TECHNICAL BULLETIN

ENVIRONMENTAL MONITORING

Introduction

Laboratory animal facilities present unique environments for microbial growth and outbreaks of infectious agents. Density of animal populations, the frequency of handling for husbandry duties and research procedures, and the constant and diverse stream of supplies into such facilities all provide opportunities for the introduction and dissemination of pathogens. Biosecurity processes have always targeted incoming animals and, to a lesser extent, inanimate supplies and materials, but quality assurance programs have traditionally focused primarily on monitoring the animals. Now, many institutions are realizing that monitoring the animals' surroundings is as vital to overall management of disease risk as is direct monitoring of the animals. Contaminants can enter a facility via many environmental routes, including incoming air circulators, feed, bedding, and water supplies, as well as via equipment for research and facility maintenance, on human staff or administrative supplies. An effective environmental monitoring program not only gives assurance that biosecurity processes are functioning properly to minimize risk from the introduction of microbes into animal facilities, but also can help in detecting and, thus, containing infection problems as early as possible.

The increased demand for immunocompromised and gnotobiotic rodents for biomedical research has led to more stringent housing methods as well as to more frequent microbial testing to detect potential exposure to undesirable microbes. While environmental monitoring is an important component of the standard operating procedures of all animal facilities, it is especially critical in immunocompromised colonies, in which traditional serological assays are uninformative. In gnotobiotic colonies, a fully integrated environmental monitoring program should continuously survey every potential transmission route, thereby supporting and allowing refinement of protocols and processes to limit microbial contamination by source materials.

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Feed and Bedding

Contaminated feed or bedding can rapidly disseminate microbial contamination throughout animal housing facilities. Sterilization with an autoclave is a common first-line strategy for disinfecting bulk dry supplies as well as cage components (1). Autoclave systems must be calibrated for specific loads and load configurations, since effective heating and pressure cycles to ensure sterility vary with both the material and type of container. Routine use of chemical indicator tape ensures that autoclave internal chamber temperatures have reached at least 80°C. However, autoclave tape only indicates exposure to heat, but not duration, steam penetration or pressure. Commercially available bacterial spore strips with calibrated biological indicators (endospores) should be placed deep within the load in autoclave runs and are required for quality control of the sterilization process (2). Sporocidal activity is the most sensitive check of autoclave effectiveness, because endospores are more resistant to heat than viruses or vegetative bacteria.

Irradiation of food and bedding supplies with high-energy gamma-emitting radioisotopes like Cobalt 60 or Cesium 137 is another large-scale disinfection process. Low doses of DNA-damaging irradiation are lethal to parasites and insects with large genomes, while it takes greater amounts of irradiation to kill bacteria due to their smaller genomes. In addition, some bacteria can form endospores that are more radiation-resistant. Viruses are the smallest nucleic acid-containing pathogens, and are more resistant to irradiation at most established doses. Finally, prion particles associated with spongiform encephalopathies lack DNA and RNA, and are resistant to irradiation (3). Overall, large-scale irradiation is very effective in eliminating parasites and most bacteria from food and bedding, but may fail to completely eliminate viruses and some bacteria from bulk materials.

It is therefore recommended that periodic sterility checks on both feed and bedding be considered in the overall surveillance program for animal facilities. Testing these materials provides direct confirmation (in addition to autoclave tape and spore strips) that your autoclave is sterilizing its load. For example, feed and bedding aliquots should be analyzed using microbiological media and subsequent plating while swabs of feed and bedding storage bins should be used to inoculate bacterial plates to check for contamination.

Cage Systems

Circulating air and cage surfaces are potential sources of microbial contamination for research animals. Many animal research facilities have switched from traditional open-top or static microisolator cages to individually ventilated cage (IVC) systems in order to limit airborne transmission of infectious agents (4-6). Rodents housed in IVCs have much lower incidence of cage-to-cage transmission of infectious agents, because each cage receives HEPA filtered air under positive pressure, and is in effect an isolated biosecure zone (7-9). In IVC systems, transmission of infectious agents is often due to contamination during husbandry activities, cage washing or experimental manipulation by researchers (10, 11).

Charles River's biosecurity experts have identified cagewashing as a probable source of spread of infection in multiple customer facilities, despite each cagewasher nominally using 180°F water. The effectiveness of cagewashers can be monitored by reduced bacterial counts after washing. Reduction in bacterial counts can be measured through RODAC (Replicate Organism Detection And Counting) plating or swabbing

(followed by plating) cages pre- and post-cagewash. Many facilities follow cagewashing with autoclaving the washed cages. In these instances, autoclave function should be monitored with spore strips, as described above.

Water Supply

Drinking water is always a potential source of microbial contamination for research animals, whether delivered from bottles or from automated animal watering systems (10). Periodic assessment of water quality should be part of any environmental monitoring program. Bacteria are the most difficult water-born microbes to control, and some can survive for years in purified water virtually devoid of nutrients (12). That being the case, even the most sophisticated treatment and filtration systems (see following discussion) are susceptible to bacterial colonization. These bacteria, such as *Pseudomonas aeruginosa*, are unlikely to be pathogenic for immunocompetent animals but are a health concern for most immunocompromised animals and a major problem in biofilm formation. Over time, many bacteria can adhere to the internal surfaces of systems and develop into biofilms, an organized structure of bacterial colonies protected by a layer of slime. Biofilm is the chief source of many of the free-floating bacteria in drinking water as pieces of the film detach and flow downstream to cage bottles or feeding valves. Excess biofilm can also lead to clogging of small apertures or valves. To control free-floating bacteria, it is important to periodically treat internal surfaces with a residual disinfectant. During automated flushing, low-residual chlorination or acidification can lower bacterial contamination in automated drinking water systems. Bacteria within the biofilm coatings, however, can withstand hundreds of times the concentration of common disinfectants such as chlorine that would kill free floating bacteria. Elimination of biofilms, therefore, may require special treatment.

All animal drinking water systems need monitoring. Untreated city water systems need monitoring for pathogens and total bacterial counts. Advanced treatment systems require monitoring for any bacteria, particularly those that can cause biofilms. There are several commercially available microbial water testing procedures. General bioburden or USP testing includes total aerobic and coliform counts in water supplies. For this testing two 100ml water samples with neutralizing agent are required. USP testing utilizes membrane filtration with media that allows for total heterotrophic bacteria counts as well as total coliform counts. More sensitive water sterility testing can also be performed by filtering water, then culturing the filter membranes, using fluid thioglycolate media and trypticase soy broth. Additionally, endotoxin (LAL) testing can detect extremely low levels of bacterial endotoxin (0.05-5.0 EU/mL) in water samples.

Animal Drinking Water Treatment and Purification

There are several strategies for disinfecting and purifying source drinking water. Most water delivery systems purify water in several sequential steps, beginning with pre-filtration to remove gross particles that can compromise the function of the watering system. Filtered water may then be purified further using any number of strategies. Reverse osmosis (RO), also known as hyperfiltration, removes particles as small as ions from a solution, and satisfies the most demanding specifications for gnotobiotic colonies. RO is capable of filtering bacteria, salts, sugars, proteins, particles, dyes, and other constituents that have a molecular weight of greater than 150-250 daltons. RO produces highly purified water that is 99.95% free of microbial contaminants. However, with time, even well-maintained RO systems can become colonized with biofilm-producing bacteria such as *Pseudomonas aeruginosa.*

Other water purification techniques include distillation, which removes a wide range of contaminants by boiling source water and collecting the resulting condensate. This process is more energy-intensive and more expensive to operate than RO systems. Distillation units may not be practical for larger animal facilities because of the volume of drinking water required. Deionized, or DI water, is purified by passing water through ion-exchange resins that filter dissolved ionized chemicals. However, deionization does not remove organic chemicals, bacteria or other microorganisms. Colonies of microorganisms can become established and proliferate on the nutrient-rich surfaces of the resin. If not regularly sanitized or regenerated, ion-exchange resins can themselves contaminate drinking water with bacteria. Finally, ultraviolet light is widely used to kill or inactivate microorganisms and prevent bacterial growth and contamination in water purification systems, and in recirculating reservoirs of drinking water. Bacterial DNA is very sensitive to UV radiation, while mold spores and some viruses, because their DNA is well-protected by capsids, are more UV-resistant. An initial germicidal 254 nm UV lamp is installed in-line in the pretreatment system, with a second UV lamp located downstream of the reservoir tank, so that as the water circulates through the tank and out, it passes through the UV light prior to distribution to the animal cages. The role of both these lamps is to control and prevent bacterial contamination in the water purification chain. Nonetheless, without proper maintenance, and unless the efficacy of these systems is monitored by bacterial surveillance, even UV treatment systems can become heavily colonized downstream of the UV bulbs.

Facility Surfaces

Overall surface testing throughout an animal facility is another important component of environmental monitoring (13). RODAC Plates are designed for surface monitoring, or swabs can be used and subsequently plated on microbiological media (14). Samples can be taken from any part of a facility that comes into direct or indirect contact with animals – including animal rooms or any surface or equipment that comes into contact with animal by-products (i.e. waste disposal, cage cleaning, carcass storage etc.). All general areas should be monitored on a regular schedule, including room walls, ceilings, counters, sink areas, floors, and door handles. In addition, items that cannot be autoclaved and have been hand-washed or washed in a dishwasher merit particular scrutiny. Although many surfaces such as walls or floors are not required to be sterile, a several-log decrease in bacterial counts on RODAC Plates can be a good marker of adequate disinfection (14). Similarly, action levels can be established, such that bacterial counts higher than a historical post-disinfection level trigger repeat cleaning and perhaps management review.

Additional surface testing strategies for environmental monitoring include commercially available kits for detecting residual indicators or markers of microbial contamination such as protein (13). For example, kits designed to detect protein yield rapid (<5 minutes) colorimetric results on test swabs in the presence of residual protein. Other residual molecules considered to be markers of microbial contamination can be monitored with test strips that assay for the presence of NAD (Nicotinamide Adenine Dinucleotide) or ATP (Adenosine triphosphate) as the bioindicator. ATP detection systems are increasingly popular for environmental surface monitoring, and utilize ATP-dependent luciferase enzyme and luciferin substrate. An enzymatic reaction releases energy as light in the presence of ATP; the resulting light is measured with a handheld light-sensitive luminometer. ATP bioluminescence has become an industry standard for rapid assessment of disinfected surfaces. The advantage of each of the kits designed to detect marker molecules is that results are virtually real-time, allowing for immediate remediation, whereas microbiologic culture methods require several days before results are received.

Post-Contamination Recovery and Room Recertification

Bringing a room back on-line following a contamination or certifying a new animal room requires thoughtful planning and appropriate environmental monitoring. Traditionally, open-air caged sentinel animals were used to evaluate the environment, however, with new technology available, there are faster, more directed means of testing.

For example, post-contamination cleanup may be monitored using one of the bioindicator kits, measuring such molecules as ATP or protein as a means of determining cleanliness (see previous discussion). Surface plating (RODAC Plates or swabs) may also be used. These strategies will give you an indication of general cleanliness, but will not provide specific pathogen information. Molecular testing allows us to obtain information on a specific pathogen. If a room was recycled for MHV, an MHV-specific environmental PCR screen would be useful for recertification. PCR-based molecular methods to detect specific pathogens offer novel environmental monitoring strategies for identifying infectious agents through surveillance of microbial harborages on cage surfaces, and in bedding and circulating air. For example, direct environmental monitoring of IVC systems is conducted with cage surface wipes and sampling of exhaust air filters from cage racks, followed by PCR detection of specific pathogens. While it may take weeks for mice to seroconvert after initial infection, PCR methods can screen exhaust air filter samples and cage surface wipe tests for the presence of a wide array of bacteria and viruses, with results available in days, or even hours. Even though PCR-based monitoring assays must be carefully controlled to avoid false positives, they are extremely sensitive. In one recent study, alginate/saline swabs and PCR methodologies were able to confirm experimentally introduced Sendai virus on cage surfaces for up to two weeks after initial exposure (9). IVC exhaust air filter sampling and PCR amplification could detect experimentally introduced mouse hepatitis virus, Sendai virus, mouse parvovirus MPV-1a, as well as *Helicobacter* (8, 15). Most commercial rodent diagnostic testing laboratories offer PCR testing for rodent viruses and bacteria. Swabs of surfaces and filters are commonly used test articles for pathogen-specific PCR. PCR is an efficient means of post-recycle testing because test samples are easy to obtain and ship, and results are usually forthcoming within a few days.

Summary

Recent improvements in animal housing such as IVC systems have reduced the incidence of cage-to-cage transmission of infectious agents. Because of the success of this type of housing, direct monitoring of the animals is less likely to detect any adventitious infections. Therefore, effective environmental monitoring programs have assumed an increased importance in monitoring facilities for the presence of unwanted microbes. Environmental monitoring also is designed to survey a myriad of potential routes of disease transmission in housing materials, such as water, food, and bedding, as well as to monitor the efficacy of disinfection or sterilization of those materials. As such, environmental testing is designed to augment and complement animal health monitoring programs of serological and PCR testing for pathogens. Importantly, recent advances in molecular techniques and sampling methods have led to environmental monitoring assays that are more rapid than traditional animal health surveillance. By implementing a comprehensive and proactive microbial monitoring program, animal facilities can minimize contamination events, and work to contain outbreaks quickly if and when they occur.

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