



360 DIAGNOSTICS™

Services

- Germ-free colonies should be monitored for extraneous bacteria and fungi, and for pathogens.
- Testing for extraneous microbes should be conducted frequently.
- We offer guidance on confirmation testing and next steps when infectious agents are detected in germ-free mice or isolators.

Health Monitoring for Germ-Free Animals

Husbandry of germ-free mouse colonies is a challenge for any animal facility. Successful maintenance and production of germ-free animals requires accurate, sensitive, and robust collection and testing methods. Accurate and sensitive detection of these agents requires aerobic and anaerobic cultures of samples obtained from mice and isolators. Comprehensive germ-free testing is necessary to ensure that animal colonies don't contain contaminating microbes.

Confirmation of Sterility

During the initial installation of a germ-free vivarium, it is important to establish an effective sterility monitoring program. It should be performed when establishing a colony to show that all aspects of a germ-free vivarium are free of pathogens. Specific tests can be utilized as needed (e.g., confirming sterility after protocol changes or when using a new reagent lot).

Item(s)	Test(s)	Sample
Feed	Rodent Feed Sterility Test	10-50 grams following sterilization, collected aseptically#.
Water	Germ-Free Water Testing <i>Includes:</i> Microbial bioburden water test with counts* with MALDI-TOF	Two 100 mL containers (containers containing chlorine neutralization supplied when requested through LTM™).
Bedding	Bedding Sterility	20-25 grams following sterilization, collected aseptically.
Enrichment – Toys	Environmental Swab (Culture)	Rub e-swab across surfaces that may contain bacteria or fungi.

EVERY STEP OF THE WAY

Item(s)	Test(s)	Sample
Enrichment – Dietary Supplements	Sterility Test – Broth Cultures	10-50 grams following sterilization, collected aseptically#.
Isolators	Environmental Swab (Culture) RODAC Plate^ with MALDI-TOF Identification* Surveillance Plus PRIA®	Run e-swab across surfaces of any enrichment item post-sterilization. Fecal pellet, body swab, and oral swab (pooled by sample type, up to 10 of each), one swab of the isolator floor, one swab of the exhaust ports EAD™ sample (for PRIA®).
Germ-Free Animals	16S rRNA PCR Surveillance Plus PRIA®	Up to 10 fecal pellets pooled in a microcentrifuge tube. Fecal pellet and body swab and oral swab (pooled by sample type, up to 10 of each).

*If contaminants are present, MALDI-TOF can be added to your order to identify the offending microbe.

#Please consult LTM™ for specific reagent testing volume and instructions.

^ RODAC plates to be supplied by the customer.

Our scientific team is [available to help you](#) develop a program with confidence.

Routine Animal Health Monitoring Program

After establishing a germ-free vivarium, routine testing supports maintenance of the facility and promotes early detection in case of a barrier breach. These diagnostic tests allow investigators to identify where breaches occur and identify the offending microorganisms.

Each test methodology plays an important, unique role in the monitoring process – they complement each other, and joint submission is strongly recommended. Culture-based testing will detect viable anaerobic and aerobic bacteria or fungi. 16s rRNA (ribosomal RNA) PCR testing determines the presence of microbial DNA, but does not differentiate between viable organisms and free-floating agent DNA. Therefore, we designed our assay to minimize the risk of false positives, ensuring non-viable DNA (as commonly found in autoclaved feed) does not confound your test results. PRIA testing is also performed to detect targeted rodent pathogens, including viruses, bacteria, fungi, and parasites that are disruptive to research or consequential for animal health.

Test(s)	Sample	Frequency
Germ-Free Culture – Standard Culture Testing <i>Includes:</i> Aerobic Culture Anaerobic Culture Fungal Culture Wet Mount	For aerobic culture testing and wet mount, up to three fecal pellets are pooled in a BD e-swab. For anaerobic culture testing, collect one freshly expelled fecal pellet into an anaerobic transport tube.	Weekly
Germ-Free Culture – Plus Culture and Molecular Testing <i>Includes:</i> Aerobic Culture Anaerobic Culture Fungal Culture Wet Mount 16s rRNA PCR	For aerobic culture testing and wet mount, up to three fecal pellets are pooled in a BD e-swab. For 16s rRNA PCR, 10 fecal pellets are pooled in a microcentrifuge tube from a common source (e.g., colony or isolator). For anaerobic testing, collect one freshly expelled fecal pellet into an anaerobic transport tube.	Monthly
Germ-Free Microbe Assessment –Molecular Testing for Bacterial DNA and Pathogens <i>Includes:</i> 16s rRNA PCR 16s Next Gen Sequencing (NGS)	For 16s rRNA PCR, 10 fecal pellets are pooled in a microcentrifuge tube from a common source (e.g., colony or isolator).	Monthly
Surveillance Plus PRIA®	Up to 10 fecal pellets per microcentrifuge tube. Oral/Body swab (one swab per cage for up to five animals or swab each animal with individual swabs. Pool the swab tips up to 10:1). EAD™ sample appropriate for housing type*.	Quarterly

*Follow the sampling protocol based on your institution's caging method, ensuring that all required sample types are submitted. Please contact us if you have any questions.

Program Assessment and Design

Our experts can help you develop and implement a monitoring program based on your specific needs. Once it is up and running, you can get in touch with our support team to assist with submission logistics, sample preparation, analyzing results, and data management.

Sample Submission

Request complimentary sample shipping supplies (e.g., swabs, vials) via Laboratory Testing Management® (LTM™), our online order management platform. You can also use LTM™ to generate submission forms and schedule your samples for submission. By creating orders via LTM™, our laboratory will know when to expect your samples for testing, and you can track your samples online throughout the entire process. To learn more about, and access, LTM™, visit www.criver.com/ltm.