charles river

Summary

To keep them free of all exogenous microorganisms, Charles River germ-free (or axenic) mice are raised in sterile isolators supplied with autoclaved food and bedding. This document describes the surveillance of our germ-free colonies for extraneous bacteria and fungi, and for pathogens.

RESEARCH MODELS AND SERVICES NORTH AMERICA

Routine Health Monitoring of Immunocompetent Germ-Free Mouse Isolators in North America

Testing for extraneous microbes is conducted frequently (Table 1), based on the potentially high incidence of this type of contamination and its significant consequences to customers. Weekly, a slurry from each isolator (consisting of feces and environmental swabs in animal drinking water) is inoculated onto various culture media, then incubated aerobically and anaerobically. Because microbial contaminants may be fastidious or non-cultivable on cell-free media (like much of the indigenous microbiota), culture-independent methods are employed. Wet mounts of the slurries collected each week are examined by phase microscopy for motile organisms. In addition, feces collected quarterly from mice in each isolator are assayed by PCR for the bacterial 16S ribosomal RNA (rRNA) gene.

Comprehensive health monitoring for pathogens is performed annually on mice from each isolator. Animal organs and tissues are grossly examined, and histopathology is carried out if lesions suggesting an infectious disease process are observed. Specimens from the gut and skin are examined microscopically for endo- and ectoparasites. Blood samples are screened for pathogen-specific antibodies by the multiplexed fluorometric immunoassay (MFIA®), with corroboration of unexpected (or indeterminate) findings mostly by indirect immunofluorescence assays (IFA).

For cultural isolation of bacteria and fungi, various microbiologic culture media are inoculated with respiratory and gut samples and incubated aerobically and anaerobically. Samples for anaerobic culture are collected from euthanized mice dissected in the anaerobic workstation. Isolates are identified both according to their colonial and cellular morphology and by MALDI-TOF mass spectrometry, and, if necessary, by PCR. In addition, swabs of the skin, oral cavity, and feces are tested by PCR for pathogens of all types.

Health monitoring reports are provided by Charles River on our website (http://www.criver.com/products-services/basic-research/health-reports) and are continuously updated as new results become available. These reports document the status of the composite of all the germ-free isolator colonies for that species, strain, or area located at a given facility.

The confirmed detection of bacterial, viral, parasitic, or fungal agents in germ-free mice or isolators would result in immediate cessation of shipment from the isolator and immediate elimination of the isolator colony. Charles River considers each isolator to be a microbiologic unit and will not test and cull individual cages within an isolator.

For assistance regarding specific information on Charles River monitoring procedures, additional data on animals, or interpretation of the monitoring information, please direct inquiries to Charles River Technical Services (877-274-8371) or email askcharlesriver@crl.com.

Table 1: Microbiological Surveillance of Germ-Free Mouse Isolators

Methodology	Test	Sample Type and Frequency: Slurry (*Weekly*)	Feces (*Quarterly*)	Animals (*Annually*)
Microbiology	Culture	X		X
	Phase microscopy	X		X
PCR	Bacterial 16S rRNA		X	
	Rodent pathogens			X
Gross and microscopic exams	Necropsy			X
	Parasitology			X
Serology	MFIA/IFA			X