

Microbiological Monitoring in Individually Ventilated Cage Systems

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Housing rodents in IVC racks has many advantages over conventional cages but also presents unique challenges related to health monitoring. The authors review the issues to consider in design of a sentinel program using IVC systems.

There exist many methods and strategies for monitoring rodent colonies for microbial agents. Several general principles are applicable when developing and assessing the efficacy of a microbiological monitoring program, irrespective of the type of caging used for rodent housing. First and foremost, the monitoring program must specifically target the infectious agents of concern. In particular, there should be consideration of the route and duration of transmission of the infectious agents, the probability that the agents will be found, and the likelihood that the agents will cause disease or affect research results. Second, the monitoring program should make the most efficient use of personnel and resources. Finally, the outcome of a successful monitoring program should aid research, not hinder it, and should result in animals that are sufficiently pathogen free for research and should facilitate exchange of animals between institutions.

Rodents in contemporary animal colonies live in several types of caging. The three types of caging systems commonly used are open-top cages, static isolator cages, and individually ventilated cages (IVCs). The type of housing used affects the ease with which infectious agents can be spread between rodents housed in separate cages. Rodents housed in open cages and serviced in the open have the highest risk of transmitting infectious agents from cage to cage via aerosols and fomites. This is one reason why this type of housing is being eliminated in many facilities.

Rodents housed in static isolator cages have a lower risk of infectious-agent transmission than do rodents housed in open-top caging, because the filter lid serves as a physical barrier to infectious-agent transmission, although transmission can occur if filter lids are not properly installed¹ or during cage changes. It has also been shown that the exhaust gases do not actu-

ally pass through the filter material in the lid but escape through the gap between the cage and the cage lid². This is the reason that many facilities have instituted the use of ventilated cage change stations for husbandry and experimental manipulations of rodents housed in static isolator cages.

IVCs differ from static isolator cages in several ways. Each cage receives a supply of HEPA filtered air at a rate of between 30 and 120 air changes per hour (ACH). This results in lower temperature and humidity as well as lower concentrations of ammonia and carbon dioxide than in static isolator cages³. Some IVC systems balance the air supply with the air exhaust system, thus permitting control of differential pressure at the cage level. Thus each cage, theoretically at least, is its own biocontainment zone. An alternative approach in some IVC systems is to seal the cages to achieve biocontainment (instead of balancing differential pressure). This approach has, however, the obvious disadvantage of being potentially lethal in the event of cage ventilation failure^{4,5}. Rodents housed in IVCs have the lowest risk of cage-to-cage spread of infection⁶⁻¹¹ (J. Schmidt, personal communication). Transmission between animals housed in IVC systems generally occurs as a result of dissemination during husbandry procedures or experimental manipulations by investigators, thus justifying the use of ventilated cage change stations for such activities. Nevertheless, it is essential to exercise great care to ensure against transmission by fomites on hands or equipment, such as watering valves, particularly when active infections are present^{11,12}.

Monitoring methods, such as placement of room sentinels adjacent to the HVAC exhaust duct, as used traditionally with mice housed in open-top caging, require reassessment with regard to their efficacy in static isolator cages and in IVC systems.

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Methods such as exposing sentinels to soiled bedding, as used traditionally with mice housed in static isolator cages, also require reassessment for their applicability in IVC systems. Moreover, we should add to our monitoring approach new methods specifically designed to exploit IVC system characteristics by the sampling of air exhausted from the IVC system. There are two ways of monitoring the exhausted air, either from individual cages or from the whole IVC rack. Small gauze filters placed on the cage exhaust opening, on the exhaust opening from a zone of the rack (e.g., a row or column of cages), or on the exhaust manifold of the entire rack can permit periodic monitoring of the effluent air. After removal from the rack, molecular PCR-based methods can detect infectious agents on the filters. Additionally, there can be continuous monitoring of exhaust air from the IVC rack (or a selected zone of the rack) that is achieved by testing of sentinels housed in a specially designed cage (BioScreen system; BioZone Inc., Fort Mill, SC, and BioZone Ltd., Margate, UK) that receives as its air supply a portion of the mixed exhaust air from several cages on the rack, before HEPA filtration (Fig. 1). The effectiveness of such a system is clearly dependent on the uniformity of ventilation of all cages on the rack so as to be confident that a low incidence of infection will be represented in the exhaust air without regard to the location on the rack of the infected animals¹².

Sampling Methods

There are many ways of detecting an infectious agent that has been transmitted to one or more rodents housed in IVCs. The most direct method of surveillance is to monitor the colony mice themselves for evidence of an infection. This method seems highly reliable on first consideration, but it presents significant drawbacks. If the animals are properly screened, considering that each cage is essentially a self-contained unit, then it is by far the least cost-effective method, because it requires the largest investment of mice, staff, and testing reagents. Moreover, the age, genotype, and experimental use of the animals may interfere with the applica-

tion of the testing methods (e.g., serology on immunodeficient animals).

The use of sentinel mice to monitor a colony decreases the number of mice needed to detect an infection within a colony, because each sentinel can serve in monitoring of many colony mice. There are three types of sentinel animals. First, contact sentinels (i.e., mice housed in the same cage as the colony mice to be monitored) are highly reliable at detecting infectious agents transmitted by all routes (air, feces, urine, wounds, contact, etc.). Second, soiled-bedding sentinels (i.e., mice housed on soiled bedding that has been removed from several cages of colony mice) are most effective at detecting agents that are transmitted in feces or urine. Third, exhaust air sentinels (i.e., mice that are exposed to rack exhaust air) are effective at detecting agents that are transmitted in respiratory excretions. Lipman and Homberger¹³ provide an overview of the advantages and disadvantages of the three types of sentinels. Finally, direct detection of infectious agents present within the environment of a colony is feasible by swabbing such surfaces as cage and cage racks, by taking appropriate samples from rack exhaust air, and so on.

Methods Used to Detect Infectious Agents

Several methods exist for testing samples collected from mice or from their environment. Traditionally, most viral infections are detected using serological assays. The advantages of serological assays are that they are inexpensive and can provide a historical picture of which agents are present in the colony (i.e., at any time after a mouse becomes seropositive, antibodies to the rel-

BioScreen™

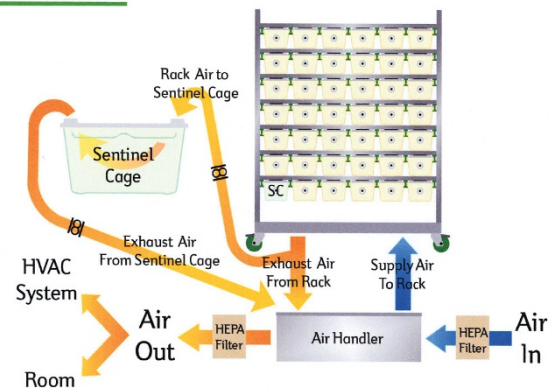


FIGURE 1. The principles of exhaust air sentinel monitoring shown in a schematic of airflow through an IVC system, with exhaust air being directed through a sentinel monitoring cage before HEPA filtration and final release.

evant agent can be detected). The primary disadvantage of serological assays is that mice infected with an agent may not become seropositive until 2 or more weeks after the infection's initiation. Therefore, in the midst of an outbreak, many mice may become infected and may potentially be transmitting virus to other mice, yet may still be seronegative. Additionally, immunocompromised mice, especially those with B-cell deficiencies, may produce few or no antibodies in response to an infection.

Recently, molecular methods have augmented our arsenal of testing methods. Molecular methods, in particular PCR and RT-PCR, detect the nucleic acids present within a bacteria or virus. In general, these methods are highly sensitive and rapid, and can be very effective means to determine which animals are infected with a particular agent. Molecular methods are particularly useful during outbreaks, when it is essential to determine rapidly (within a few hours) the location of infected mice within a rack, so as to develop a strategy for containing the spread of the agent. The limitations of molecular methods are that they are relatively expensive, they can yield both false negative and false positive results, their high sensitivity makes them prone to cross-contamination, and many substances found in blood, feces, and other animal tissues can function

as inhibitors of the enzymes used in these assays, causing false negative results. Finally, the nucleic acids detected may be present in a noninfectious form of the agent, such as in inactivated material present on the surface of cages or change stations.

Detection of bacteria usually follows culture of fecal, cecal, nasopharyngeal, or other tissue samples on specialized agar plates. Specific identification of pathogenic bacteria after culture usually involves a combination of enzymatic and fermentation tests. Microscopy is the usual method for visual detection in diagnostic specimens of endoparasites and ectoparasites.

Variables That Can Affect the Efficacy of Monitoring in IVC Systems

Infectious Agent-Specific Variables

There are several infectious agent-specific variables that affect the ease with which an

agent can be detected in mice housed in an IVC system. The first influence is the route(s) by which the agent is shed from the animal (Table 1). Agents that are transmitted only through animal-animal contact, such as mites, are very difficult to detect in mice housed in an IVC system. It is possible to detect these agents directly in colony animals or using contact sentinels. One can effectively detect agents that are transmitted via the fecal-oral route, such as mouse parvovirus (MPV), using contact sentinels, sentinels exposed to soiled bedding, or molecular testing of feces from colony animals, but exhaust air sentinels are not effective at detecting these¹². In contrast, the use of contact sentinels, exhaust air sentinels, or molecular testing of cage surfaces is effective in detecting agents that are shed in respiratory secretions, such as Sendai virus, but sentinels exposed to soiled bedding are ineffective at detecting these¹². The methods used to sample the cage or rack surfaces for air-

borne infectious agents (cage wipe material or protocols, filter material, or placement location) may influence the effectiveness of detecting the agent of interest. For example, our lab detected Sendai virus RNA on the cages of experimentally infected mice for 2 weeks using calcium alginate swabs wet with saline, whereas another group was able to detect Sendai virus RNA on the cages of experimentally infected mice for only 3 days using alcohol wipes^{12,14}.

A second major influence that affects the ease with which an infectious agent can be detected in mice housed in an IVC system is the infectious-agent load to which the sentinel mice are exposed. Infectious-agent load depends on the duration of shedding, on the concentration of agent shed, and on the stability of the agent after it is shed (Table 1). Detection of agents such as murine rotavirus (epizootic diarrhea of infant mice, or EDIM), which causes an acute infection in adult immuno-

TABLE 1. Infectious agent-related factors

Infectious agent	Agent size	Agent stability	Persistent shedding	Agent incidence	Mode(s) of transmission
RNA viruses					
Lymphocytic choriomeningitis virus	110–130 nm	Low	Yes	Low	Urine, saliva, milk
Mouse hepatitis virus	80–160 nm	Low	No	High	Feces
Mouse rotavirus	75 nm	High	No	High	Feces
Pneumonia virus of mice	100 nm	Low	No	Low	Respiration
Sendai virus	100–200 nm	Low	No	Low	Respiration
Reovirus 3	65 nm	High	No	Low	Feces
Theiler's encephalomyelitis virus	28–30 nm	High	Yes	Low	Feces
DNA viruses					
Ectromelia virus	290 nm	High	No	Low	Contact, feces
Mouse adenovirus 1	67–74 nm	High	Yes	Low	Urine, feces
Mouse adenovirus 2	67–74 nm	High	No	Low	Feces
Mouse cytomegalovirus	175–200 nm	Low	Yes	Low	Saliva, urine
Mouse minute virus	20–25 nm	High	No	Low	Feces, urine
Mouse parvovirus	20–25 nm	High	Yes	High	Feces
Bacteria					
CAR bacillus	4–12 µm	High	Yes	Low	Respiration
<i>Citrobacter rodentium</i>	1.5–2 µm	Low	No	Low	Feces
<i>Clostridium piliforme</i>	8–10 µm	High	Yes	Low	Feces
<i>Helicobacter</i> spp.	5–10 µm	Low	Yes	High	Feces
<i>Mycoplasma pulmonis</i>	0.3–2 µm	Low	Yes	Low	Respiration, genital
<i>Pasteurella pneumotropica</i>	1–2 µm	High	Yes	Low	Respiration
<i>Pneumocystis carinii</i>	2–8 µm	High	Yes	High	Respiration
<i>Salmonella</i> spp.	2–5 µm	High	No	Low	Feces
Parasites					
<i>Aspicularis</i> eggs	89 µm	High	Yes	High	Feces
Mites	195–500 µm	High	Yes	Low	Contact
<i>Syphacia</i> eggs	118–155 µm	High	Yes	High	Perianal contact

competent mice, with shedding of virus for ~5 days, can be very difficult. Direct tests for the agent in the animal or the environment must occur during the short window of shedding and therefore are quite unreliable at detecting a low-level infection within a colony. Detection of EDIM using serology and sentinels exposed to soiled bedding has the limitation that the timing of the soiled-bedding transfer must coincide with the short period of shedding to be effective. In IVC systems, a biweekly cage-changing schedule, with biweekly addition of soiled bedding to sentinel cages, has the disadvantage that the bedding transferred may or may not contain infectious virus. For agents that cause acute infections, methods that monitor the mice continuously over an extended period, such as seroconversion of colony mice, contact sentinels, exhaust air sentinels, or long-term placement of gauze filters in the exhaust air stream, are most effective. Frequent monitoring, using sentinels exposed to soiled bedding or short-term placement of gauze filters in the exhaust air stream, can also be effective. Periodic monitoring methods, such as sentinels exposed to soiled bedding, and continuous monitoring methods, such as exhaust air sentinels, are both likely to be effective at detecting agents that cause chronic or persistent infections.

The concentration of an excreted infectious agent influences the difficulty of its detection. Agents, such as MPV, that cause a chronic infection with low-level fecal shedding, can be difficult to detect. Detection of these agents necessitates testing an adequate sample size. A sample size that is too small or a monitoring protocol that samples a nonrepresentative group of cages can result in sporadic detection of the agent within a colony. For example, in a recent study, mice that received a single dose of soiled bedding from a group of cages in which 20% of the cages housed MPV-infected mice seroconverted, whereas those that received several doses of soiled bedding from a group of cages in which only 5% of the cages housed MPV-infected mice did not seroconvert¹². Agents, such as mouse hepatitis virus (MHV), that are gen-

erally shed at high concentrations, are much easier to detect using all monitoring methods, but the reliability of detection is still a problem¹⁰⁻¹².

The stability of the infectious agent in the environment also influences the difficulty of its detection (Table 1). For agents that persist as infectious particles in the environment for only short periods of time, such as most enveloped viruses, detection is more difficult than for agents that are stable as infectious particles for long periods in the environment, such as parvoviruses or pinworm eggs. Labile agents can be missed if the timing of exposure by contact, exhaust air, or soiled bedding does not coincide with the period of shedding. Even though many enveloped viruses, such as coronaviruses, lose their infectivity upon drying and rupture of the envelope, the nucleic acids inside the viral particles remain detectable¹⁵. Therefore, molecular assays can detect the recent presence of agents even after they lose their infectivity and transmissibility to mice within the colony.

For monitoring methods that detect infectious agents in the exhaust air, the size of an agent should theoretically affect its detectability. Exhaust air and particulates should carry smaller agents such as viruses more effectively than larger agents such as parasites (Table 1). For example, MPV and *Helicobacter hepaticus* both cause chronic intestinal infections and are shed in feces, yet MPV, a 20-nm virus, was detectable on filters placed in the rack exhaust air, whereas *H. hepaticus*, a 5- to 10- μ m bacterium, was not¹².

Equipment-Related Conditions

There are several system-specific conditions that may influence the efficacy of microbiological monitoring of mice housed in an IVC system. The air change rate that is achievable in an IVC rack is much higher (30–120 ACH) than that achieved in static isolator cages (>5 ACH)². A recent study showed that the higher air change rate in an IVC system compared with static isolator cages resulted in lower relative humidity³. Furthermore, the mean

bedding weight gain per mouse was 50% greater in static isolator cages than in the IVC system. The less humid cage environment present in the IVC system could result in the dehydration and inactivation of many infectious agents. This effect, together with the dilution of organisms in the exhaust air by the higher ventilation rate, could lead to decreased transmission of agents to colony and sentinel mice. The decreased intercage spread of an infectious agent means that infections are sporadic and confined to just a few cages at a time, and it is therefore essential to use an adequate sample size when monitoring mice housed in IVC systems. Several studies have shown that the high airflow achieved in IVC systems results in lower ammonia levels than those seen in static isolator cages at 5–7 days after cage change^{3,16-18}. The lower ammonia levels measured in IVCs are probably the direct result of lower relative humidity, because high humidity has been linked to proliferation of urease-positive bacteria that can convert urea to ammonia¹⁹. Furthermore, the type of bedding used in IVC systems can affect the rate of ammonia accumulation, because some bedding types may contain endogenous ureases²⁰. The frequency of cage change generally depends on the time required for intracage ammonia concentrations to rise to a level considered irritating to the mucous membranes of husbandry personnel (generally >25 p.p.m.)²¹. Because ammonia levels rise more quickly in static isolator cages, it is generally necessary to change static isolator cages at least once a week, whereas mice housed in IVC systems may need changing only once every 2 weeks. Less frequent cage changing decreases demands on personnel time, decreases the quantity of bedding used, increases cage longevity, and may decrease pup mortality²², but it can also decrease the efficacy of soiled-bedding monitoring. If husbandry personnel add soiled bedding to sentinel cages only during routine biweekly cage changes, then this reduces the number of times that soiled bedding will be added to the sentinel cage in the IVC system as compared with static isolators, and agents pre-

sent in the soiled bedding have a longer time in which to become noninfectious before being exposed to susceptible sentinel animals. It will be necessary to optimize soiled-bedding sentinel monitoring protocols used in IVC systems so they can accurately detect agents if they are present. This means following a strict protocol to avoid bias by regularly sampling all cages on the rack, and one should also consider supplemental sampling of soiled bedding between cage changes. Both of these have implications for labor and may have the negative effect of increasing the probability of cage-to-cage transmission during soiled-bedding collection.

The rate at which a particulate accumulates on a filter may also affect the sensitivity of filter-based tests. Agents such as MPV are present at low concentrations in exhaust air. Recently, we reported that MPV DNA was not detectable on filters placed on the rack exhaust filter for 24 h, but MPV DNA was detectable on filters placed on the rack exhaust filter for 2 weeks¹². The positioning of filters within the IVC rack can also influence the chance that an agent will be detected. For example, MPV, MHV, and Sendai virus were all detectable on filters placed on the lids of cages housing infected animals for 24 h at 6 days after inoculation, but only MHV and Sendai virus were detectable on filters placed on the rack exhaust filter for 24 h at 6 days after inoculation¹². This indicates that the closer to the source of the infection, where the infectious-agent concentration in the exhaust air is highest, the greater the likelihood of detecting it. It will be necessary to determine for each IVC system the optimal positioning of test filters and the time required to accumulate adequate particulates, which serve as fomites to transport infectious agents through the air, and should consider the ventilation pattern of the system. Theoretically, any location within the exhaust system of the IVC rack that a small (1 cm²) filter can be temporarily placed can serve as a test site. The type of bedding used in IVC cages could affect the rate of particulate generation and accumulation on filters and therefore could

affect air-testing efficacy. Aspen chip bedding has been found to be substantially less dusty than wood fiber or straw pelleted bedding²³. Theoretically, the greater the amount of dust generated from bedding, on which agents can be carried through the air, the greater the chance of detecting the agent on a filter.

The accuracy of monitoring methods involving exhaust air sampling is dependent on the uniformity of the airflow achieved in the IVC rack and thus on the IVC rack design. When infections are present in only a small number of cages on an IVC rack, as is often the case, the reliability of exhaust air monitoring, whether using gauze filters placed on the rack prefilter or using exhaust air sentinels, is likely to be highly dependent on the uniformity of exhaust airflow within the rack. Nonuniform airflow resulting in large variations in exhaust airflow from individual cages may be a cause of inaccurate or unreliable sampling of cages housing infected mice seen in some studies (B. Tiemann, personal communication). Furthermore, some IVC systems have a single manifold that is used for supply air only, and exhaust air simply escapes from the cage into the room. In such IVC systems, it may be impossible to appropriately sample exhaust air either by using gauze filters or with exhaust air sentinels.

One can operate IVC racks using either a positive or negative air pressure differential. Under a positive air pressure differential (bioexclusion mode), the supply fan for the rack pushes more air into the rack than is pulled out by the exhaust fan, resulting in a small amount of air leaking out of the cages into the animal room. Under a negative air pressure differential (biocontainment mode), the supply fan pushes less air into the rack than is pulled out by the exhaust fan, resulting in a small amount of air being sucked into the cages from the animal room. Theoretically, different air pressure differentials might affect the efficacy of air monitoring methods, but in our experience the efficacy of microbiological monitoring was equivalent under positive and negative air pressure differentials¹².

It is important to remember that in an IVC system, the primary means of infectious-disease spread within a rack occurs during husbandry procedures or experimental manipulations. During an outbreak, monitoring exhausted air from individual cages using cage-top gauze filters, or monitoring exhausted air from the entire IVC system using filters placed on the rack exhaust manifold, can be highly efficient in determining the extent of an infection within a rack or within a facility, and in confirming elimination of an agent after such an outbreak. Both of these filter-based methods do not require handling of potentially infected mice and can produce results within a few days. This can be very valuable when handling animals for sentinel bleeding or for collection of soiled bedding could lead to spread of the infection.

Conclusions

Rodent microbiological monitoring continues to be a highly dynamic, nonstandardized, and continually evolving process. Monitoring strategies and approaches to diagnostic sample collection are confounded regularly by the convergence of innovative husbandry refinements, new developments in a wide array of caging systems, evolving pathogens, and novel rodent genotypes. In this environment of constant change, and often in the face of relatively meager financial resources for assessment of these mitigating influences, the validation of monitoring approaches has proved to be a challenge. Consequently, we recommend a multifaceted approach to monitoring for infectious agents in IVC systems. Each monitoring program should consider both infectious agent-related and equipment-related variables. The routes of transmission for the infectious agents to be monitored, the duration and infectious-load shed, and the stability of the agent in the environment should all inform the decision as to which approach to monitoring is appropriate. Likewise, the frequency of testing should specifically target the battery of agents to be tested and the type of caging used. It is also essential to understand and consider airflow rates and uni-

formity of airflow in each type of IVC system. Until techniques and technology, still to be developed, simplify the monitoring process, a fully integrated program of microbiological monitoring should make use of a sensible combination of soiled-bedding sentinels, contact sentinels, and, where possible, exhaust air monitoring. Even a broad sentinel-based monitoring program, however, is unlikely to be fully reliable and completely sound. Consequently, the most comprehensive monitoring program should also include health evaluations of colony rodents that present as clinical cases.

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