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Introduction
Quality Assurance/Surveillance monitoring programs used in contemporary rodent colonies are vital for prevention of clinical disease and also for prevention of adventitious infections (coming from another source) that could interfere with research. Critical decision-making about animal experimentation often relies on the quality and reliability of monitoring materials and methods. This priority must also be achieved with efficiency and at an affordable cost. The purpose of rodent colony health surveillance is to detect adventitial contamination (bacteria, viruses or parasites) at an early stage. Most monitoring protocols use specific pathogen-free immunocompetent animals as sentinels. They are amenable to serological, bacteriological and parasitological testing and act as sensors to document the microbiological status of a given colony. Sampling is typically set up to detect the agent in sentinel animals that have been exposed either via direct contact or through soiled bedding.

Trained animal technicians, husbandry and research personnel are all valuable members of the animal health care team and are essential for a quality preventive medicine program. Their enthusiasm and interest should be cultivated, because they represent extensions of the veterinarian within the facility. It is paramount that they be trained in the fundamentals of observing and reporting animal health-related problems. Since husbandry supervisors and staff commonly observe the animals on a daily basis, it is critical that they are properly trained in recognizing clinical disease and maintaining the integrity of the microbial barrier.

Good preventive medicine is the product of:

1. Proper animal source information
2. Quarantine and testing
3. Clinical disease investigation
4. Periodic colony surveillance
5. Contamination containment (it will happen)
6. Microbial barrier maintenance
7. Organized record keeping
Definitions

**Axenic**: Derived by hysterectomy; reared and maintained in plastic isolators with germ free techniques; demonstrably free from all forms of associated life.

**Barrier-maintained**: A microbially-defined animal that has been removed from the isolator and placed in a barrier. These animals should be repeatedly tested to monitor for presence of the deliberately given organisms and presence of accidentally acquired organisms.

**Conventional**: An animal with unknown and relatively uncontrolled microbial burden generally reared under open cage and unrestricted animal room entry conditions.

**Defined microbially associated**: An axenic animal that has been intentionally associated with one or more microorganisms, usually to normalize the gut.

**Gnotobiotic**: As above, except that any additionally acquired forms of life are fully known. These should be in fewer number and nonpathogenic.

**Rederivation**: process used to obtain specific pathogen free mice from a strain with an infection problem or one of unknown health status. Rederivation procedures depend on the pathogen involved and may include treatment with antimicrobial agents, burning out of acute infections, cross-fostering following cesarean section, in vitro fertilization (IVF), or embryo transfer.

**Specific pathogen free**: Animals are free from a defined set of pathogenic organisms that may cause clinical or subclinical disease. The health status will depend on individual lists of exclusion organisms. It is important to realize that SPF is not a standardized definition and that mice from different facilities may have different pathogens.

**SPF versus Conventional Housing**

A well-maintained animal research facility provides an environment suitable for productive research. The physical and biologic environments should be controlled within limits necessary for the collection of valid data. It is necessary to balance the cost related to premature loss of an experiment against the cost of controlling various environmental variables [1].

Selection of facility design and operational methods should be based on the following considerations:

- Characteristics of the animal species, strain, or stock: Cost, availability, sensitivity to cold, fighting, hypersensitivities, immune status, and research uses
- Length of study - acute vs. long term
- Microbial quality of the animals

**Housing Options in Research Facilities Include:**

1. Conventional - wire lid (open air) cages and no facility/room entry procedures
2. Open air cages in some type of barrier facility where entry procedures are in effect
3. Microisolator® Cages (MI) maintain barrier at the cage level. This is the best option in research facilities where researchers need frequent access to the animals (Fig 1).
4. MI cages with autoclaved caging, equipment, and food and water - this is especially important for immunocompromised rodents and special transgenic colonies.
5. Special containment (biohazard, chemical, radioisotope, quarantine). Air pressure is negative compared to hallway to minimize environmental contamination; most products are typically autoclaved upon removal from the animal room. Ideal situations for cubicles or plastic flexible film isolators.

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**Figure 1. Examples of filter-top microisolator caging.** - To view this image in full size go to the IVIS website at www.ivis.org.

Individual cage level barrier systems (now called microisolator cages) were developed over 30 years ago and are commonly used to maintain pathogen-free rodents for biomedical research. Cage level barriers have been broadly adopted in research institutions because reliable compliance with special entry procedures has been difficult to achieve. With cage level barriers, potential breaks in the containment will be limited to specific cages and should not jeopardize the remaining animals within the room. Pathogens cannot migrate across a dry filter top cover. **Caution**: if the filter becomes wet, the integrity of the filter can break down! **IMPORTANT**: All barriers will become contaminated sooner or later! The more preparation that is made, the easier it is to manage an outbreak.
Animal Cage Changing Stations - Most animal cage changing stations have HEPA-filtered, horizontal mass air displacement flowing toward the operator. They provide product protection, but not operator protection. These hoods are not biosafety cabinets. For biocontainment, one must use a biosafety hood that provides HEPA-filtered vertical mass air displacement (Fig 2). This protects the operator as well as the product (animals). The vertical flow biosafety hoods have the additional advantage of limiting the dissemination of animal allergens throughout the room (as the horizontal hood is simply blowing them out into the room). For more information about biosafety cabinets, see: Office of Health and Safety - Biosafety

![Figure 2. Biosafety cabinet class II type A2.](www.ivis.org)

Animal Isolation Cubicles (usually designed to hold a single rack of cages per cubicle) - Animal facilities that have cubicles provide more isolation for research projects (Fig 3). These are most effective for studies that use a small number of animals. They can also be used for isolation or quarantine of animals without tying up larger spaces. The best design allows for positive or negative air pressure in each cubicle. Each cubicle then can be used for quarantine or biocontainment (negative) or protection of SPF animals (positive).

![Figure 3. Isolation cubicles, each with a separate air supply.](www.ivis.org)

Plastic Flexible Film and Rigid Isolators - These containment systems offer portability and are very effective for isolating an animal completely from potential environmental microbial contaminants. They do have some disadvantages, mainly: Requirement for trained technicians, usually have a higher daily cost to maintain, limited size that reduces animal capacity, and increased difficulty with handling/conducting experimental manipulations.

Rodent Source Monitoring
The highest risk for introducing an infection into a rodent colony is through the introduction of live animals. Every time rodents are introduced into the facility, it puts the resident rodent population at risk. These risks are cumulative. The inadvertent introduction of a singly infected animal could cause a disease outbreak that could have not only a significant financial impact, but also cost in lost time, lost research data etc. Prevention of contamination is the key, as clearing an introduced pathogen may take years. However, without the introduction of new animals most of the current research would not be possible. It is therefore necessary to minimize the risk by thorough screening the health status of all incoming animals.

Vendor Surveillance - Rodent quality assurance begins with defining the microbiological and parasite status of animals or tissues prior to their entrance into the research/breeding facility. Most rodents are purchased from commercial vendors, who have improved their barriers and health surveillance programs to the point that their colonies are free of the specific pathogens for which they are surveying. These vendors regularly provide summaries of the health status of their colonies and are not monitored independently by receiving facilities. Animals from these sources can usually be introduced directly into the animal rooms. However, vendor production rooms do become contaminated and animals are often shipped before surveillance programs detect the contamination. It is important to understand that surveillance data usually lags the present state of the colony by 4 - 8 weeks, sometimes up to 3 months. This may be because of many factors including the interval of testing and time for sample collection, processing, analysis, and reporting. Additionally, if sentinel animals are used, an antibody response to novel pathogens is not detectable for at least 2 weeks following the initial exposure, further delaying results.

Also, suppliers may have inconsistent SPF exclusion limits that may be more or less restrictive. Occasionally, a pathogen may be excluded from their health surveillance program, thus outbreaks of this pathogen are not contained or reported. Past examples of this have been Pasteurella pneumotropica, rat and mouse parvovirus, and Helicobacter sp. Major vendors
currently make their recent surveillance results available on line for convince.

Charles River Laboratories (http://www.criver.com/health/index.jsp)
Harlan (http://www.harlan.com/us/index.htm)
Taconic (http://www.taconic.com/healthr/hthrefer.htm)
Jackson Laboratory (http://jaxmice.jax.org/health/index.html)

Rodents from most well known commercial sources can be moved directly from the receiving dock to barrier housing rooms without opening the boxes or checking the animals. A few barrier facilities quarantine all rodents on arrival.

**Rodent Shipping Concerns**

**Contamination can occur during shipping.** The safest shipping method is on the producers’ dedicated trucks for shipping only SPF animals. Shipping via air constitutes a significant risk. Contamination during shipment can only be detected by quarantining the group of animals for sufficient time for antibody to develop and then looking at serology, cultures, necropsies, parasite checks etc. For long-term studies, many pharmaceutical companies bring the group into a barrier room, hold them for 3 - 5 weeks and then run a battery of tests to assure clean animals to start the project. The level of scrutiny on arrival depends on cost considerations, previous history with the vendor, current microbiologic level at the institution, and pathogen containment procedures.

The exterior surfaces of the shipping crate or rodent boxes (Fig 4) should always be considered to be contaminated. Improper disinfection of the exterior before unpacking can lead to contamination of the entire cohort!

![Figure 4. Rodent shipping box from the vendor. - To view this image in full size go to the IVIS website at www.ivis.org . -](image)

**Quarantine**

**Quarantine of Rodents from Non-Commercial Sources** - Due to the explosive increase in transgenic and knockout mouse strains over the past few years and the rise in national and international collaborations, a growing number of rodents (mostly mice) are being introduced from non-commercial sources, including other universities, research institutions or small biotechnology companies. While most of them maintain a health-monitoring program, they usually do not maintain closed colonies and they do not have a financial interest in providing only high quality animals. To assure that no unwanted organisms are introduced along with these animals, quarantine rooms are very helpful to isolate groups until the health status of every shipment is confirmed. Quarantine length and testing strategy depend on:

1. Originating colony- level of barrier security
2. Originating colony- level of surveillance testing
3. Length of stay at the new facility
4. Destination of the rodents at the new facility

The program at the originating colony should be verified by contacting the clinical veterinarian at the originating institution. A copy of the latest pathogen screening results done at the originating colony should be obtained. Some common questions to ask include:

1. What is the contamination history of the colony?
2. What is being surveyed? How often?
3. Are husbandry and access methods secure?
4. Are there contaminated colonies in the facility?
5. What pinworm detection method is being used?

**General Approach to Quarantine** - Health reports from the originating colony should be obtained to determine which adventitious agents are present. Most facilities use serology to test for all viral agents and some bacterial pathogens. See
Biology and Medicine of Mice and Biology and Medicine of Rats chapters in Laboratory Animal Medicine and Management for a summary of major pathogens in rodents. Pinworm detection presents more of a problem since some use a tape test to detect *Syphacia*, but fail to perform postmortem cecal exams to detect *Aspicularis*.

The quickest approach to screening new imports is to request several extra cohorts from the exporting facility for sacrifice. The imported group can be surveyed by screening representative animals from the group. If none are available, animals are typically held in quarantine and sentinels are placed. Sentinels are screened after a minimum of 3 - 5 weeks exposure to potential pathogens through dirty bedding transfer or direct contact.

**Note:** for immunodeficient rodents or novel transgenic lines with unknown immunologic changes, relying on serology alone for pathogen detection is STRONGLY discouraged. False negative may be quite common.

Another method that significantly expedites release of new animals is to automatically treat incoming animals for pinworms through feeding commercially available fenbendazole-medicated feed. Ivermectin can be used to treat both ectoparasites and pinworms. Once treatment is completed, serology can be performed on a cohort of mice to detect viral pathogens. Most bacterial pathogens can only be detected by culturing at necropsy.

**Note:** several transgenic strains show increased hypersensitivity to Ivermectin [2,3]. If unsure as to safety, it is strongly recommended to dose a few trial mice before treating the entire line.

Decisions about which agents to screen for are unique to each institution. Factors to consider include risk tolerance, research use of the imported mice, length of time to be housed at your facility, and financial considerations. Ultimately a balance must be reached between these factors. Regardless of the final choice, it is wise to consider a plan of action in advance of unexpected positive results for each agent (i.e., Are you screening mice to obtain prevalence data? Will you treat, isolate, rederive, or euthanize affected mice?) For more options, see the Containment and Elimination of Pathogens section below.

**Rodents destined to be euthanized within a few days or weeks** - These rodents are usually moved directly to a quarantine cubicle or conventional room and used for research. Additionally, access to quarantine should be limited and special arrangements made between the investigators and the staff. It is all too common for investigators to serve as inadvertent fomites, transferring pathogens between the laboratory and the animal facility. Generally, a startup meeting between the veterinary staff and the investigator is a good idea for discussion of the intended procedure in the context of protecting the facility colonies from pathogens. If the investigator decides later to keep these animals, the quarantine process should start at that time.

**Rodents destined for conventional rooms** - These rodents can be moved directly to a conventional room for housing if there are no concerns of contaminating the existing colonies. However, it is prudent to perform a simplified examination to minimize the risk associated with importing novel pathogens.

**Typical screening protocol for conventional rodents**

- Gross examination of tissue
  (if suspicious lesions are detected, tissues are processed for histology)
- Examination of pelage for ectoparasites:
  - *Myobia* sp.
  - *Myocoptes* sp.
  - *Radfordia* sp.
- Examination of cecal contents for internal parasites:
  - *Giardia muris*
  - *Spironucleus muris*
  - *Aspicularis tetraptera*
  - *Syphacia obvelata*
- Serology for common pathogens:
Rodents destined for SPF/barrier housing - "Clean" animals with a history of extensive health testing, negative results, and housed in a tight barrier at the originating colony:

Generally, these animals are assumed to be free from major pathogens and are generally screened using two methods:

1. Examination of group cohorts
2. Placement of sentinel mice for 3-5 weeks.

Typical screening protocol for SPF rodents

- Gross examination of tissue
  (if suspicious lesions detected, tissues are processed for histology)
- Examination of pelage for ectoparasites (see above)
- Examination of cecal contents for internal parasites (see above)
- Serology for common and uncommon pathogens:

```markdown
**Mouse**
- Mouse hepatitis virus
- Mouse parvovirus
- Mycoplasma pulmonis
- Sendai virus

**Rat**
- Rat coronavirus/Sialodacryoadenitis
- Mycoplasma pulmonis
- Sendai virus
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**Mouse**
- Cilia-Associated Respiratory Bacillus (CAR Bacillus)
- Ectromelia virus
- Encephalitozoon cuniculi
- Epizootic Diarrhea of Infant Mice Virus
- Hantaan virus
- Lactate dehydrogenase-elevating virus
- Lymphocytic choriomeningitis virus
- Minute virus of mice
- Mouse adenovirus FL/K87
- Mouse cytomegalovirus
- Mouse hepatitis virus
- Mouse Parvovirus
- Mouse pneumonitis virus
- Mouse Thymic Virus
- Mycoplasma pulmonis
- Pneumonia virus of mice
- Polyoma virus
- Reovirus type 3
- Sendai virus
- Theiler's mouse encephalomyelitis virus (GDVII)

**Rat**
- CAR bacillus
- Encephalitozoon cuniculi
- Hantaan virus
- Kilham rat virus
- Lymphocytic choriomeningitis virus
- Mouse adenovirus FL/K87
- Mycoplasma pulmonis
- Pneumonia virus of mice
- Rat coronavirus / Sialodacryoadenitis
- Rat cytomegalovirus
- Rat Parvovirus
- Reovirus type 3
- Sendai virus
- Theiler's Murine Encephalomyelitis Virus
- Toolan's H-1 virus
- Bacterial culture of nasopharynx and cecum
  - Nasopharangeal wash
    - *Bordetella bronchiseptica*
    - *Corynebacterium kutscheri*
    - *Klebsiella pneumoniae/oxytoca*
    - *Pasteurella pneumotropica*
    - *Pseudomonas aeruginosa*
    - *Streptococcus (beta hemolytic)*
    - *Streptococcus pneumoniae*
  - Cecum culture
    - *Klebsiella pneumoniae/oxytoca*
    - *Pasteurella pneumotropica*
    - *Pseudomonas aeruginosa*
    - *Salmonella* sp.
- Fecal PCR
  - *Helicobacter hepaticus / bilis / sp.*

The above comprehensive lists are often modified as needed dependent on institutional goals. For more information about these pathogens, the reader is directed to Biology and Medicine of Mice and Biology and Medicine of Rats chapters in Laboratory Animal Medicine and Management.

- Rodents with marginal (not equivalent to Charles River Assessment Panel) health reports, missing or incomplete serology, known infection with adventitious agents, or a weak barrier at the originating colony:

  Generally, these animals are assumed to be contaminated until proven otherwise and can be processed through several methods:
  1. Examination of group cohorts
  2. Placement of sentinel mice for 3-5 weeks
  3. Automatic rederivation

  If no extra animals are available, sentinel animals can be placed in the same cage to maximize exposure on arrival. The animals should be housed in individually ventilated cages under BSL 2 conditions to exclude transmission of infectious agents between the different quarantine groups. After 3-5 weeks the sentinels are removed, necropsied and samples are submitted for pathology, microbiology/parasitology and serology for all relevant rodent pathogens. Total duration of quarantine for a clean group is approximately 6-8 weeks.

  All animals known to be contaminated should be rederived before being released into a barrier animal facility. Rederivation procedures depend on the pathogen involved and may include treatment with antimicrobial agents, burning out of acute infections, cross-fostering following cesarean section, *in vitro* fertilization (IVF), or embryo transfer. Rederivation of mice infected with viral pathogens is typically done by using either embryo transfer or cesarian delivery and fostering to SPF females (see chapter on Common Surgical Procedures in Rodents in Laboratory Animal Medicine and Management). The contaminated group should be held in quarantine or a cubicle during this process. It is important to ensure that clean breeders are successfully produced before the remaining contaminated animals are euthanized. A step-by-step description of cesarean rederivation in the mouse can be viewed at: Caesarian Rederivation in the Mouse.

**Sentinel programs**

One of the most common ways to assess the health status of laboratory rodent colonies is to use sentinel animals. These animals are independent of the research colonies and are maintained for the expressed purpose of testing for the presence of pathogens (either directly or via antibodies to those pathogens). At most institutions, sentinel animals are euthanized to facilitate sample collection, to enable collection of sufficient quantities of serum, and to perform direct cecal examinations (for *Aspiculuris* pinworms); however, sentinel animals can be maintained for multiple sampling periods to provide additional historical evidence for circumstances associated with seroconversion.

Sentinels are typically housed in the same fashion as the colony animals in the room. For rodents in cages with open tops (no microisolation filters) this housing method for sentinels works well, and the sentinels are usually exposed to pathogens.
that are spread by aerosol as well as those spread by fomites. For rooms featuring microisolation cages, housing the sentinels in these cages creates an obstacle to their exposure to any pathogens present in the room. For this reason, many facilities that house rodents in microisolation cages transfer soiled bedding from the colony animals into the sentinels’ cage. This system works reasonably well for pathogens spread by fecal-oral contamination [4,5] but less so for pathogens typically transmitted by aerosol, such as Sendai virus [5,6] and CAR bacillus [7]. Another option used is to house sentinel animals in cages with open tops, even though the colony animals are in microisolation cages. In this case, the sentinels are essentially surveying the room environment, but not necessarily the status of the colony (which could be effectively protected from environmental pathogens by the filtered cage). A third option for animals in microisolation cages is to house sentinels with colony animals ("nose-to-nose" sentinels). This method ensures that the sentinel will experience the same pathogen exposure as the colony member, regardless of whether the pathogen is spread by aerosol or direct contact. However, it introduces the risk that the sentinel rodent could contaminate the colony animal(s), and thus is seen as a less desirable approach. It also requires that the sentinels have a different coat color from the colony animals for easy identification.

The source of sentinel animals can be either unused colony members, or rodents purchased (or bred in-house) with the expressed purpose of serving as sentinel animals. The advantage of using colony members is that it eliminates the risk of introducing a pathogen via the housing of extramural sentinels. The disadvantage of their use is that the strain chosen may respond differently to a pathogen than other colony members, especially in a colony of genetically modified animals. Purchase or breeding of sentinel animals can assure that an appropriately immunoresponsive strain is chosen, but adds the risk of possibly causing a contamination, in addition to detecting it. In either case, animals used for sentinels must be old enough to have functional immune systems without interference from passively transferred maternally derived antibody. In general, mice 8 - 12 weeks are used. As younger animals are less expensive, it may be cost-efficient to purchase slightly younger animals (e.g., 5 weeks) as sentinels, knowing that they will be 11 - 13 weeks old at the time of sample collection.

The number of sentinels per cage, and the number of colony cages per sentinel cage, are commonly debated parameters of a rodent health surveillance system. A mathematical formula \[ N = \log \left( \frac{1 - \text{probability of detecting infection}}{1 - \text{assumed infection rate}} \right) \] was historically used to calculate the number of sentinels that would be needed to detect an infection at a certain confidence limit, given an assumed infection rate [8]. This formula, however, assumes random spread of infection, which is no longer a valid assumption for animals housed in microisolation cages. Ultimately, the number of sentinels per cage and the number of colony cages per sentinel cage are dictated by cost efficiency and risk tolerance. Each animal, and each cage, bears a cost to the institution in purchase costs, per diem rates, charges for sample testing, and additional technical labor. This cost must then be balanced against the need for sufficient samples to generate meaningful information that can be used to appropriately guide colony management.

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<th>Rodent Colony Monitoring - Key Points</th>
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<td>Sentinel animals are valuable tools for colony screening</td>
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<td>New arrivals should be separated from the existing colony</td>
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<td>Balance risk/cost</td>
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<td>Pathogen detection typically lags original incident</td>
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<td>DO NOT PANIC</td>
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**Testing Methodologies**

The most common means by which to assess the presence of viral pathogens is to examine serum for the presence of specific antibodies. At the present time, most viral antibodies are detected using an enzyme-linked immunosorbent assay (ELISA) system. At present, the vast majority of mouse viruses surveyed by major commercial laboratories are assayed primarily using ELISA (15/17 for one site). Exceptions would include mouse thymic virus, for which the test is the indirect immunofluorescence assay (IFA), and lactate dehydrogenase-elevating virus, whose presence is detected by elevation of LDH in the serum and not by an anti-viral immune response. For those viruses detected by antibody, most have confirmatory tests done by either IFA or hemagglutination inhibition (HAI) methodology.

Gradually replacing antibody assays for pathogen detection is the use of polymerase chain reaction (PCR) to detect DNA sequences characteristic for the pathogen. An advantage of PCR is that it does not require the host to have a completely functional immune system to enable detection. The disadvantage is that it is still in development for many pathogens, it is extremely sensitive (such that false-positive results can occur from nucleotide sequences remaining from previous assays),
and it only detects active infections, whereas serologic analyses also identify convalescent infections [9]. Pathogens for which PCR is the current test of choice include the Helicobacter spp. Fecal samples are used as the medium for detection of Helicobacter DNA sequences. PCR analysis is also available for many of the viruses assessed by serological means; tissues required for submission vary with regard to the tissue tropism of the virus (e.g., mesenteric lymph nodes for mouse parvoviruses, kidney for LCMV and K virus).

For most bacterial species other than Helicobacter, culture on artificial media is still the basic means by which to determine the presence or absence of the pathogen. Nasopharyngeal swabs are typically taken from sentinels to look for Pasteurella pneumotropica and intestinal samples are taken to survey for Citrobacter rodentium and Pseudomonas aeruginosa, among others.

For the presence of pinworms, the anal tape test or direct cecal examination is most commonly used. Anal tape tests do not require euthanasia of the host mouse, but it is only possible to detect Syphacia sp. pinworms by this method. This is because Syphacia sp. pinworms deposit their ova on the perineum of the host mouse or rat, thus being readily available to adhere to the placement of the cellophane tape. On the other hand, Aspiculuris tetraptera pinworms deposit ova within the large intestine of the host, and so rodents infected by this species would yield false-negative results by the anal tape method [10]. Many institutions now euthanize the sentinels and harvest ceca for direct examination for adult pinworms. This has the disadvantage of requiring the death of the host rodent, but it detects both species of pinworm as well as any infections that might be "sterile" through immaturity of the worms or single-sex infestations.

Some institutions prefer to run in-house diagnostics, while others outsource to various vendors. Advantages of in-house testing (flexibility, speed with which results are known) should be balanced with economies of scale. In general, the more samples to run the cheaper it is to run them. Many institutions opt for a hybrid approach by running commercially available ELISA tests in-house and sending out sera for more comprehensive analysis at predetermined intervals. Two major commercial laboratories specializing in rodent pathogen screening are:

- Charles River Laboratories (http://www.criver.com/products/diagnostics/)
- University of Missouri Research Animal Diagnostic Laboratory (http://www.radil.missouri.edu/)

Other academic and commercial veterinary laboratories are listed at: http://netvet.wustl.edu/vspecial.htm#clinpath

**Containment and Elimination of Pathogens**

Once pathogens are detected in an animal research facility there are multiple options for containing the "outbreak" and for restoring the colony to its original pathogen-free status. It is most important to realize that results obtained in tests of sentinels (and to a lesser degree, colony members) are historical determinations. That is, the infection has been already present for a while in the colony, possibly even for several months. As such, decisions should be made calmly and judiciously. Rapid, knee-jerk responses to the discovery of positive results often accomplish little, except to generate panic (authors’ personal observation). In some situations, the presence of the pathogen may be inconsequential to the ongoing research, and simply "quarantining" the housing room and waiting for the end of the natural course of the experiments may be the most prudent option. Thoughtful, rational evaluation of the circumstances associated with the contamination (often in consultation with the affected investigators) is the best way to start the process of pathogen containment and elimination.

Containment practices should be based upon the extent of the contamination, the biology of the offending pathogen, the nature of the facility barrier, and the housing options at the institution. With regard to the extent of contamination, widespread contaminations need to be handled differently than those that are isolated to one room, or to one rack within a room, or to one investigator within a room. Contaminations that affect multiple rooms may require facility-wide procedural changes to achieve containment, and may leave scientists and veterinarians with fewer options than infections with narrower distribution. The "strength" of the results from the sentinels can also be a determining factor. If all of the sentinels within a cage fail to yield the same result, a decision must be made as to whether to treat the colony as contaminated (even though not all sentinels seroconverted), initiate immediate repeat testing, or consider the result as a false positive and reassess with the next round of sentinels.

In addition, containment decisions will be affected by the pathogen itself. Is it a pathogen for which you can readily treat (e.g., pinworms)? Is it readily contained by standard disinfection and control methods? What are the consequences of its persistence to the research project and the animals’ health? Is it a pathogen from which most vendors assure their animals
will be free (i.e., how likely is it you could reintroduce it with the next shipment)? Is the pathogen zoonotic and thus a risk to employees?

The characteristics of the barrier also affect containment practices. A pathogen barrier can be at the level of the cage (microisolation), room, and/or building, and it can be structural, procedural, or (most likely) a combination of both. Barrier practices that need to be considered in containment decisions include whether supplies are autoclaved into the barrier, whether room ventilation systems can be changed from positive to negative pressure, whether animals leave the barrier for experiments or stay in internal procedure rooms, whether there is an established clean/dirty traffic pattern, and whether the cage wash facility is pass-thru or one-sided.

The housing possibilities available in a contamination also influence the approach to containment. The issue of housing includes primary enclosures (microisolation vs. open-top cages) as well as room capabilities. The ability to isolate contaminated populations is always helpful, whether it be in the initially identified room (preferred), or in an alternate location. Circumstances that are conducive to relocating contaminated populations include the availability of remote sites that effectively sequester the infected rodents, and/or the availability of space in another contaminated room that enables consolidation of infected populations and thus the disinfection and "re-opening" of a clean room.

The following are some basic guidelines for the containment of pathogens that the authors have followed and endorse:

1. Microisolation caging is an effective means to limit the spread of infections. It has been demonstrated to be effective in published reports [5,11] and also from the authors' personal experience. If a contamination occurs within the microisolation caging system, the system should be maintained. If a contamination occurs in open-air, unfiltered caging, conversion to microisolation caging would be a significant step in containing the pathogens. Conversion of MHV-contaminated mouse colonies from open-air cages to microisolation systems has eliminated the virus, even without using disinfection practices (e.g., opening cages in HEPA-filtered laminar flow hoods only, use of chlorine dioxide disinfectant) commonly associated with microisolation caging (authors’ personal experience).
2. Moving contaminated animals is not always the best idea. By moving contaminated animals, the infection could be spread rather than contained. Corridors could be contaminated during the transport, and pathogen-naïve roommates or neighbors could become contaminated accidentally. Relocation of contaminated animals should always be done judiciously; the advantage of moving them should clearly outweigh the disadvantage of possibly widening the zone of infection [12]. The ability to truly isolate a population and to consolidate several contaminated populations are two justifications that the authors have used for relocating infected rodents.
3. One should consider increasing pre-entry precautions and the level (amount) of protective clothing worn. Protective clothing would include disposable gowns and double shoe-covers (assuming that a pair is already worn in the barrier facility). While there are no definitive studies indicating that protective clothing assists in pathogen containment, it may cause the occupant to ponder the rationale behind the protective clothing and prevent he or she from engaging in a more harmful practice.
4. Controlling and limiting access to contaminated housing areas is another practice that merits consideration when a pathogen barrier has been broken. Limiting traffic will reduce the possibility of contaminants being spread to areas outside of the room or facility, and will also decrease the opportunity for the introduction of additional pathogens. If animals are permitted to leave and re-enter the facility for experimentation, consideration should be given to curtailing and/or regulating this practice as much as possible. Development of procedure rooms within animal housing space could serve to diminish contamination spread.
5. If possible, contaminated animal housing rooms should be made negative to the external corridor. At many institutions, this can be accomplished by increasing the flow of exhaust from the animal housing room. If the only option to change air pressure relationships is to decrease supply airflow, care must be taken that the total air supply does not fall below the accepted standard of 10 room air changes per hour.
6. Once an animal housing room (or rack within a room) becomes contaminated, the order in which the care staff changes the cages within their daily routine needs to be modified. Contaminated racks and rooms should always be entered and handled last in the course of a day’s work, at least as pertains to working with species that could be infected by the offending pathogen.
7. The circumstance of a contamination is an opportune time to revisit with husbandry and research laboratory staff members to review proper facility practices, especially if practices are modified as a result of the contamination. Continuing education and training in proper technique will help to contain the current contamination, and hopefully prevent (delay) future occurrences.
One should base pathogen containment decisions on both published information and institutional experience. No two facilities are alike in either design or operation. Because a containment strategy worked (or did not work) at one location does not guarantee that the results will be the same at another institution. Husbandry practices, reasonable containment options, and the needs and preferences of the scientific investigators all vary between institutions. Practices listed above should be used for guidance and not as mandatory control policies.

Once the offending pathogen is contained, a plan for its elimination from both the colony and the facility must be developed. In the authors’ experience, the first consideration is to ensure that the status assumed from the sentinels represents the condition of the actual experimental colony. Because the results from sentinels reflect historical pathogen presence, one can have situations in which a serologically negative colony is represented by seropositive sentinels. The most common reason for this “false-positive” situation is that the contaminated colony animals that shed pathogens to the sentinel animals were euthanized for experimental purposes, and since replaced with new, uninfected colony members. This is especially true when microisolation caging is used, as pathogen spread from cage-to-cage is significantly diminished, and initially infected mice can essentially be an "island" of contamination within the colony until that cage is ultimately removed.

Probably the most expedient and cost-effective means by which to accomplish colony re-evaluation is to test the serum of individual colony animals (one per cage) for the offending (viral) pathogen. While this has some inherent risks (e.g., possible loss of colony members to procedural injury or anesthetic death, cage representatives not reflective of the status of cage mates), it can yield results in less than two weeks time (often in days with in-house ELISA testing) and is typically an accurate representation of the health status of all cage residents. If none of these colony animals are positive for the offending pathogen, then elimination has already been accomplished. In the authors’ collective experiences, this has been the most common result when colonies housed in microisolation cages are surveyed after detection of a viral contamination by sentinels.

In those cases when survey of colony members yields seropositive animals (or when eradication decisions need to be based on sentinel results without colony surveillance) the method of elimination depends upon the specific pathogen identified, the nature of the research affected by the contamination, and the financial implications of the eradication program. With regard to the pathogen, important characteristics that impact eradication include whether the pathogen is pharmacologically treatable (such as bacteria and pinworms), whether it is easily removed from the environment (enveloped vs. non-enveloped virus), whether it is eliminated by the host or can remain subclinical or latent, and whether or not it is zoonotic.

The nature of the affected research impacts eradication in that short-term studies can often be allowed to complete their course without additional eradication measures, and readily available strains can be euthanized and quickly replaced. However, eradication becomes more challenging for breeding colonies and studies that utilize rare genetically manipulated lines. If such colonies have frozen embryos or isolated high-barrier nucleus colonies, then options exist for euthanasia of the contaminated colony and relatively rapid replacement. If not, then suspension of breeding for a proscribed period may be possible (again, depending on the offending pathogen), or rederivation by cesarean section or embryo transfer may be required.

The third factor in selection of elimination practices is the financial impact of the various alternatives. Are eradication and replacement costs borne by the principal investigator, or are they built into the per diem system as an expected cost of operation? Does treatment of the infection cost more than replacement animals? Financial arrangements and expected costs need to be evaluated as part of the rationale for pursuit of an eradication plan.

For all contaminations, regardless of the pathogen, the authors recommend that members of the veterinary staff contact investigators with information about the pathogen and possible action plans. This information includes the type and characteristics of pathogen, known consequences of infection to animal health and research parameters, options for further evaluation of the colony, and options for elimination of the pathogen. This information exchange should be completed within a few days, or with a modest delay to complete information gathering and to enable assumption of operational changes (e.g., change direction of airflow, change order of room entry). Once investigators have been informed, a notice can be posted on the room door regarding the contamination and any changes in entry procedures.

The action plan selected would be a joint decision between the veterinary staff and affected investigator(s). Upon agreement of the course of action, the plan is enacted for containment and eradication. The final step of the plan would include the provisions for follow-up testing and eventual restoration of specific pathogen-free status. In most cases this final stage
would be the outcome of the next round of surveillance testing; however, in some cases specific testing prior to follow-up
surveillance may be appropriate (e.g., special colonies from which external shipment is commonplace, colonies surveyed
less frequently than every three months).

For contaminations caused by pinworms, the most significant decision to be made by the veterinary and scientific staffs is
the method of treatment. Common options include feeding rodent chow impregnated with fenbendazole [13,14] or
administration of Ivermectin, either by injection, topically [15] or in the drinking water [16]. Consideration should be given
to treating all boxes within a room, even those for which sentinels were negative (to prevent cyclical infections between
different colonies within a room). At one author’s institution, all caging is replaced and the room (ceilings, walls, floors) is
washed down twice during the treatment period. However, there have been published reports that room treatment may not
be necessary to ensure complete eradication of pinworm infestation [14]. Replacement of sentinels should be based on the
life cycle of the offending pinworm species; it may be necessary to delay placement of new sentinels to enable any surviving
pinworm ova to complete a life cycle before sentinels are placed (since sentinel detection of pinworms requires two
generations: the development of adult worms in the intestinal tracts of colony animals, and the development of their eggs
into the next generation of adults in the sentinels).

For viral contaminations the most dramatic approach is to euthanize all colony animals represented by the positive sentinels.
For colonies that are easily replaceable, either through purchase or rederivation via frozen embryos, this may be the most
cost effective approach to eradicate the contamination. More commonly, the approach to viral outbreaks is to test individual
colony animals for antibodies to the detected pathogen and euthanize cages that are positive (if microisolation caging is
used). For some viruses (e.g., Sendai, SDAV) the practice of ceasing all new entries (via both purchase and breeding) for a 6
- 8 week period has been demonstrated to be effective [17]. In the authors’ experience, this method has worked, but
inconsistently. A variant of this eradication plan for self-limiting viral infections is to re-establish a breeding colony with
seropositive animals only [18] thus yielding mouse pups that are initially protected by passive maternal antibody, but
eventually are seronegative because the infection has “burned out” during the weeks of gestation and nursing. For both the
breeding cessation and seropositive breeder plans, it is important to realize that these eradication methods require
immunocompetent mouse strains in which behavior of the infecting virus is consistent. Genetically manipulated mice with
slightly altered immune function may not be effectively cleaned by these methods because the host animals can harbor
and/or shed viruses longer than the immunocompetent strains and stocks on which these methods were first proven.

For many valuable genetically-altered strains, the most practical approach to eradicate viral contamination is rederivation.
The classic method of rederivation is by cesarean section of the dam, with passage of the uterus through a disinfectant
solution, and ultimate transfer of the pups to uncontaminated, pseudo-pregnant foster mothers. This method works
effectively for just about all viruses except those passed vertically (e.g., LCMV) or in the genome (e.g., some strains of
mammary tumor virus). Alternatively, embryo transfer technology has been used to impregnate the foster mothers with
fertilized embryos from the contaminated strain (usually taken at the 1 - 2 cell stage), thus also eliminating any vertically
transmitted viruses. Low-technology rederivation (i.e., transfer of pups on the day of birth to waiting, lactating foster
mothers) is possible and can be effective [19], but frequent testing of the offspring is essential to cull any mice in which
horizontal viral transmission from dam to pup occurred during the first few hours after birth.

An important consideration for virus eradication plans is to what extent environmental decontamination is necessary. The
use of microisolation caging has tempered the need to thoroughly decontaminate animal housing rooms, since the housing
technology operates under the principle that anything outside of the animal’s cage should be considered as contaminated.
However, many institutions still practice routine room disinfection after viral and helminth outbreaks. At one author’s home
institution, room disinfection is now a standard practice in association with decontamination from parvovirus outbreaks, but
not necessarily those of other viruses. This is primarily because of the hardy nature of parvoviruses and the concern that
they could survive on room surfaces for an extended period, even after routine surface disinfection methods.

In conclusion, several references are available that provide excellent guidelines for establishing and maintaining effective
"Questions and Answers" programs [9,20,21]. A cooperative effort from both animal care staff and investigators is needed
to protect research animals for adventitious infections and to control/eliminate pathogen outbreaks.
References


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