



TITLE: Gnotobiotic Sentinel Colony Surveillance

SOP Category: Gnotobiotic

CMR SOP #: 4.25

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Approval: LaTisha V. Moody, DVM, DACLAM

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SCOPE:

This document describes the procedures to be followed when performing full colony surveillance testing for all germ-free mice. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University Gnotobiotic facilities.

OBJECTIVE:

The objective is to describe the process of direct testing and collection of samples from flexible-film isolators and Allentown Sealed Positive Pressure (SPP) individual ventilated cages (IVC) for all germ-free (axenic) mice to ensure they maintain their germ-free (GF) status. Colony surveillance testing is performed triannually typically in January, May, and September on all GF mice in the gnotobiotic core excluding experimental animals.

PROCEDURES:

A. Collecting samples

1. For SPP cages:
 - a. Fecal samples will be collected for processing primarily by the Molecular Resources Facility (MRF). Other diagnostic laboratories may be used if necessary.
 - b. You must collect at least two fecal pellets per cage but no more than five fecal pellets per tube.
 - c. Bring the cage(s) to be sampled onto the sterilized surface according to *SOP # 4.22 Sterilization of BSC surfaces and gloves*.
 - d. Using either the 2-person or one person method, unwrap and aseptically bring in sterilized pouch(es) of fecal collection tubes into the sterilized BSC.
 - e. Bring the cage(s) into the sterilized BSC using either chosen method.
 - f. Collect two fecal samples according to the *SOP # 4.23 Routine Sterility Testing of GF Mice Colony*.
 - i. Note: one fecal sample tube will be submitted to the external diagnostic lab (i.e. MRF or IDEXX) and the other fecal sample will be used for in-house microbiological culture testing.

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- g. Remove samples from the BSC, write the date the sample was collected on the tube and label samples as described in the table below:

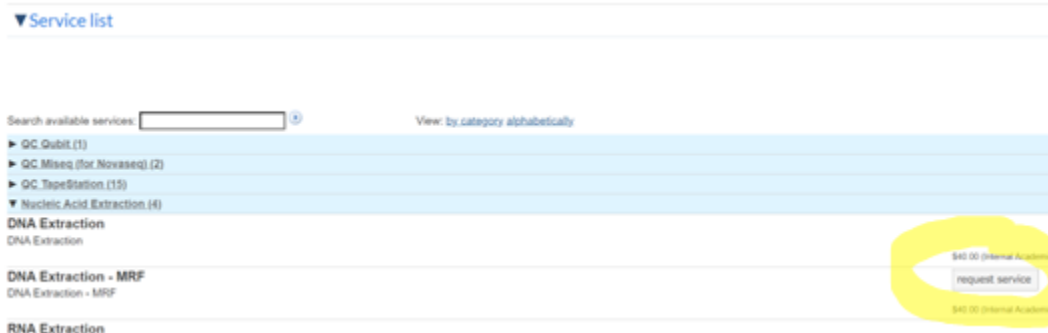
Description	Sample Label Examples
Fecal pellet for PCR	Moo 39482 Moo Iso 3
Isolator swab for microbial aerobic culture	Moo Iso 3-swab

2. For Flexible-film Isolators:

- You will need two fecal collection tubes per isolator. You must collect at least two fecal samples per isolator but no more than five fecal pellets per tube.
- Fecal samples will be submitted for PCR testing using the MRF diagnostic lab. Collect fecal samples from any cage(s) inside the isolator. One fecal tube will be submitted to the external diagnostic lab MRF, and the other fecal tube samples will be used for in-house testing.
- When the samples are removed from the isolator, write the date the sample was collected on the tube.

B. How to Submit Samples to Molecular Resource Facility (MRF)

- Login to iLab <https://rutgers.ilab.agilent.com/landing/196> and click on Genomics Center.
- Click on Request Services. Under the Service list, click on Nucleic Acid Extraction drop down selection of our Service list (insert image). Click on the request service button for DNA Extraction - MRF.



- Fill out the iLab form with the appropriate information and press the button “submit request to core”. Fill out any more information inside the form once the request is submitted if needed.
- Print out the MRF qPCR Sample submission Form and fill out the sample IDs for each sample. Write legibly and put the date on the submission form. The MRF Sample Submission form can be found in the One Drive: CMR/Newark - Test / Gnotobiotics / IDEXX & Colony Surveillance Reports / Molecular Resource Facility Submission Form.

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5. Complete the paper submission form with the sample ID, species, date, and project description. Scan a copy of the paper submission form and upload the form into the iLab request and email MRF at mrfadm@njms.rutgers.edu to alert them that samples will be dropped off at a designated time.
 - a. MRF hours of operation are Monday-Friday 10am-12pm and 2-4pm.
6. Pack your labeled samples into a Ziplock bag and place them on ice packs in a box.
7. Retrieve one positive control fecal sample saved from colonized mice.
8. Label positive control as *Conventional 1* then pack into a separate Ziplock bag on ice inside the box.
9. Close the lid of the box.
10. Fold the paper copy of the submission form into thirds and tape it to the box with the corresponding samples.
11. Drop off samples to MSB level F-635-637. Samples can be handed to anyone in the laboratory. The main contact in the lab is Nathaneal (Nate) Joseph or can email mrfadm@njms.rutgers.edu.
12. PCR results will be emailed to gnotobiotics@research.rutgers.edu within 1 week.

C. Recording Results

1. Access the correct year for the *Triannual Sentinel Colony Surveillance* excel sheet from OneDrive – Test / Gnotobiotics / IDEXX & Colony Surveillance Reports / Sentinel Results (IDEXX and MRF).
2. Select the corresponding month tab found at the bottom of the excel sheet.
3. Record sample information as stated in the excel sheet. Enter the IDEXX case number found on the IDEXX Sample Submission form if submitting to IDEXX or MRF if submitting to the Molecular Resource Facility.
4. Input UPS tracking number found on the UPS packing slip receipt if shipping to IDEXX.
5. When the results are received, input finalized results with any notes attached to each sample. Samples can be resubmitted and tested as requested.
6. Any confirmed positive cages may be sent to Idexx Bioanalytics for identification, notify the PI and lab members via email.

D. How to Submit Samples to Idexx Bioanalytics

1. Idexx Bioanalytics diagnostic laboratory can be used for screening GF mice or identification of contaminants at the *genus* level using MALDI-tof or sequencing.
2. Log in on [IDEXX BioAnalytics](https://www.idexxbioanalytics.com) website using the login information listed below.
3. Follow instructions for submitting order to IDEXX in IDEXX & Colony Surveillance Reports / Submitting Samples for IDEXX & UPS Shipping Label.
 - a. Go to www.idexxbioanalytics.com
 - b. Click on client login
 - c. Enter Username [cmr2021] and Password [beanbag]
 - d. Click on Submit a case
 - e. Click on Health monitoring
 - f. Fill in sample ID, species, investigator, building, area, room for first sample

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- g. Click + button to add additional rows
 - h. Fill in information for the next sample
 - i. Repeat until all information for all samples is entered
 - j. Check boxes and click Add Services
 - k. Click on Microbiology
 - l. Choose feces as sample type
 - m. Choose Germ-free complete profile
 - n. Click add selections
 - o. Click close
 - p. Click order/billing details
 - q. Enter purchase order #
 - r. Enter Study ID
 - s. Click on Submission review
 - t. Click on Submit
 - u. Click on View, then click on View again
 - v. Print
 - w. Fold paper and put in plastic bag with samples, then seal bag
4. After printing the submission form, fold the paper and put it in a plastic bag with samples, then seal the bag. Pack your labeled samples into a Ziplock bag and place them on ice packs in a styrofoam box. Fold and insert IDEXX Submission form inside the styrofoam box. Tape the box to seal it.
 5. You can drop off the package into a UPS drop box (most pickup before 5:30pm) OR schedule a UPS pickup per instructions found in Submitting Samples for IDEXX & UPS Shipping Label
 - a. Go to www.UPSCampusShip.com
 - b. Enter Username [spencesm@rutgers] and Password [NkCMR650]
 - c. Choose IDEXX Bioresearch
 - d. Weight = 0.25#, Dimensions = 7 x 6 x 5", Packaging type = other packaging, Value = \$1
 - e. Choose UPS Next Day Air
 - f. Choose Rutgers mailroom to pay
 - g. Click Next
 - h. Click Ship Now
 - i. Print out packing slip
 - j. Cut out packing slip and tape on outside of Styrofoam box
 - k. Cover the entire packing slip with packing tape so it does not get wet
 - l. Place in UPS drop box outside of MSB or other location
 - m. Results are typically emailed within 2-3 weeks
 - n. All samples submitted to IDEXX *must* be shipped Monday-Wednesday and avoid shipping on days with inclement weather to prevent delays in transport.