian or veterinary technician usually observes every animal every day, but this is impractical in larger colonies with many cages of experimental mice. The animal caretaker or other individual responsible for daily observation and routine husbandry care of experimental mice is often the primary individual who detects sick mice. Animal caretakers must be trained to observe mice each day for wellness as an important part of routine animal care. Certain mouse behaviors (e.g., daytime sleep in groups) and the use of high-density caging and/or poorly transparent plastic cages may make adequate observation of each mouse difficult. The best opportunity to observe the health of each mouse occurs during routine cage changing. Although the handling time for each mouse is brief, well-trained animal caretakers can observe both mouse behavior and physical appearance as the cage is opened and as individual mice are transferred to a clean cage. Normal mice react with curiosity to the opening of the cage, appear sleek and active once aroused from sleep, and actively investigate and mark the new cage once they have been placed into it. Sick mice may remain inactive or may demonstrate a lack of balance when disturbed from sleep. During mouse transfer, skin wounds or swellings may be noticed as the mice are lifted and transferred to the clean cage. Experienced caretakers may detect more subtle signs such as weight loss, dyspnea, abnormal postures/gaits, or dehydration during routine handling. A mechanism must be in place for the caretaker to report any observed abnormalities to the veterinary staff and possibly to the scientist as well. Optimally, the mechanism for reporting sick mice will also produce written documentation for follow-up care.

Training of animal caretakers should include not only how to differentiate normal from abnormal but some understanding of whether animals are expected to appear abnormal due to genetic or experimental manipulation. In particular, carcinogenicity and infectious disease studies or colonies of animals having noticeably abnormal phenotypes should be discussed with the caretaker so they understand what is expected, what is not expected, and what degree of abnormality is acceptable for that particular study or colony.

3. Observations by Scientists

During the course of experimentation, scientists may also notice mice that appear unwell. In addition, the evaluation of experimental results may provide important clues that mice are ill or have been infected by an agent that has affected the animal’s physiology even in the absence of noticeable clinical signs. A mechanism should be in place to facilitate the reporting of sick mice to the veterinary care staff by scientists. After observation of mice and investigation into potential causes, veterinary staff should convey their conclusions or suspicions to the reporting scientists. Further discussions can then follow regarding potential diagnostic or therapeutic options as well as the potential impact of either the illness or the treatment on ongoing experiments.

B. Reporting of Sick Mice

1. Reporting Mechanisms

A convenient, efficient written mechanism should be used to identify, report, and record sick mice. This mechanism must be available both to veterinary and caretaking staff and to scientific staff. It should include identifying information for the animal cage and must allow the observed clinical signs, course of the disease, and follow-up to be recorded. A variety of effective mechanisms have been developed at research institutions. Most mechanisms include the sequential numbering of reports and visibly marking the cages of sick mice for ease of identification by scientists, veterinary technicians, and veterinarians. Successful forms include multipart cards and multicopy forms that provide information to all interested parties. Colored cards or colored plastic tags are often used to help find the desired cage in large rooms with hundreds of cages. A written record of mice reported as sick is important to facilitate detection of patterns, which may indicate the presence of disease or an unexpected phenotype or experimental outcome. These records also provide documentation of the quality of delivered health care for inspectors or site visitors from regulatory or accrediting agencies.
### TABLE 11-1

**CLINICAL SIGNS OF ILLNESS AND POTENTIAL DIAGNOSES BY BODY SYSTEM IN MICE**

| Body System     | Clinical Signs                                      | Potential Diagnoses                                                                 |
|-----------------|-----------------------------------------------------|-------------------------------------------------------------------------------------|
| General         | Hunched, inactive, weak, unkempt coat, weight loss or poor growth | Nonspecific signs of severe illness                                                 |
| Respiratory     | Sneezing, labored breathing, nasal discharge, cyanosis | Pneumonia (Sendai virus, *Pneumocystis carinii*, or *Mycoplasma pulmonis*)         |
| Ophthalmic      | Purulent exudate                                     | Bacterial conjunctivitis                                                           |
|                 | Corneal opacity, shrunken globe of eye               | Damage due to poor retro-orbital bleeding technique                                |
| Neurologic      | Head tilt, circling                                  | Bacterial (esp. *Mycoplasma*) or idiopathic otitis media                           |
| Integumentary   | Bleeding lesions                                    | Fight wounds (especially hindquarters, tail)                                      |
|                 | Nonbleeding lesions with hair loss                   | Ulcerative dermatitis (especially neck, shoulders)                                |
|                 | Lesions or necrosis of tail tip                      | Idiopathic inflammation                                                           |
|                 | Loss of fur or whiskers without skin lesions         | Ulcerative dermatitis                                                             |
|                 | Swellings                                            | Overgrooming ("barbering")                                                       |
| Gastrointestinal| Diarrhea                                             | Scars from previous injuries                                                      |
|                 | Prolapsed rectum                                     | Vascular occlusion of tail due to poor bleeding technique                         |
|                 | Weight loss, overgrown incisors                      | Cage injury (tail caught between cage and lid)                                     |
| Urinary         | Enlarged bladder, inactivity                         | Overgrooming ("barbering") by cage mates                                        |
| Reproductive    | Prolonged gestation, weakness                        | Spontaneous tumors, especially mammary or lymphatic                               |
|                 | Tissue protruding from vulva                         | Experimentally induced tumors                                                     |
|                 | Runted or dead pups                                  | Abscesses                                                                          |
|                 |                                                      | Mouse hepatitis virus (primarily pre-weaned mice)                                  |
|                 |                                                      | Mouse rotavirus (EDIM) (primarily pre-weaned mice)                                 |
|                 |                                                      | Pinworms (rare)                                                                   |
|                 |                                                      | *Helicobacter* or *Citrobacter* infection                                          |
|                 |                                                      | Idiopathic                                                                        |
|                 |                                                      | Dental malocclusion                                                                |
|                 |                                                      | Urethral obstruction (males)                                                      |
|                 |                                                      | Dystocia                                                                          |
|                 |                                                      | Uterine prolapse                                                                  |
|                 |                                                      | Viral infection (EDIM, MHV, others)                                                |
|                 |                                                      | Lactation failure                                                                 |

2. **Initial Observations and Actions**

After mice are reported sick, initial observations may be made by veterinary technicians with expertise in rodent health or by laboratory animal veterinarians. Many common conditions of mice, such as fighting, barbering, or dental malocclusion, can be readily diagnosed by veterinary technicians who can contact scientists and proceed to take appropriate action. Other conditions require the involvement of a veterinarian trained in laboratory animal medicine and may require additional testing such as serology, bacterial culture, or necropsy examination before the problem can be diagnosed and resolved.

The actions that need to be taken after a mouse is reported as sick will depend on the clinical signs, experimental use, and/or value of the mouse. Some conditions, such as hair thinning due to genetic influences or excessive grooming without dermatitis may require no action. Other conditions, such as the presence of small, well-tolerated subcutaneous tumors, may be expected as part of the experimental use or the genotype of the animal. The action to be taken in these cases will depend on what is approved in the animal care and use protocol. For example, if the animal use protocol predicts that mice will appear ill as a consequence of an experimental infection and that moribund mice will be humanely killed by the scientists, mildly ill mice may be reported to the scientist without taking further action.

Other scenarios may require prompt and proactive response. For example, if several cages of mice were unexpectedly found dead and others were found moribund, immediate contact with the scientist, euthanasia of moribund mice, necropsy of mice, and investigation of husbandry conditions would be warranted. Husbandry conditions to be investigated would include the availability of water and the stability of an appropriate room temperature. The experimental manipulations to which these mice had been subjected must be considered if the mice were not naïve, and it is important to verify what was actually done rather than relying solely on the written experimental protocol.
Thus, it is essential that the veterinary staff understand the planned use of the mice, any anticipated illness, and any endpoints (such as euthanasia) that have been included in the approved animal care and use protocol. Just as veterinarians in clinical practice care for pet animals within the context of their relationship with their owners, laboratory animal veterinarians must respect and work within the context of the scientist-animal relationship in experimental settings. In addition, laboratory animal veterinarians are expected to keep in mind the principles of humane care and use and ensure that unexpected or unnecessary distress or suffering of experimental animals is minimized throughout experimental processes.

C. Staff to Provide Health Care for Mice

It is evident that the establishment of a high-quality health care and QA program for experimental mice requires the involvement of trained laboratory animal professionals. The numbers, types, and percent effort of these individuals varies based on the number of mice for which care is to be provided. Quality health and husbandry care always requires the involvement of trained animal caretakers and a veterinarian with experience and/or training in laboratory animal medicine. Veterinary technicians can also serve an important role in direct health care and by performing diagnostic testing. In an institution with large numbers of mice, a veterinary pathologist with training and/or experience in the diagnosis of laboratory animal diseases can be expected to make valuable contributions to the care of mice, as well as to serve as a valuable resource to scientists involved in discovering or documenting new mouse phenotypes. In smaller institutions, laboratory animal pathology services are often provided by a commercial laboratory, although this option limits the broadest contributions of veterinary pathologists to the care of mice.

III. QUALITY ASSURANCE (QA) PROGRAMS FOR MICE

A. Rodent QA Is an Essential Tool to Manage Experimental Mouse Colonies

A QA program is an essential tool for management of modern colonies of experimental mice. Infectious agents can cause clinical disease, disrupt biomedical research projects, compromise the validity of experimental results, and interfere with collaborative efforts between institutions (Baker 2003; Patterson et al. 1998; Rehg and Toth 1998). In addition to the individualized care described previously, many decisions regarding proper management of mouse colonies must be made based on knowledge of the infectious agents that are present in, or excluded from, the colony in general. A QA surveillance program is designed to detect and document, at preset intervals, the absence or potential presence of any of a number of infectious agents. A QA program also will include additional sampling when an infectious outbreak is suspected. If active contamination by a previously excluded agent is detected, or if new information suggests that a preexisting endemic infection can no longer be tolerated or contained, the QA program will include options for containment and eradication of these unwanted infectious agents.

1. Terms Commonly Used Regarding the Microbial Status of Mice

A common understanding of several terms is essential for further discussion of rodent QA programs. The terms “barrier maintained,” “pathogen free,” or “specific pathogen free” (SPF) are commonly used in reference to the health status of mice. Although there have been suggestions that standards to define these terms be developed, there is no common agreement as to the exact pathogens that should be absent from a pathogen-free or SPF mouse colony (Jacoby and Homberger 1999; Lindsey 1998). Part of this lack of consensus is because many agents show variable pathogenicity when they infect mice of different ages or strain backgrounds, so the need for their exclusion is ultimately dependent on the type of research for which the mice will be used. Most agents infecting mice do not cause overt clinical signs or deaths in otherwise healthy mice with fully competent immune systems. However, even in the absence of any clinical signs, infectious agents can affect organ systems and physiologic parameters that alter research outcomes (Baker 2003). Although it might seem prudent to exclude all known infectious agents from colonies of experimental mice, factors such as the time, labor, and monetary costs of eradication may make this an unrealistic goal in many research institutions. The risk of adverse impact by the agent must be balanced against the cost of its eradication and/or exclusion.

The health status of study animals should always be described in manuscripts submitted for publication. Because the terms pathogen free or SPF have no specific definition, they should always be accompanied by a description or list of the specific agents excluded from the colony.

2. Genetically Engineered Mice Complicate Effective Management of Murine Health

An additional factor in managing modern colonies of experimental mice is evidence that the process of transgenesis or selective breeding can result in mice with unexpected variations in susceptibility to, or elimination of, infectious agents as compared to “normal” mice even when overt immunodeficiency is neither suspected nor clinically apparent (Rehg et al. 2001). This type of relative or “cryptic” immunodeficiency may be undetected until the mice are actively monitored by sentinel surveillance or polymerase chain reaction (PCR) testing for their ability to eliminate murine viral agents, such as
mouse hepatitis virus (MHV), which can be completely eliminated by "normal" mice (Pullium et al. 2003; Rehg et al. 2001). Contemporary viral strains also may be undergoing changes as they adapt to the physiologic environment within host animals that express novel phenotypes and to the selective pressures of modern housing systems and QA monitoring programs. These factors must be taken into consideration when designing an appropriate surveillance regimen and when planning a response to infectious disease outbreaks.

3. Costs for Murine QA

Because the rodent QA program is an essential tool for the management of colonies of experimental mice, costs for the program should be built into the per-diem or daily housing costs (Jacoby 2003; Jacoby and Lindsey 1997; Small 1986). The failure to build these costs into “mandatory” costs, or any option that allows QA sampling to be optional or “cost added” will leave the veterinarian or manager without essential information and will severely compromise the quality of the animal care program. Additional costs will be incurred and may be high when excluded infectious agents gain access to mice and need to be eradicated. Costs for eradication of pinworms can range from over $15 per cage for 8 weeks of treatment utilizing feed containing fenbendazole to $3 per cage using alternating ivermectin and piperazine in the water for 8 weeks (B. Karolewski, personal communication). Eradication costs for outbreaks with viral agents such as MHV can be expected to vary greatly but have been calculated in one instance, using the test and cull method, to be approximately $26 per cage (M. Batchelder, personal communication). As per the Guide for the Care and Use of Laboratory Animals, preventive medicine, quarantine, surveillance, diagnosis, control, and treatment of disease are essential components of a program of adequate veterinary care for research animals (ILAR 1991, 1996). Methods to provide all of these components vary between institutions and may include the use of in-house or commercial diagnostic necropsy, serology, parasitology, PCR, and microbiology testing. The types of and number of staff needed to provide a rodent QA program will vary from institution to institution and will be highly dependent on the numbers and types of rodents in-house but should include involvement at some level of a laboratory animal veterinarian with expertise in rodent disease, a laboratory animal pathologist, and veterinary and laboratory technicians.

4. Components of a Murine QA Program

An effective rodent QA program includes several components, such as the exposure and routine testing of sentinel or colony mice from each housing area, testing mice submitted for diagnostic necropsy examination, maintenance of a list of approved commercial vendors, procedures for importation and quarantine of mice from other sources, screening of tissues or biologic materials that might potentially transmit infectious agents to mice, and methods of eradication of infectious agents after they have gained entrance to mouse colonies. Aspects of these topics are covered in this chapter. Other relevant topics, such as the potential effects of infectious agents on research results, are beyond the scope of this chapter and are covered elsewhere in this book and in other resources (Baker 2003).

B. Programs for QA for Existing Colonies of Mice

1. For What Agents Should We Test and Why?

The agents selected for testing should reflect the prevalence of infectious agents in modern colonies (Jacob and Lindsay 1998), should include testing for excluded exotic murine agents and zoonotic agents, and should emphasize agents that are known to affect animal health or alter research results (ILAR 1991, 1996; Rehg and Toth 1998). Each QA program should also be customized based on the types of research being conducted and the health history of the existing murine colonies. If feral rodents are present, additional testing may be advisable for agents commonly carried by feral rodents that are not common in laboratory mice such as murine cytomegalovirus and mouse thymic agent. A consensus has not been reached in the United States regarding recommendations for QA testing for mice. Groups in Europe have made recommendations regarding health monitoring but not all institutions may follow these voluntary guidelines (Niklas et al. 2002).

Table 11-2 summarizes infectious agents of mice that are often monitored as part of a rodent QA program, the relative importance of testing for each agent, and the reason(s) that the presence of these agents is monitored. References to support the key reasons for monitoring these agents are provided.

2. How Often Should Murine Colonies Be Tested for the Presence of Infectious Agents?

Ideally we would like to know what agents infect or have infected every mouse, all the time, but this is not a realistic expectation. Testing cannot be done continuously, even though it is done very frequently in many contemporary colonies. Immunocompromised animals, isolator housing, and research needs can complicate a testing program. Additionally, logistical and financial limitations frequently require compromises between optimal quality and costs. Successful QA programs strike a cost/benefit balance between the desire to test all animals at high frequency and the reality of these constraints. It is common practice to test more frequently for agents that are common, readily transmitted, and have the potential for significant impact on the colony, while assaying for other agents on a less frequent schedule. The frequency of testing should also take into consideration the planned experimental use of the animals and how critical it is to exclude selected agents.
# Monitoring Infectious Agents of Experimental Mice

| Agent                                      | Importance* (Key Reasons)* | Method(s)† | Comments                                                                 | References                                |
|--------------------------------------------|----------------------------|------------|--------------------------------------------------------------------------|-------------------------------------------|
| **VIRUSES**                                |                            |            |                                                                          |                                           |
| Mouse adenoviruses                         | Moderately important (1)   | ELISA, IFA |                                                                          | Lipman et al. 1999                       |
| Murine cytomegalovirus                     | Moderately important (1, 2)| ELISA, IFA |                                                                          | Lipman et al. 2000                       |
| Ectromelia virus                           | Essential (3, 4, 5, 6)     | ELISA, IFA |                                                                          | Small 1986                               |
| Hantavirus                                 | Rarely tested/optimal (2, 7)| ELISA, IFA | Test in non-*Mus* murine species and in mice imported from other countries |                                           |
| K-virus (mouse pneumonitis virus)          | Rarely tested/optimal (1)  | ELISA, HAI |                                                                          | Nicklas et al. 1993                      |
| Lactate dehydrogenase elevating virus      | Rarely tested/optimal (6)  | Serum chemistry, PCR |                                                                          | Small 1986                               |
| Lymphocytic choriomeningitis virus         | Essential (2, 6, 7)        | ELISA, IFA |                                                                          | Nicklas et al. 1993                      |
| Mouse paroviruses (MMV, MPV-1, MPV-2)      | Moderately important (5, 6) | ELISA, IFA, HAI, PCR | Virus may persist in lymphoid tissues                                    | Nicklas et al. 1993                      |
| Mouse hepatitis virus (MHV)                | Essential (4, 5, 6)        | ELISA, IFA, PCR | Time to seroconversion to MPV may be variable                            | Small 1986                               |
| Mouse mammary tumor viruses                | Rarely tested/optimal (1)  | ELISA, IFA, HAI |                                                                          | Nicklas et al. 1993                      |
| Mouse norovirus (MNV)                      | Rarely tested/optimal (9)  | Multiplex fluorescent immunoassay, PCR | Recently described agent with no clinical disease in immunocompetent and most immunodeficient mice | Karst et al. 2003                         |
| Mouse polyomavirus                         | Rarely tested/optimal (2)  | ELISA, IFA, HAI |                                                                          | Carty et al. 2001                        |
| Mouse rotavirus (EDIM)                     | Essential (4)              | ELISA, IFA |                                                                          | Small 1986                               |
| Mouse thymic virus                         | Rarely tested/optimal (1, 2)| IFA |                                                                          | Broeders et al. 1994                     |
| Pneumonia virus of mice                    | Moderately important (4)   | ELISA, IFA, HAI | ELISA and IFA tests do not distinguish among reovirus subtypes 1, 2, and 3 | Wagner et al. 2003                       |
| Reovirus-3                                 | Rarely tested/optimal (1, 2, 6)| ELISA, IFA, HAI |                                                                          | Small 1986                               |
| Sendai virus                               | Essential (4)              | ELISA, IFA, HAI | Clinical disease rare; shedding of virus is sporadic and potentially long lasting | Wagner et al. 2003                       |
| Thiel's murine encephalomyelitis virus (GD-VII) | Moderately important (4) | ELISA, IFA |                                                                          | Brownstein et al. 1989                   |
| **PARASITES**                               |                            |            |                                                                          | Razengurt and Sanchez 1993               |
| Pinworms (*Aspiculurus, Syphacia*)          | Essential (4, 5)           | Cecal or fecal exam, tape test |                                                                          | Mohn and Philipp 1981                    |
| Fur mites (*Myobia, Myocoptes, Radfordia*)  | Essential (4, 5)           | Hair or pelt exam, skin scrape or pelt digestion |                                                                          |                                           |
| **BACTERIA**                                |                            |            |                                                                          |                                           |
| Cilia-associated respiratory bacillus      | Rarely tested/optimal (5)  | ELISA, IFA, histology with special stains |                                                                          | Matsushita et al. 1987                   |
| *Citrobacter rodentium*                    | Rarely tested/optimal (4)  | Culture |                                                                          |                                           |

*Importance reflects the significance of the agent. Key Reasons include benefits and risks associated with the agent.
†Method(s) include ELISA, IFA, PCR, and other diagnostic techniques.

Continued
### TABLE 11-2
**MONITORING INFECTIOUS AGENTS OF EXPERIMENTAL MICE—cont’d**

| Agent                        | Importance (Key Reasons) | Method(s) | Comments                                                                 | References |
|------------------------------|--------------------------|-----------|--------------------------------------------------------------------------|------------|
| Clostridium piliforme        | Rarely tested/optional (4) | ELISA, IFA, PCR, culture, test for toxin | Primarily a pathogen in athymic nude mice | Clifford et al. 1995 |
| Corynebacterium spp.         | Rarely tested/optional (8) | IFA, culture |                                                                            |            |
| Helicobacter spp.            | Moderately important (5) | Culture, PCR | Fluorogenic nuclease PCR more sensitive than culture                      |            |
| Klebsiella pneumoniae        | Rarely tested/optional (4) | Culture |                                                                            |            |
| Mycoplasma pulmonis          | Moderately important (4, 5) | ELISA, IFA, culture | Serologic tests used more commonly than culture | Lai et al. 1987 |
| M. arthritidis               |                          |           |                                                                            |            |
| Pasteurella pneumotropica    | Moderately important (4) | Culture, PCR |                                                                            |            |
| Pseudomonas aeruginosa       | Rarely tested/optional (6) | Culture |                                                                            |            |
| Salmonella enterica          | Rarely tested/optional (7) | Culture |                                                                            |            |
| Staphylococcus enteritica    | Rarely tested/optional (6, 8) | Culture |                                                                            |            |
| Streptococcus group B        | Rarely tested/optional (8) | Culture | Primarily a pathogen in athymic nude mice                                 |            |
| FUNGI                        |                          |           |                                                                            |            |
| *Encephalitozoon cuniculi*   | Rarely tested/optional (1) | ELISA, IFA |                                                                            |            |
| *Pneumocystis marina*        | Rarely tested/optional (8) | PCR, histology with special stains in immunosuppressed animal |                                                                            |            |

*aKey to Importance codes:
Essential = Agents known to cause clinical disease and/or affect research results; zoonotic agents; exotic agents excluded from research rodents in the United States.
Moderately important = Rare agents; agents unlikely to cause disease or affect research, but which are generally excluded from SPF colonies (often included in commercial screening panels); common agents that are often tolerated (e.g. Helicobacter).
Rarely tested/optional = Extremely rare agents; opportunistic or “nonpathogenic” agents that could possibly cause disease in immunodeficient animals.

*bKey to Reason codes:
1 = Included in many commercial panels of tests.
2 = Exposure may indicate presence of feral or loose rodents.
3 = Exotic, excluded from research rodents in the United States.
4 = Causes clinical disease of immunocompetent mice, including deaths or clinical illness.
5 = Affects research results, with or without causing clinical disease.
6 = Agent shown to be carried in tumor cells or other biologics.
7 = Zoonotic, infects humans.
8 = Causes clinical disease in immunodeficient mice.
9 = Recently described, effects on research unknown; not part of standard commercial panels.

*cKey to Methods:
ELISA = Enzyme-linked immunosorbent assay.
IFA = Immunofluorescence antibody.
HA1 = Hemagglutination inhibition.
PCR = Polymerase chain reaction.
For example, experimental results in areas such as infectious disease research or immunology are especially susceptible to variability if mice have concurrent infections. Mouse parvoviruses have been shown to alter the proliferation of immune cells (Engers et al. 1981; McKisic et al. 1993, 1998), and MHV is suspected to cause long-lasting alterations in immune function (Cook-Mills et al. 1992; Cray et al. 1993); therefore, groups of mice for such studies should be tested frequently to make sure that they do not harbor these agents. For critical studies, each cohort of animals may need documentation that they were not exposed to certain infectious agents.

In practice, many successful murine QA programs utilize serology to confirm the absence of common viral and mycoplasmal agents from barrier colonies on a quarterly or, less commonly, semiannual basis. Animals housed under conventional conditions with open-topped cages may be tested less often, sometimes as infrequently as once per year. Areas at high risk of exposure or where an infectious disease might have a significant effect on research outcome should be tested on a more frequent basis. The site-specific history of previous findings and intervals between status-changing events can also be used to develop a rationale for the frequency of testing (Shek and Gaertner 2002).

In addition to routine testing of representative animals at a pre-set schedule, mice undergoing diagnostic necropsy should also be evaluated for evidence of current or prior infection with murine agents.

Although mice arriving from approved commercial vendor colonies are not usually tested prior to permanent housing, quarantine and extensive testing for mice arriving from other institutions, and nonapproved commercial vendors is an essential component of the QA program. Alternatively, incoming animals could be rederived by embryo transfer. Similarly, previously untested tissues or biologic materials that will come in contact with mice should be screened for infectious agents prior to use in the colony.

Some facilities perform validation testing of representative animals that are obtained from outside vendors for use as sentinels. Although this is not a widely used practice, it can help ensure that subsequent detection of infectious agents in sentinels is a reflection of the sampled population, not something that was preexisting on arrival. Because one concern is that negative test results on arrival may miss an incubating infection, it has even been suggested that representative mice be held from each arriving sentinel shipment in an isolated area for testing if seroconversion is detected in the sentinels that were distributed. Another option is to bring in pregnant mice as a source of sentinel offspring that are distributed only after the dam tests negative at weaning (Pullium et al. 2004).

3. Which Animals Should Be Tested?

QA programs generally rely on testing of either sentinel animals or colony animals or both. Sentinel animals are mice verified or assumed to be free of the pathogens of interest and are obtained specifically for testing after exposure to colony animals, either directly or indirectly via soiled bedding. Although the sentinel method spares colony animals, and allows a small number of animals to represent the colony as a whole, a sentinel program may fail to detect some agents due to lack of transmission or lack of susceptibility. For example, some agents are poorly transmitted by air or by soiled bedding, and may not infect sentinels exposed by these routes. Immunocompetent sentinels may not develop detectable infection with agents such as Pneumocystis carinii and Corynebacterium bovis, which can cause significant disease in immune-deficient mice. The strain and age of animals chosen as sentinels may also affect their susceptibility to certain agents such as mouse parvovirus (MPV) (Besselsen et al. 2000).

Mice used as sentinels are most commonly young, outbred mice because such animals are relatively inexpensive and would be expected to exhibit a reliable immune response after exposure to infectious agents. Sentinel mice are generally female, which reduces the likelihood of fighting. Although the use of athymic nude mice as sentinel animals has been suggested, no real advantages have been demonstrated over immunocompetent sentinel mice in most situations (Clarke and Perdue 2004). The source of the sentinel mice (commercial vendor barrier or in-house colony) should be screened on a regular basis to ensure that the sentinels themselves are not the source of any positive test results (Pullium et al. 2004).

Methods of incorporating sentinel and/or colony animal testing into the QA program are described later.

a. Sentinel Animals Exposed to Aerosols and/or Soiled Bedding

Purposeful exposure of sentinel mice to soiled bedding is the most common method used to monitor for the presence of excluded infectious agents in populations of mice. The sentinel mice are housed on pooled bedding collected from the cages of colony mice. Although housing the mice in the same room could expose the mice to airborne infectious agents without the addition of soiled bedding, bedding exposure increases the likelihood of detecting endemic agents (Thigpen et al. 1989). It is recommended that the exposure period includes a period of time for seroconversion of not less than 3 weeks (Rehg and Toth 1998; Small 1986).

Experience has shown that not all infectious agents are readily transmitted by bedding transfer. This includes labile agents such as enveloped viruses (Sendai virus, MHV), which may not survive for more than 1–2 days in bedding. If not continually shed, such viruses may have already become inactive before the bedding is transferred to sentinels (Artwohl et al. 1994). Pasteurella pneumotropica survives for only 120 minutes on mouse hair and is not readily transmitted by soiled bedding (Scharmann and Heller 2001). Other agents, such as mouse parvoviruses, are more resistant to environmental conditions and can be expected to survive in soiled bedding and to be spread routinely to bedding sentinels (Jacoby and Smith et al. 2003;
Smith et al. 1993). Helicobacter spp. are also transmitted to bedding sentinels (Whary et al. 2000). Pinworm eggs are readily shed and are thought to survive for prolonged periods, whereas mites attach their eggs to hair shafts and close contact is usually required for transmission. Thus bedding sentinels can be expected to acquire or seroconvert to some infectious agents under some conditions, but should not be expected to be reliably exposed to all potential infectious agents.

When bedding transfer is used to expose sentinels, it is important to optimize exposure of the sentinels to any agents potentially in any cage in the room. This is made more difficult because cage changing is periodic, not daily, usually occurring at intervals such as 7 or 14 days. Because bedding height must remain at a level lower than the water source to avoid cage flooding problems, only a limited number of samples and limited volume of soiled bedding per sample can be transferred to the sentinel cage at each interval. Studies have not yet been done that can give clear guidance on the minimum quantity of bedding that needs to be transferred from each cage at each interval. Sampling errors, in the form of false-negative tests, are possible even in the best programs.

Further complicating the use of bedding sentinels to detect infection in rooms of mice is the effect of housing colony animals in isolation cages. The use of either static or ventilated isolation cages will slow the spread of infectious agents within the room. Although the obvious advantage of this type of rodent housing is that it will slow cross-contamination during an outbreak, it must be realized that it will also decrease the exposure of sentinel animals (Compton et al. 2004). Strategies must be put place to compensate for this fact, and the time to detection may be prolonged. Ventilated isolation cages are also often changed less frequently than are static isolation cages, yielding fewer opportunities for transfer of infectious agents and longer intervals during which infectious agents may become inactive before bedding is transferred. Considering these limitations, it is possible that rooms with isolation caging may harbor small numbers of cages containing mice that are infected with agents of concern that will be a real challenge for the QA program to detect. A method of screening ventilated racks by exposing sentinel mice to unfiltered exhaust from all cages on a ventilated rack prior to high-efficiency particulate (HEPA) filtration has been evaluated (Compton et al. 2004). This method proved more effective than soiled bedding for detecting some agents (Sendai virus) but less effective for others (mouse parvovirus) and may be useful as an adjunct to other screening methods when ventilated caging is used.

b. Sentinel Animals Exposed by Direct Contact Rather than relying on bedding transfer to sentinels housed separately, sentinel animals may be co-housed directly with the colony mice being monitored. This method has both advantages and disadvantages. The use of contact sentinels avoids most of the limitations inherent in the use of bedding sentinels due to more intimate contact with the colony animals and prolongation of contact time. In addition, a sentinel seroconversion can be directly linked to a specific colony cage. However, this one-to-one relationship also means that a much larger number of sentinels would be needed to monitor the same number of colony cages. Care must be taken that the sentinel animal is clearly identified. If males are present, fighting may occur due to the introduction of a new animal. Some facilities utilize ovarietomized females to avoid the possibility of pregnancy and birth by the sentinel animal, but this is not necessary if contact sentinels are euthanatized or transferred to another cage within 3 weeks after the initiation of exposure to males.

One of the principle uses of contact sentinels is the testing of individual cages of mice to detect shedding of infectious viral agents. This is usually indicated because the animals are known or suspected to be immunodeficient and therefore would not reliably develop detectable antibodies. Genetically engineered mice, even if not overtly immunodeficient, may have a partial or “occult” immune deficiency, which may reduce or eliminate their antibody response to one or more agents. Even if some immune response is mounted, it may be insufficient to protect against disease or to eliminate the virus, resulting in sporadic viral shedding. The presence of infected immunodeficient mice within a colony can thus prevent the rederivation of mouse colonies by burnout (immune clearance) or test-and-cull methods. Direct sentinel contact can determine on a cage-by-cage basis which colony animals are shedding infectious virus.

The exposure plan for contact sentinel mice must allow enough time for initial infection and replication of the agent and for the sentinels to mount an immune response (if serologic testing is planned). This generally means the animal may be bled for serologic testing 3 weeks after initial exposure. Not all 3 weeks must be spent in contact with the colony animals; some programs use 1 week of contact time followed by 2 weeks of holding time. A period of 3 weeks is also considered adequate to allow patency of pinworm infestations, but may be inadequate to amplify a low-level infestation with fur mites. The time interval for seroconversion to some viral agents, such as paroviruses, may be variable, and a longer time period may be required.

c. Testing of Colony Animals as Part of the QA Program

1) SICK OR DECEASED ANIMALS Routine testing of mice submitted for diagnostic necropsy is an essential component of the QA program. Testing of diagnostic cases for common viral, parasitic, and bacterial agents provides information essential to case management and often provides early warning when a colony has become infected with an unwanted agent. When isolation cages are used, testing of colony mice submitted for diagnostic work-up provides information about what agents may be present in the colony that are not readily transmitted via soiled bedding.

2) Variations in Experimental Results Problems or inconsistencies in experimental data, as detected by scientists, may also indicate the presence of a subclinical infection
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(McKisic et al. 1993; Small 1986). When scientists encounter variations that could be attributed to the presence of an excluded infectious agent, sampling should ideally include mice from the investigator's colony. Supplemental testing of the room sentinels may also be useful.

3) FOLLOW-UP TESTING AFTER AN OUTBREAK IS DETECTED

Testing of colony animals is often used to determine the prevalence of an agent within the room population when sentinels have tested positive. The most common approach is to bleed and test one mouse per cage because all mice in the cage can be assumed to have been exposed if a sample mouse is seropositive. Because colony animals are valuable and often irreplaceable, the minimum amount of blood should be drawn utilizing a minimally invasive method. This type of testing is often needed to plan the disposition of an affected colony or to select infected animals for a test-and-cull eradication plan.

4. How Many Animals Should Be Tested?

In the past, mice were usually housed under open-top or conventional conditions, and statistical sampling formulas were suggested to determine how many animals need to be tested to detect an infection with a specified confidence level based on population size (ILAR 1991; Small 1986). These formulas assumed random and uniform transmission within a population in the animal room, a condition that was true when highly transmissible agents were present and filter tops, isolation cages, and filtered changing stations were not used. However, current housing methods are designed to impose significant barriers to cage-to-cage transmission of infectious agents. These changes in housing practices have partially invalidated previous assumptions and reduce confidence that these formulas are still optimal for determination of how many animals should be sampled. Establishing the number of animals to be tested requires consideration of many factors (including cost/benefit ratios) and must ultimately be estimated largely on the basis of professional judgment (Rehg and Toth 1998).

At this time, the most common approach is to dedicate one bedding sentinel cage to each 50–70 (or fewer) colony cages. In practice, this often means that one cage of bedding sentinel mice is maintained per low-density rack, or one cage is kept per side of a high-density (90–140 cages) rack. A similar 1:50 to 1:70 ratio of sentinel cages to colony cages is suggested for other housing configurations. It is recommended that sentinel cages be stocked with at least two mice per cage because the other mouse is available for use if one mouse in the cage dies. The second mouse can also serve as a confirmatory backup in case there is an equivocal test result or if additional tests (such as antigen detection or PCR analysis) require a second sample.

5. When Should Animals Be Tested?

There is no single schedule that is ideal for testing sentinel animals. Younger animals are more likely to have patent infections with parasites such as pinworms, whereas older animals have had more time to become exposed agents such as mites and some viruses that are transmitted more slowly. On the other hand, evidence suggests that older animals may seroconvert less reliably to some infectious agents such as MPV (Besselsen et al. 2000). Some programs perform nonterminal testing (bleeding, parasite testing) repeatedly on the same animals over a period of several months before a final comprehensive terminal screen. This allows comparisons to be made over time, and the same animal can be retested if results are questionable. Others will perform terminal testing on only some of the animals in each cage at each time point, leaving the cage mates available for repeat testing or later time points. Regardless of testing schedule, it is recommended that replacement sentinels be placed in the room at least 3 weeks prior to removing sentinels for terminal screening, so there is no gap in exposure should retesting of the room be desired.

6. What Tests Should Be Used, and What Are the Strengths and Limitations of Each Type of Test?

All diagnostic tests have the inherent possibility of returning a false-negative result (test was negative, but agent was present) or a false-positive result (test was positive, but agent was not present). When discussing the value and interpretation of diagnostic tests, the meanings of the terms sensitivity and specificity must be clear. A sensitive diagnostic test will have a low number of false-negative results. This type of test is useful for preliminary screening of a population to ensure that the chance of undetected contamination is minimized. A specific diagnostic test will have a low number of false-positive results and will clearly distinguish the agent of interest from other common or related infectious agents. This type of test is useful for confirming the results of preliminary screening tests before major actions are taken. Fortunately, many of the modern tests employed for QA monitoring are both sensitive and specific. However, it is strongly suggested that the testing and response plan be designed so that more than one type of test be used to maximize the validity of the interpretation (ACLAD 1991). Immunologic, molecular, and physical methods all have a place in identifying the presence of infectious or parasitic agents. Each type has strengths and weaknesses and can return false-positive or false-negative results (Compton and Riley 2001).

a. TESTING FOR VIRAL AGENTS

1) SEROLOGIC TESTING

Testing for antibody indicates if the animal has mounted an immune response (seroconverted) to the agent in question. Although testing for antibody is the most frequent type of test utilized, several pitfalls should be kept in mind. First, immunodeficient animals, including occultly immunodeficient genetically engineered mice, may not seroconvert, so interpretation of results from colony animals will need to be carefully considered in light of what is or is not known about immune status of the colony animals. Second, the presence of antibody does not indicate currently active infection...
nor does it necessarily indicate recent infection. Because it
takes a minimum of 7–10 days after exposure before antibody
is present, many viral agents have short periods of production
and shedding before protective immunity develops and the
infection is cleared. An animal may then remain seropositive
for weeks to months in the absence of active viral shedding.
This is an advantage of serologic testing because seroconversion
is generally a lifelong marker of prior exposure, but a
disadvantage in that serology does not accurately reflect infect-
ity. In addition, clearance after seroconversion cannot always
be assumed, because immunodeficient mice (including genetically
modified animals with “cryptic” immune abnormalities)
may produce ineffectual antibody without eliminating viral
agents (Hickman 2004; Pullium et al. 2003; Rehg et al. 2001).
For reasons that are not understood, seroconversion to MPV in
some groups of mice can take an extended period of time to
develop or show other variability (Besselsen et al. 2000; Jacoby
and Smith 2003). Many colony health coordinators have
learned that the assumption that all mice in a cage will share the
same seroconversion status is not valid for MPV. Lastly, some
mouse strains and some individual mice have nonspecifically
reactive sera for unknown reasons, and serologic testing of
these mice may yield a high rate of false-positive reactions.
When this manifests as broad cross-reactivity to many agents in
a panel it is somewhat easier to identify, but the use of alternative
tests for confirmation may be needed to identify untrustworthy
results when the sera shows false reactivity to only one or a few
agents. These factors all complicate the interpretation of sere-
logic results when testing mouse colonies and sentinel mice.

a) Enzyme-Linked Immunosorbent Assay (ELISA) ELISA
testing of serum is the most commonly used method to deter-
dine if a mouse has been exposed to an infectious agent
(Kendall et al. 1999A; Smith 1983). IFA testing is used less fre-
quently as a primary screening method because it is more
expensive and difficult to automate, the test interpretation
requires trained personnel and is more subjective than ELISA,
and an epifluorescence microscope is needed. For IFA testing,
cultured cells infected with the agent of interest attached to
a microscope slide are exposed to the test serum. After time is
allowed for binding of antibodies, the test serum is washed off
and a probe such as fluoresceinated goat anti-mouse antibody
is used to attach a detectable marker to bound antibody. After
washing, the slide is examined under a fluorescence micro-
scope and the reader looks for infected cells showing a
fluorescence pattern characteristic for that agent. For example,
coronavirus (MHV) replication occurs in the cytoplasm and the
technician would discount any fluorescence that was not
located in cellular cytoplasm, whereas parvovirus (MPV,
MMV) replicates in the nucleus and the technician would dis-
count any fluorescence not located in the nucleus. IFA testing is
both sensitive and specific, but there is cross-reactivity between
viral serotypes (such as MMV and MPV) due to the presence of
conserved nonstructural proteins in the infected cells.

b) Immunofluorescence Antibody (IFA) IFA testing of
serum may be used as a primary or confirmatory method to
determine if a mouse has been exposed to an infectious agent
(Kendall et al. 1999A; Smith 1983). ELISA testing is used less fre-
quently as a primary screening method because it is more
expensive and difficult to automate, the test interpretation
requires trained personnel and is more subjective than ELISA,
and an epifluorescence microscope is needed. For IFA testing,
cultured cells infected with the agent of interest attached to
a microscope slide are exposed to the test serum. After time is
allowed for binding of antibodies, the test serum is washed off
and a probe such as fluoresceinated goat anti-mouse antibody
is used to attach a detectable marker to bound antibody. After
washing, the slide is examined under a fluorescence micro-
scope and the reader looks for infected cells showing a
fluorescence pattern characteristic for that agent. For example,
coronavirus (MHV) replication occurs in the cytoplasm and the
technician would discount any fluorescence that was not
located in cellular cytoplasm, whereas parvovirus (MPV,
MMV) replicates in the nucleus and the technician would dis-
count any fluorescence not located in the nucleus. IFA testing is
both sensitive and specific, but there is cross-reactivity between
viral serotypes (such as MMV and MPV) due to the presence of
conserved nonstructural proteins in the infected cells.

c) Hemagglutination Inhibition (HAI) Assays HAI assays
are not generally used for primary testing but are very useful
for confirmatory testing and are a sensitive method of serotyp-
ing (Kendall et al. 1999C). This test relies on the ability of
some viruses to agglutinate red blood cells. When serum con-
taining virus-specific antibody is added to the reaction mix, the
antibody will coat the viral particles and prevent agglutination.
The reaction is performed in V-bottom wells. If no agglutina-
tion occurs, the red cells collect in a “button” at the bottom
of the well and will stream if the plate is tilted. Agglutinated
cells do not stream but blanket the bottom of the well. Serial
dilutions of the serum are used to determine a quantitative titer.

2) Testing for Viral Genetic Material Polymerase chain
reaction (PCR) is used to detect the presence of viral genetic
material (DNA or RNA) in sample materials. Samples such as
feces, tissues, or environmental samples can be tested by this
method. Knowledge of the genetic code of the target virus
allows the construction of extremely specific primer pairs that
complement unique segments of the viral genome. Under con-
ditions of controlled temperature variation, in the presence of a
thermostable Taq polymerase, multiple cycles of DNA replica-
cation result in massive amplification of viral target sequences.
The presence or absence of a gene product of the expected size
is confirmed, most commonly by use of acrylamide gel elec-
trophoresis (Kendall and Riley 1999D). If the agent is an RNA
virus, the use of a RNA-dependent DNA polymerase (reverse
transcriptase) allows use of the PCR technique by first convert-
ing RNA genetic material into complementary DNA, which
can then be amplified and detected by standard PCR methods
(RT-PCR) (Kendall and Riley 2000).

PCR testing has its own advantages and disadvantages when
compared to serologic testing. Because viral genetic material is
the target, PCR tests would be expected to be negative in animals that have experienced and then cleared an infection. Unless the target, PCR tests would be expected to be negative in animals the agent is endemic in the colony. One exception is MPV, which is commonly found in tissues such as mesenteric lymph nodes and spleen both early in an infection, before a serologic response is detectable and for an extended period of time after seroconversion has occurred (Besselsen et al. 2000; Shet 2003). Selection of the appropriate tissue to sample, distribution of the virus within the sample, possible exposure of the sample to conditions that favor DNA or RNA degradation, and the potential for contamination during collection or in the laboratory must all be considered when interpreting PCR results. One must also keep in mind that positive results reflect presence of genetic material, not necessarily infectious virus. PCR tests may require euthanization of the animal to collect appropriate tissue samples. Finally, PCR testing requires specialized equipment, isolated laboratory space to separate testing functions, and scientific expertise for interpretation.

Despite the disadvantages, PCR’s specificity and sensitivity have earned it a useful place in the detection and especially the confirmation of viral infections. Newer methodologies such as real-time, quantitative, and multiplex PCR may further increase the diagnostic applications and value of molecular techniques.

b. TESTING FOR BACTERIAL AGENTS Several bacterial agents are commonly excluded from experimental mouse colonies because they cause illness or are known to alter experimental outcomes.

1. Mycoplasma pulmonis and M. arthritidis Mycoplasma pulmonis and M. arthritidis have largely been eliminated from commercially available laboratory mice because they cause illness and alter immune function of mice (Jacoby and Lindsey 1998). However, they remain present in some research colonies, so testing for and exclusion of these agents is indicated. Because culture of mycoplasmal species is difficult and slow compared to culture of other bacteria, serologic testing by the ELISA method is most commonly used to detect exposure of mice. Unlike the situation with many viral agents, immune clearance of some bacterial and most mycoplasmal agents does not occur, and seropositive animals should generally be assumed to have lifelong infection even in the absence of clinical signs (Cassell et al. 1986). Although IFA testing using mycoplasmal-infected cultured cells can be done, this technique is not commonly used. PCR testing is useful to test cultured cells or biologic materials for the presence of mycoplasma. Most mycoplasmal species detected in cultured cells are of human origin, not M. pulmonis or M. arthritidis, and are therefore unlikely to be transmitted mouse-to-mouse, although they may be detrimental if injected into immunodeficient mice. Testing for mycoplasmal species in mice is especially important if mice are imported from noncommercial institutions that have limited QA testing because these agents were common at one time and may persist in isolated pockets of mice. Similarly, mouse antibody production (MAP) testing of cultured cells, discussed later in this chapter, is especially important if the cell lines originated when mycoplasmal infections were more common.

2. Helicobacter hepaticus and H. bilis Helicobacter hepaticus and H. bilis have been recognized as being common in laboratory mouse colonies and have been shown to have the potential to interfere with studies of the liver (Donovan 1993) and gastrointestinal tract (Whary and Fox 2004). Because these agents were not well understood until recently and were not excluded from many existing institutional colonies, they are common in mice housed in many locations. Commercially obtained mice often arrive free of Helicobacter but are colonized after placement into endemically infected colonies. Although some institutions exclude these agents and include testing for Helicobacter spp. in their QA programs, many other institutions assume that most rooms contain infected animals and do not test for the presence of Helicobacter as part of rodent QA. To date, serologic testing for pathogenic Helicobacter species has not been of adequate specificity for routine use. This is due to the presence of antibodies against other intestinal flora that cross-react with Helicobacter (Whary et al. 2000). Because of its high sensitivity and the ability to develop species-specific primers for the limited number of species that are known to be pathogenic, PCR testing is the preferred method and is commercially available (Hodzic et al. 2001). Freshly obtained fecal pellets and/or cecal scrapings from sentinels or colony mice are the test material of choice. Helicobacter organisms are readily transmitted by infected bedding, making bedding exposed sentinel testing a valid method to test colony status (Livingston 1998; Whary et al. 2000).

3. Pasteurella pneumotropica Pasteurella pneumotropica is an opportunistic pathogen of mice that can cause infection in multiple sites, especially the respiratory and reproductive tracts, and is excluded from most commercial colonies and some institutional colonies. Pasteurella is readily cultured on appropriate bacterial isolation media from samples obtained by aseptic flushing or swabbing of infected tissues. There is some evidence that a combination of enrichment culture of nasopharyngeal swabs followed by PCR may be more sensitive than either culture or PCR alone (Wang et al. 1996; M. Batchelder personal communication). Serologic tests, tissue PCR, or antemortem samples are not commonly used as part of a QA program.

QA practices for other bacterial species vary widely, but the most comprehensive programs routinely monitor sentinels for the presence of pathogenic, opportunistic, or zoonotic agents known to infect mice, as listed in Table 11-2.

c. TESTING FOR PARASITIC AGENTS QA testing of mice includes testing for both internal and external parasitic agents. The most common parasites in modern colonies of experimental mice are pinworms and fur mites (Jacoby and Lindsey 1998). Although much less common, other parasites may be
detected during testing for pinworms or mites performed for diagnostic or QA purposes.

1) INTERNAL PARASITES The most common internal parasites in mice are pinworms of the genera *Aspiculuris* and *Syphacia*. Three types of diagnostic techniques are used: (1) tape tests of the perineal skin for eggs, (2) fecal flotation for eggs, and (3) examination of the intestinal contents for adult pinworms. Eggs of *Syphacia obvelata*, the mouse pinworm, can be detected by microscopic examination of a piece of transparent tape after it is applied to the perineal skin. Detection of *Aspiculuris* pinworms requires either fecal flotation examination or direct examination of the intestinal contents because *Aspiculuris* does not deposit eggs on the perineal region. Despite elimination of pinworms from most commercial mouse colonies and the availability of effective treatment measures such as fenbendazole, ivermectin, and/or piperazine (Boivin et al. 1996; Coghlan et al. 1993; LeBlanc et al. 1993; Zenner 1998), pinworms remain common in institutional colonies (Jacoby and Lindsay 1998). It is especially important to use methods adequate to detect *Aspiculuris* pinworms on mice arriving from other institutions when the method of testing for pinworms at the source is unknown. Undetected infection is not uncommon in incoming quarantine mice, and may be the result of overreliance on tape tests at the facility of origin (D. Gaertner, personal communication). Tape or fecal sampling exclusively of adult mice may yield false-negative results because infected mice shed pinworm eggs for a limited amount of time after infection and the number of eggs declines as age-related immunity develops. For this reason fecal flotation or tape testing of adult animals should always be accompanied by examination of the intestinal contents for adult worms at necropsy.

2) EXTERNAL PARASITES *Myobia musculi*, *Myocoptes musculus*, and *Radfordia affinis* are the most common fur mites that infect mice (Jacoby et al. 2002). Fur mite detection usually entails close examination of the fur for mite eggs (nits) or for hatched mites. Nits are found attached to the hair shafts, whereas mobile nymphal and adult mites may be observed anywhere from the skin surface to the tips of the hairs. The most common methods include plucking fur from the nape of the neck or removal of the dorsal area of the pelt at necropsy followed by cooling and low power microscopic examination. Cooling encourages the mobile mites to travel to the tips of the hairs where they are more easily observed. These methods are adequate if individual mice are heavily infested but may yield false-negative results if the infestation is mild. It has been reported that *Myocoptes* tend to localize on the ventral abdomen and inguinal area, and a light infestation with this species may also be missed if only the neck is examined (Burdett et al. 1997). A more demanding method that is less susceptible to false negatives is the use of a pelt digestion technique using KOH (M. Batchelder, personal communication; adapted from a standard technique to clear keratin debris from skin scrapings). False-positive results may occur when testing previously infested animals after treatment, as nonviable nits may remain on the hair shafts. Differentiation of viable and nonviable nits is generally straightforward and clearly important in evaluating treatment efficacy (Burdett et al. 1997).

None of these methods will detect colony contamination if infested mice are not selected for testing, so it is important to plan the testing of sentinel and/or colony animals appropriately. Detection of fur mite infestation solely by relying on sentinel mice exposed to soiled bedding may result in missed infestations (Otto and Tolwani 2002). In addition to testing bedding sentinel mice, colony mice with hair loss or pruritus should also be routinely tested for mites by skin scrape or fur pluck and microscopic examination.

7. How Should Animals Exposed to Hazardous Chemicals or Biologic Agents Be Monitored?

The desirability of monitoring and methods used to monitor animals used in hazardous experiments depends on multiple factors. These include the source and intended use of the animals, the risk of inadvertent exogenous infection, and the hazardous substance or agent that is being used. Decisions regarding whether and how to best monitor the animals should be made on a case-by-case basis through discussion between the investigator, veterinary staff, and institutional environmental health and safety staff.

C. Quarantine of Mice

Quarantine and testing of mice arriving from a noncommercial source (e.g., an academic institution) is a crucial component of a QA program for mice. Prior to shipping, source institutions should provide pertinent health information regarding the colony from which animals will be shipped. However, this is rarely sufficient to allow the approval of direct importation of animals into an established colony. The historical summary of previous testing provided often provides an incomplete picture because it does not include all of the agent-specific information needed by the receiving institution or because the data provided are out-of-date. There may also be concerns about the accuracy and validity of the test results provided, because the health monitoring program may be quite different from what is in place at the receiving institution. Even if the colony of origin truly is free of contamination with excluded agents, animals may be exposed to infectious agents during transport as a result of cross-contamination from animals in other shipments or from feral rodents in loading docks or holding areas. Although it can delay the availability of imported mice for research and utilizes additional resources, a formal program for quarantine isolation and pre-release testing of animals arriving from noncommercial vendors is considered essential.
1. Quarantine Process

A structured, stepwise approach to quarantine evaluation is recommended. Shipping via a dedicated transport vehicle that contains only animals from a single source and is thoroughly decontaminated between shipments would be ideal; however, this option is not always readily available. Inspection of shipping crates on arrival for obvious damage may reveal an increased risk of contamination during transport. Animals should also be visually inspected on arrival for any signs of ill health related to preexisting conditions, trauma, or exposure to adverse conditions during shipping. Infectious agents rarely present as a clinical problem, so to rapidly identify potential problems at the beginning of the quarantine period, it may be useful to collect serum (for serologic assays), feces (for fecal flotation and/or PCR testing), anal tape impressions, and skin scraping or fur samples (for parasite examination) or other nonterminal samples directly from the shipped animals during the initial phase of quarantine isolation. These data can be used to verify that the pre-shipping documentation was accurate and would allow adjustment of the quarantine and/or rederivation plan if animals arrive infected with agents other than those expected based on pre-shipping health reports. Testing done very shortly after arrival (e.g., within 1–3 days) will generally detect agents present in the mice prior to shipping, because most agents would require a period of incubation, amplification, or seroconversion before they are detectable (with the possible exception of highly sensitive PCR assays). If unexpected agents are identified during quarantine, a comparison of test results from this early testing with those done later in quarantine (e.g., 2–5 weeks later) can help differentiate preexisting infection in the colony of origin from exposure that occurred during shipping. It is considered common courtesy that the recipient institution informs the source institution of any unexpected findings so that they can re-examine their own QA results and possibly retest the colony of origin.

2. Quarantine Housing and Isolation

Animals in quarantine need to be housed in an isolated area and the animal husbandry procedures utilized need to maximize the likelihood that infectious agents could be spread between groups of mice undergoing quarantine. Optimally, quarantine groups will be small (less than five cages) so that all cages can be tested for each excluded agent; however, the relocation of entire laboratory groups between institutions and the shipment of the associated animals as a large group may make this impractical. Shipments of entire colonies are sometimes broken up into two or more smaller shipments to avoid loss of irreplaceable lines should a shipping disaster occur. These groups can be quarantined separately, or all shipments may be combined and quarantined as a single group. The best possible isolation for quarantined groups of animals would be the use of one room per imported shipment in an “all-in, all-out” system, but more space-efficient approaches are often necessary, including the use of isolation cubicles, semirigid or flexible film isolators (Rehg and Toth 1998), or careful use of microisolation caging (Otto and Toiwani 2002). As an added precaution against the possibility of an internal or external parasite infection remaining undetected or causing cross-contamination of other shipments, many institutions prophylactically provide feed containing a therapeutic level of fenbendazole to eliminate pinworms (100 to 150 parts per million in the diet) (Boivin et al. 1996; Coghlan et al. 1993) and utilize an acaricide. Useful acaricides include permethrin-impregnated cotton nesting material (currently marketed as Mite Arrest) (Mather and Lausen 1990) or dichlorvos-containing pellets (Fraser et al. 1974). Treatment against pinworms and fur mites ensures that these agents do not gain access to colony animals despite the possibility of false-negative testing results from imported animals, contact sentinels, or bedding sentinels.

3. Testing During Quarantine

The testing regimen for animals in quarantine should be based on the list of excluded agents for the recipient animal facility and the type of research for which the animals will be used. At minimum tests should include serology for excluded viral and mycoplasmal agents and tests for internal and external parasites. The addition of gross necropsy examination (generally performed on exposed sentinels unless a surplus of shipped mice exists) is strongly suggested, whereas histopathology is rarely indicated except as a follow-up to abnormal gross findings.

Although some institutions rely exclusively on exposure and testing of bedding-exposed sentinels or direct testing of the incoming mice, these methods are not optimal. The first problem with such a plan is that not all infectious agents are readily transmitted to noncontact sentinels by transfer of soiled bedding, especially in the relatively short span of the quarantine period (Thigpen et al. 1989). The second issue is that serologic testing of the incoming mice will only be instructive if those mice have a “typical” physiologic response to infection and mount a “normal” antibody response. In many cases the incoming mice have a unique mutant genotype that has only been partially phenotyped, and a brisk seroconversion to common agents cannot be presumed. The fact that idiosyncratic abnormal immune and physiologic responses exist in genetically engineered strains has been discussed in the context of MHV contamination (Rehg et al. 2001), and the potential ramifications of this phenomenon can be used to justify the use of known immunocompetent contact sentinels as the primary test animal for groups of quarantined mice. A practical means to allow for thorough diagnostic testing without jeopardizing the well-being of the small number of valuable (sometimes irreplaceable) animals shipped is to establish co-housing exposure of a contact sentinel animal with each cage of arriving animals, or with a percentage of representative cages if the shipment is large. With careful management to prevent them from giving birth while in contact with shipped mice, the use of
young female contact sentinels will minimize concerns about fighting as a result of foreign animal introduction (Otto and Tolwani 2002). Contact sentinel animals may be removed to separate cages after 1–2 weeks of exposure to the shipped animals and complete testing of contact sentinel animals can then be performed 3 or more weeks after the initial exposure to the shipped animals. Surveillance performed on the incoming mice still does have a place in the quarantine plan, because they are the main subjects of interest. Testing of shipped mice is most useful when the test is noninvasive and directly assays for the presence of an agent, for example, feces, anal tape impressions, and skin scrapings or fur plucks can be used to test for endo- and ectoparasites and *Helicobacter* spp. (by fecal PCR).

In addition to scheduled testing, any animal (sentinel or incoming) that dies during quarantine should be submitted for postmortem examination. Consideration should also be given to bleeding surviving cagemates for serologic assays after allowing an appropriate period for seroconversion.

D. Testing of Biologic Materials

Testing of biologic materials prior to administration to mice is an essential component of a QA program. Cultured cell lines, tumor cells, serum components, antibody preparations, and other materials of biologic origin may carry murine viruses or bacteria capable of infecting the recipient mice (Bhatt et al. 1986; Collins and Parker 1972; Lipman et al. 2000; Nicklas et al. 1993). Resulting infections may profoundly affect research both in the implanted animals and indirectly in other research animals if an outbreak spreads. Nicklas et al. (1993) reported that 70.4% of mouse tumors propagated in vivo were positive for one or more murine viruses, with lactate dehydrogenase-elevating virus being the most common. Other viruses detected were reovirus-3, lymphocytic choriomeningitis virus (a zoonotic concern), MMV, and MHV; *M. pulmonis* was also identified in one specimen. Other authors have reported outbreaks of ectromelia virus traced to contaminated commercial mouse serum (Dick et al. 1996; Lipman et al. 1999, 2000).

Biologic materials that should be tested include any that have originated from rodents or which have been exposed to rodents directly (in-vivo passage) or indirectly (e.g., via tissue culture media additives). Once tested, subsequent passages in clean mice (free of agents of concern to the institution) may be permitted, but retesting is recommended should an outbreak occur in the facility. Unfortunately, the risks of transmission of viruses to mice by these biologic materials are not always understood nor appreciated by scientists, and an active educational program is usually needed to ensure that untested materials do not expose mice and unintentionally introduce infectious agents into a colony. As a way to increase compliance, many institutions make investigators aware of this requirement at both the facility level (policies and procedures disseminated by the veterinary care group) and the protocol level (IACUC approval requires testing).

1. Methods to Detect Viral Agents in Biologic Materials

The MAP bioassay (De Souza and Smith 1989; Rowe et al. 1959) and commercially available multiple-agent PCR panel tests are used to test incoming biologic materials. If isolation space is available at the home institution, MAP testing can usually be done in house. If isolation space is limited or unavailable, materials to be tested are frequently sent to commercial testing laboratories for either MAP or PCR testing.

a. Mouse Antibody Production (MAP) Although the original protocol for MAP testing was relatively complex, the basic principle is that immunocompetent pathogen-free mice are injected with the test material and held in isolation for at least 3 weeks, a period that allows time for seroconversion to infectious agent(s) that may present in the sample. After the holding period, mice are bled and serum is tested for antibodies for a panel of agents of interest. An alternative option for testing is to isolate experimental mice being given an untested preparation for the first time and to place immunocompetent contact sentinel mice within the cage(s). After an appropriate incubation period, the sentinels are tested to determine if a viable and replicating agent of concern was present in the test material, because it should have also infected the contact sentinel mice. Because the sentinels are not directly injected with the original material but would be given secondary exposure to agents shed by the experimental mice, at least 1 additional week of pretesting exposure (i.e., an exposure time of 4 weeks or greater) should be provided.

One potential pitfall of MAP testing is that the use of aggressive tumor cell lines or hybridomas may cause morbidity or mortality so quickly that seroconversion of injected animals and/or effective transmission of potential infectious agents to sentinel mice will not develop. In these cases it will be necessary to utilize a standard MAP testing regimen that includes freeze-thaw cycles to kill the cells and release viral particles. Regardless of method, all MAP testing should be done in quarantine isolation.

b. Polymerase Chain Reaction PCR testing is rapidly replacing classical MAP testing because it offers some significant benefits, including high sensitivity (Booza et al. 2003; Yagami et al. 1995), shorter turn-around time, and no need for additional animals. Aggressive tumors can be tested without the risk of early mortality. A fluorogenic nuclease RT-PCR has been suggested as useful for detecting agents such as LCMV, murine coronaviruses and parvoviruses, PVM, and Sendai virus in biologic materials (Besselsen et al. 2002; Redig and Besselsen 2001; Wagner et al. 2003). Disadvantages include the potential for false-positive results from nonviable DNA fragments when biologic materials or animals receiving biologic materials are tested. In addition, PCR testing has not yet been validated to be accepted by the Food and Drug Administration (FDA) as a replacement for traditional MAP testing for good laboratory practice (GLP) studies.
essential component of a QA program. However, despite our best
unwanted infectious agents.
methods are key factors to minimize the introduction of
movements and the utilization of optimal barrier housing
fact. For these reasons, strict control of animal and personnel
prevent problems by eliminating the initial contamination
before routine testing identifies the event. During this period,
serial dilutions and further passage can be used to confirm the diagnosis. One must be careful interpreting
elevated LDH enzyme in the case of animals injected with
viable tumor cells, which may invade and damage tissues,
releasing endogenous LDH. To avoid these complications, the
use of a fluorogenic nuclease RT-PCR has been reported
between cages and rooms. The use of isolation-type caging,
precautions that are in place to limit cross-contamination
equipment will not prevent cross-contamination if proper hus-
HEPA-filtered change stations and "clean to dirty" personnel
animal care and research staff. Of course, it is always better to
be used to eliminate these agents from mouse colonies.
Because the frequency and extent of QA testing is almost
always limited by financial resources, an infectious agent
often remains undetected for a period of weeks or months
before routine testing identifies the event. During this period,
the rate of spread of the infectious agent will be determined by
the biology of the agent and by the efficacy of the standard
precautions that are in place to limit cross-contamination
between cages and rooms. The use of isolation-type caging,
HEPA-filtered change stations and "clean to dirty" personnel
traffic can be very effective, but the mere presence of the
equipment will not prevent cross-contamination if proper hus-
bandry practices and barrier precautions are not followed by
animal care and research staff. Of course, it is always better to
prevent problems by eliminating the initial contamination
event than it is to rely on detection and containment after the
fact. For these reasons, strict control of animal and personnel
movements and the utilization of optimal barrier housing
methods are key factors to minimize the introduction of
unwanted infectious agents.

IV. INFECTIOUS OUTBREAK SOURCES
AND MANAGEMENT

Successful rodent QA management must take into account
both the routes by which unwanted infectious agents can be
introduced into experimental colonies and the methods that can
be used to eliminate these agents from mouse colonies.

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always limited by financial resources, an infectious agent
often remains undetected for a period of weeks or months
before routine testing identifies the event. During this period,
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prevent problems by eliminating the initial contamination
event than it is to rely on detection and containment after the
fact. For these reasons, strict control of animal and personnel
movements and the utilization of optimal barrier housing
methods are key factors to minimize the introduction of
unwanted infectious agents.

A. Sources of Unwanted Infectious Agents

1. Introduction of Contaminated Animals from Noncommercial Sources

Quarantine of mice arriving from noncommercial vendors is an
essential component of a QA program. However, despite our best
efforts there is always the possibility that quarantine proce-
dures may fail to detect the presence of an infectious agent that
was present in the colony of origin or acquired during transport.
For example, the arrival of large groups of mice into quarantine
may lead to detection failures because not every incoming cage
of mice can be reasonably monitored using contact sentinels.
As mentioned previously, the exclusive use of bedding sen-
tinels to detect potential infectious agents during quarantine
may yield detection failures because not all agents are consist-
tently transmitted using soiled bedding. Finally, some
infectious agents may be at a very low incidence, for example,
a single cage of mice out of a larger shipment may be infested
with fur mites and the exposure and sampling methods used
during quarantine may fail to detect such a limited infestation.
Failure of quarantine may not be noticed immediately, because
barrier precautions such as isolation caging and the use of
HEPA-filtered change stations may slow the spread of the
undetected contaminant for a time until it reaches a prevalence
detectable by the sentinel testing program or clinical illness
becomes apparent.

2. Introduction of Contaminated Biologic Materials

Untested biologic materials to which experimental mice
are exposed can be a source of colony contamination (Nicklas
et al. 1993). Exposure can be direct, such as when older sam-
pies of a tumor cell line infected with an excluded agent are
thawed and injected into mice. More difficult to trace are out-
breaks resulting from indirect exposure, such as one that
occurred in 1999. In that case, ectromelia virus was transmit-
ted to mice when contaminated commercially available
imported mouse serum was used as a medium supplement for
culturing cells that were later injected into mice (Dick, et al.
1996; Lipman et al. 1999; Lipman et al. 2000). Each institu-
tion needs to educate scientists regarding the need to test
biologic materials, even if they may expose mice only by an
indirect route. It is also important to realize that commonly
used tissue culture repositories do not routinely test for all
murine agents. When novel biologic materials are proposed
for use, a thorough risk analysis should be done to determine
if there is a potential for contamination with murine agents and
whether the harvest and preparation of the material would be
expected to inactivate any viable agents that might be present.
When in doubt, it is best to test a representative aliquot
directly by MAP or PCR testing. Methods for testing cells and
other biologic materials have been discussed elsewhere in this
chapter.

3. Introduction of Contaminated Animals from Commercial Vendors

The selection of commercial vendors that provide high-quality
animals and utilize secure modes of packaging, transport, and
delivery is another integral part of the QA program. Ideally,
every mouse arriving from outside the institution would
undergo quarantine and testing, but in practice the requirements of scientists are too great and resources such as quarantine space and financial support are too limited to quarantine most mice arriving from commercial vendors (Rehg and Toth 1998; Small 1986). There is a small but real possibility that mice arriving from approved, commercial vendors can introduce unwanted infectious agents into animal housing locations. In most cases, outbreaks are self-reported by vendors as soon as they are detected, and communication with the vendor and review of acquisition records can help identify the most suspect recent shipments to contain the problem. In some cases there has been suspicion that vendor animals are carrying infectious agents, at low levels or sporadically, even when the vendor’s internal testing is negative. Clinical studies have been designed specifically to determine whether this might be the source of some outbreaks (Pullium et al. 2004).

4. Vermin

Feral or loose rodents are a ready source of unwanted infectious agents, and insect pests can facilitate cross-contamination. Any facility, independent of location (rural, suburban, urban), can inadvertently house its own subpopulation consisting of feral mice that may have entered from outside the building or may have escaped from research colonies and managed to survive. Vermin-proof designs should be included when new facilities are built, but many older buildings have been and will remain infested with mice. Once they are present in the building, it is nearly impossible to completely eliminate feral rodents. Structural features such as dropped ceilings, interstitial spaces, or ceiling access ports are necessary to provide engineering and maintenance personnel access to electrical, ventilation, and plumbing systems, but they also provide conduits and safe havens for resident rodents. Feral mice have been found nesting inside sheetrock walls and insects have been found nesting inside hollow doors and wall bumpers, so all aspects of design must be considered during design and renovation.

Effective professional vermin control programs are an essential component of animal care for all facilities. Traps should be provided and monitored routinely for mice as well as insects. If mice are trapped alive, it is useful to euthanatize and test them as part of the QA program. Results of such testing should be interpreted carefully, taking into account the location where the animal was trapped, the appearance of the animal (e.g., non-wild-type coat color) and the likelihood of its being an escapee or an intruder. If feral rodents are known to inhabit a facility, it is essential that both colony and sentinel animals be housed in covered or isolation cages because the presence of food and mice in open cages will attract nocturnal rodent visitors. Incursions by feral rodents into animal rooms where sentinels are kept in open-top cages may result in positive QA findings that reflect the agents present in the vermin rather than those agents truly present in the experimental mouse colony being monitored.

5. Nearby Research Colonies

Although all of the possibilities listed previously must be considered, experience has shown that the most likely source of contamination affecting “clean” animal colonies (SPF, barrier-maintained, etc.) at any particular institution is a “dirty” colony (conventional, nonbarrier, etc.) kept within the same institution. For a variety of reasons, including the need to utilize bulky equipment in laboratory spaces, many research institutions maintain both barrier and conventional colonies of mice. These distinctly different populations may be maintained in close proximity in some facilities (occasionally within the same corridor), or they may be housed in separate buildings or on different campuses. When a single facility houses both populations, the movement of people and animals is difficult to fully control, especially during off hours, and unapproved traffic or transfers can allow infectious agents to gain access to mice in barrier rooms. Because infectious agents such as MHV and Sendai virus can be assumed to be transmitted not only by infected mice but also by exposure to contaminated fomites (e.g., bedding particles) or recently contaminated surfaces, it only takes one error in procedure to transmit an infectious agent into a barrier colony. Even when kept physically separate, there will be varying degrees of operational overlap that could cause cross-contamination when factors such as staffing, storage, materials transfer, supervision, and inspection are considered.

From a research standpoint, the current emphasis on collaborative and multidisciplinary studies, shared resource cores, and prompt transfer of research-related materials (including mutant mice) makes it likely that even physically separated facilities at a single institution will be involved in interrelated experimental projects that could cause a cross-contamination event.

Although some facilities have been designed to operate as fully contained environments from which mice never leave, more often mice must at some point be transferred from a centralized housing area to procedural space in a laboratory area or a transient housing location in an approved satellite area. Pest control and proper feed/bedding storage to exclude vermin and cross-contamination can be much more difficult in these areas due to the relative lack of separation from contiguous spaces. In addition, scientists housing mice at distant locations may be less aware of or less concerned with the operational procedures needed to protect mice from infectious agents. These distantly housed mice may then serve as the nidus for infection of mice housed within the animal facility if there is even occasional personnel or animal traffic between the central facility and the decentralized areas.

On those occasions when the source of an outbreak can be identified, the contaminating event usually involves some type of human error. Animal care staff, although caring and well-meaning, are not experts in infectious diseases and may make
mistakes. The working environment between management and staff members must be supportive, so that staff can self-report mistakes to management if they realize that proper procedures were not followed. Scientific staff at all levels (technician, student, fellow, faculty) may also make mistakes in following barrier rules. Education of research staff is often made more difficult by language difficulties and differences in cultural viewpoint. New investigators may be overeager to begin working with animals before they have had the opportunity to undergo thorough training in barrier techniques. The “publish or perish” culture of modern science may lead scientists to consider taking short-cuts around facility rules when programs such as quarantine or biologic testing appear to needlessly delay completion of their research. This may lead to circumvention of proper procedures such as “sneaking in” mice without prior quarantine clearance or to smaller violations such as not wearing coveralls or gloves in evening hours when observers are scant. Procedural lapses of this type are difficult to identify, and if they result in a contamination event the source of the outbreak will likely not be determined. Absolute compliance with facility standard operating procedures and barrier precautions is needed to completely eliminate the possibility of introduction of unwanted agents; however, this level of compliance is often unattainable. Compliance failures remain a significant cause of outbreaks of infectious diseases in experimental mice.

B. Options for Action When an Unwanted Agent Is Detected

1. Always Confirm Initial Positive Test Results for Viral Agents

In the case of a single positive test or an equivocal positive result, confirmatory results should be obtained before any drastic action is taken. Confirmation should include testing of the same sera (or that of a cagemate) by another method within the same laboratory (e.g., confirming ELISA results by running an IFA test), or by arranging for testing at a different laboratory (Rehg and Toth 1998). There are some indicators that can be used to make a presumptive positive diagnosis without waiting for additional test results, such as finding more than one sentinel cage from the suspect area that is positive or obtaining a positive test in a screening assay known to be very specific (e.g., historically low false-positive rate). Caution should be taken in relying on a single positive ELISA test, especially if other mice in the same cage were not seropositive. Interpretation of nonserologic tests is more straightforward, because they assay directly for the presence of observable organisms or genetic material. However, bacterial isolates can be mischaracterized, artifacts in the sample can be misidentified as parasite components, and PCR samples may become contaminated or results may not be repeatable. Therefore, it is still important to verify the accuracy of test results prior to making decisions that will have a major impact on the colony and the research programs involved.

2. Eradication Options

A number of options exist once it has been confirmed that an unwanted infectious agent is present in a colony of experimental mice. These methods vary in their complexity and in their likelihood of success. It must be stressed that the success of some methods has appeared to decrease over time, possibly as a result of changes in the mouse populations found in contemporary colonies. For example, the breeding cessation (burnout) strategy relies on the assumption that many viral agents do not persistently infect mice but are eliminated from the host by immune clearance mechanisms before or at the time of seroconversion. This may be the case with normal, immunocompetent mice, but a large percentage of mice in modern colonies are either known to be immunodeficient (genetically or as a consequence of experimental manipulation) or are poorly characterized and unique mutant strains. Although the mice themselves may appear normal, genetic manipulations can result in subtle alterations in immune responses and abnormal host-viral interactions that can apparently reduce the ability of mice to eliminate viral infectious agents (Pullium et al. 2003; Rehg et al. 2001). Burnout will not work if mice thought to be immunocompetent are actually partially immunodeficient and do not clear the infection. Likewise, a test-and-cull program that relies on the detection and removal of exposed animals based on seroconversion will fail if immunodeficient animals become infected but do not mount a detectable immune response. Not only will such animals not be removed but they are also more likely to shed virus persistently and serve as a nidus of continued infection.

The options for rederivation of colonies of experimental animals infected with viruses, Helicobacter and Mycoplasma are listed previously. These eradication strategies attempt to break the chain of transmission between shedding and naive animals (Table 11-3).

In the case of parasites, medications can be used to directly eliminate an active infection from the animals. Colonies of mice infected with pinworms can be successfully treated using feed containing fenbendazole (Boivin et al. 1996; Coghlan et al. 1993), application of ivermectin (Baumans et al. 1988; West et al. 1992), or water containing ivermectin or ivermectin and pirimazine (Lipman et al. 1994). Colonies of mice infested with mites may be treated utilizing ivermectin (Levee 1994), permethrin (Mather and Lausen 1990), or dichlorvos (Fraser 1974). Extensive testing (even including pelt digestion) might be required to conclude that a fur mite infestation has been fully eliminated. These antiparasitic drugs are not without potential side effects, and potential toxicity should be carefully considered, especially with vulnerable or irreplaceable mice (Toth et al. 2000). For example, therapeutic doses of ivermectin may be fatal to animals lacking an effective blood-brain barrier.
| Method                          | Strengths                                      | Weaknesses                                                                                                                                                                                                 | References          |
|--------------------------------|------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Colony depopulation and        | Highly effective                               | Not an option if mice cannot readily be replaced.                                                                                                                                                         | Wallace et al., 1981|
| replacement                     |                                                | Requires specialized equipment and skills. Research colony must be restarted from small number of rederived animals. During active infection, some viruses could theoretically be transmitted vertically. Foster dams should be tested for presence of contaminating agent(s) when pups are weaned, prior to release of offspring. | Reetz et al., 1988  |
| Embryo transfer                 |                                                |                                                                                                                                                                                                           |                     |
| Cesarean section and           | Highly effective                               | Requires specialized equipment required, but some surgical skills are needed. During active infection, some viruses could theoretically be transmitted vertically. Foster dams should be tested for antibody against contaminating agent(s) when pups are weaned, prior to release of offspring. | Singletary et al., 2003|
| foster on clean dams            |                                                |                                                                                                                                                                                                           |                     |
| Birth and immediate cross-     | Reported to be effective for *Helicobacter* and | Not routinely used for viral agents or *Mycoplasma* infections. No specialized equipment or surgery needed.                                                                                              | Truett et al., 2000  |
| fostering onto clean dams      | some viruses                                   |                                                                                                                                                                                                           | Lipman et al., 1987  |
| and culling                    |                                                |                                                                                                                                                                                                           | Duysen et al., 2002  |
| Test every cage in the colony  | Useful for most viral agents when mice are     | If housing conditions and practices are not effective in retarding spread of the infecting agent, test and cull may be ineffective. Culled mice will include recovered immune mice as well as mice currently infectious. Must stop breeding temporarily. Multiple rounds of testing are required. Most often used when the prevalence of infection is relatively low and mice are valuable. |                     |
| and cull seropositive mice     | immunocompetent                                |                                                                                                                                                                                                           |                     |
| Use of seropositive mice to    | Useful for MHV when mice are immunocompetent   | Relies on development of protective immunity and complete elimination of virus, which may not be reliable in genetically engineered or deficient animals.                                                                 | Smith et al., 2002   |
| start a new breeding colony    |                                                |                                                                                                                                                                                                           |                     |
| Expose all mice, followed      | Useful for most viral agents when mice are     | Not useful for agents known to produce sporadic long-term shedding or that are not eliminated from host. Relies on development of protective immunity and complete elimination of virus, which may not be reliable in genetically engineered or immunodeficient animals. | Weir et al., 1987   |
| by temporary cessation of      | immunocompetent                                |                                                                                                                                                                                                           |                     |
| breeding and burnout           |                                                |                                                                                                                                                                                                           |                     |
| Antibiotic therapy             | Eliminates agent directly                      | Has been attempted to eliminate agents such as *Helicobacter* and *Pasteurella*, but results in field settings have been mixed.                                                                            | Foltz et al., 1996   |
|                               |                                                |                                                                                                                                                                                                           | Goelz et al., 1996   |

(Skopets et al. 1996). Because of the possibility of false-negative tests for parasites, rederivation options such as colony eradication and replacement, embryo transfer, and cesarean section with cross-fostering should be considered because these methods would be expected to be fully effective.

A final option is to choose to tolerate the infectious agent in the colony of mice. Investigators must be fully informed of the presence of an undesirable infectious agent and what is known about its potential effects on their research. Presence of the agent may also hinder their ability to collaborate with investigators in other institutions, which may reject shipments of potentially exposed animals. This option must be undertaken only with the overall risk to other mice in the institution in mind and may not always be an institutional option even if it is the preferred option for a particular research group.

V. LIMITATIONS OF OUR CURRENT KNOWLEDGE AND FUTURE DIRECTIONS

Providing mice for research that are free of infectious agents or clinical disease is a constantly changing challenge. On the one hand, more sensitive, more specific, and less expensive diagnostic methods are continually being developed. Automation of serology testing, utilization of specific recombinant antigens, qualitative and quantitative molecular techniques, and multianalyte profiling have or will have significant effects on our ability to detect infectious agents in a timely and cost-effective manner. At the same time, the presence of infectious agents is becoming less tolerable in research colonies due to effects on experimental results, the need to
share animals between institutions, and the susceptibility of valuable genetically engineered animals to traditionally non-pathogenic agents. “New” infectious agents are being uncovered, which may be truly “new” or newly mutated variants, or simply preexisting agents that we did not previously have the tools to detect. Traditional methods of eradication such as burnout and depopulation are either no longer effective or no longer tolerable with the large number of unique genetically engineered mouse lines now common in research facilities. A “herd health” approach to animal disease is being replaced by an approach more geared to salvage of as many animals as possible, and treatment of individual valuable mice is becoming more common than ever before.

As infectious agents, detection and treatment methods, and the research environment evolve, the veterinary and husbandry staff caring for research animals must strive to keep themselves educated and informed. Animal health and QA programs are not static but are always a work in progress and must also evolve in response to the challenges and needs of the research community.

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