



THE S E N T I N E L

ARE YOU IN DANGER OF A BIOSECURITY BREAKDOWN?

Don't find out after the fact – Proactive Environmental Monitoring can alert you to weaknesses within your Biosecurity program.

Here at Charles River, we take extraordinary measures to ensure the biosecurity of our animals. State of the art barrier room and isolator technology; food, bedding, air and water treatments as well as dedicated delivery systems are just a few ways we strive to keep our animals virus antibody free (VAF). We've implemented these procedures to provide our customers with peace of mind; so when Charles River animals arrive at your facility, investigators can undertake studies without concern or hesitation.

As a commercial rodent production company Charles River has exacting biosecurity standards and there are basic tenets of these standards that are widely applicable and easily implemented in biotech, pharmaceutical and academic laboratory animal facilities. Charles River would like to share some simple practices you can adopt to help keep your facility microbiologically clean. We would also like to impress upon you the importance of your QA programs in assuring biosecurity.

Facility Integrity & Cleanliness

A structurally sound facility is the first step in decreasing the risk of contact transmission of pathogens from wild or feral rodents to animals within your colony. Potential areas of harborage and routes of ingress/egress should be eliminated. Items attractive to wild or feral animals such as food, bedding and garbage should be stored off the floor in covered containers. Routine visual inspections of the facility's interior and exterior should alert you to areas in need of attention.

Sterilization & Disinfection

Sterilization and *Disinfection* are the two processes for reducing the risk of pathogen transmission from the movement of laboratory equipment and supplies through an animal facility. *Sterilization* is the most complete of the two methods theoretically resulting in the elimination of all microorganisms while *Disinfection* may only target certain microorganisms and/or if the bioburden is higher than recommended, may not result in the total

elimination of potential pathogens if not controlled rigorously. Bacterial spores, free-living stages of parasites (i.e., pinworm eggs and protozoan cysts) and non-enveloped viruses are very resistant to inactivation and may not be eliminated by such disinfection procedures. While the disinfection of equipment and supplies usually suffices for VAF colonies we recommend that gnotobiotic colonies (i.e., immunocompromised animals) receive equipment and supplies that have been sterilized to decrease the risk of opportunistic infection.

Food & Bedding

Food and bedding are easily sterilized through heating using saturated steam-heat (autoclaving). Care must be exercised to achieve uniform steam penetration and temperature throughout the load. Pulsed vacuum cycles should be used to promote rapid and uniform steam penetration as well as short autoclave times to preserve the nutritional value of the food. Spore strips and autoclave tape may be included in each run to serve as indicators of heat treatment and bioburden reduction. Load configuration and composition should be kept constant and the cycle calibrated and/or validated regularly.

Irradiation may also be used to sterilize food and bedding. Gamma radiation, usually emitted from a ⁶⁰CO source, is a type of ionizing radiation able to pass through solid objects. Durable, water-resistant packaging labeled with the treatment date should facilitate the storage and timely usage of irradiated supplies. Note that gamma irradiation is not effective against small viruses (such as parvoviruses) and certain radioresistant bacteria.

Water

UV irradiation is effective for the disinfection of drinking water. UV light inactivates microorganisms by damaging DNA, rendering them non-viable. However, UV irradiation should not be relied upon as the sole treatment for water intended for gnotobiotic rodents because comparatively small viral and bacterial pathogens as well as those capable of highly efficient DNA repair may be resistant to UV treatment. Water may also be disinfected through chemical processes such as chlorination or ozonation.

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Chemical disinfectants inactivate microorganisms by acting as denaturants that disrupt structural protein and lipid, as reactants that form or break covalent bonds or as oxidants.

Filtration removes microbes from water. Filters work by both entrapping and adsorbing particles (i.e., depth filters) or excluding them based on membrane pore size (i.e., screen filters). Screen filtration is used for water disinfection and can be categorized by the minimum size of particles retained: microfiltration – range 0.1-10.0µm, ultrafiltration – range 1,000 –1,000,000 MW, or reverse osmosis – low molecular weight molecules, including salts. Microfiltration of water retains bacteria, fungi and their spores but cannot be relied upon to exclude viruses. Removal of viruses from water can be also be achieved by ultrafiltration and reverse osmosis. Treatment methods can and should be combined. Regardless of the disinfection methods used, a routine systems check should be implemented. Post-treatment bacterial and chemical analysis can be performed on drinking water, to measure the system's performance. This should be done near or at the point of use.

Surfaces

Chemical disinfectants are commonly used to decontaminate a room, isolator or the surfaces of supplies and materials and containers being brought into an SPF colony or removed. By linking the physiochemical characteristics of microorganisms with their susceptibility to chemical inactivation, you can choose the best product for eradicating a particular pathogen from the environment. For example, disinfectants containing phenolics and quaternary ammonium compounds disrupt lipid membranes and are more potent against lipophilic, enveloped viruses (i.e., Sendai, MHV) than hydrophilic, non-enveloped viruses (i.e., MPV, RPV).

For optimum effectiveness, solid surfaces should be sanitized before disinfection to remove organic matter that may hinder the process. Disinfectants should be used according to the manufacturer's specifications. Bacterial plates (RODAC) and ATPase monitors can be used to monitor surface disinfection by assaying for residual bacteria post treatment. In addition, molecular-based PCR testing can be utilized to detect the presence of viruses on surfaces and membrane filters. Viral PCR testing may be of particular importance in monitoring room decontamination, allowing you to assess the efficacy of the clean up. Interpreting the results of surface monitoring can be complex.

Pathogen Transmission

Insects & Personnel

Insects may act as biological and mechanical vectors carrying rodent pathogens into an animal facility. The most effective means of controlling insects are to deter their entry. If they exist within a facility, an entomologist should be consulted to establish an extermination plan that minimizes or obviates chemical insecticide application as pesticides may alter rodent physiology.

Facility personnel can also act as mechanical vectors. Access to colonies should be limited and a "clean to dirty" flow pattern should be established for people and supplies. Gowning and gloving should be prescribed as appropriate to the risks imposed as well as implementation of mechanisms to limit animal-human contact (i.e., animal manipulation in laminar flow hoods). Animal technicians should be prohibited from having pet rodents and visits to the facility limited to people that have not had recent contact with other laboratory animals.

Special Considerations for New Facilities

Once a new facility has been established, prior to populating the room, sentinel animals should be used to identify potential pathogens in the environment. Following adequate exposure, four to six weeks, sentinels should be tested for bacterial and viral pathogens. This prescreening exercise allows you to populate with the assurance the environment is clean.

For further information please visit the Charles River web-site; specifically, ***A Guide to Research Rodent Housing*** at www.criver.com/techdocs/biosecurity_housing.pdf and ***Recovering from a Microbiological Contamination in your Animal Facility*** at www.criver.com/pdf/contamination.pdf. If you have any questions regarding our biosecurity initiatives or environmental testing procedures please call Charles River Technical Assistance at 1-800-338-9680.

References:

1. Shek, W.R., and Gaertner, D.J., (expected 2001): QC for Laboratory Rodents and Lagomorphs. In: Laboratory Animal Medicine, 2nd Edition, edited by J. Fox.
2. White, William (2001): The Use of Laboratory Animals in Toxicological Research. In: Principals and Methods of Toxicology, 4th Edition, edited by A. Wallace Hayes, pp. 773-818.


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