

**TITLE: Verification of Germ-free Status for GF imports****SOP Category: Gnotobiotics****CMR SOP #: 4.26****Page: 1 of 3****Effective Date: 2/18/25****Approval: *LaTisha V. Moody, DVM, DACLAM*****Revisions: 3/24/22, 6/1/22, 2/6/25****SCOPE:**

This document describes the procedures to be followed when importing germ-free (GF) animals from approved and non-approved vendors. Approved vendors for GF mice include Taconic, Jackson and Charles Rivers Laboratories. Non-approved vendors are from any other source not listed under the approved vendors list. This SOP applies to all Shipping Coordinator (SC), Animal Care Staff Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University Gnotobiotic facilities.

**OBJECTIVE:**

The objective is to screen all GF imports arriving at the gnotobiotic facility to ensure axenic status. The Principal Investigator (PI) or designee requests GF rodents to be imported from approved or non-approved sources.

**ROLES & RESPONSIBILITY****PI Responsibility: The PI requesting to import animals must:**

1. PI must alert the gnotobiotic staff that a GF import request will be submitted.
2. Submit an Animal Import Request in the iLab Operations Software. This service can be requested through the Rutgers iLab webpage:  
<https://rutgers.ilab.agilent.com/landing/196>
3. Ensure that the requested strains are listed on the import request as it is documented on their IACUC-approved protocol.
4. If a Material Transfer Agreement (MTA) is required, the PI must provide confirmation of completion prior to import:  
<https://research.rutgers.edu/researcher-support>

**Shipping Coordinator (SC) Responsibility:**

1. Review the iLab submission for completeness and check PI's protocol for strain verification.
2. Submit estimated charges associated with the import, including supply charges, technical time, and third party services through iLab for approval.

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3. Once estimated charges are approved by the PI, SC will change the status of the request to processing, then contact the sending institution to obtain:
  1. Facility and health monitoring program description of gnotobiotic facility.
  2. Details on how animals will be shipped.
  3. Health reports within  $\leq 1$  month of import request date. Samples must be collected from the originating isolator or IVC cage including:
    1. Microbiology (bacterial and fungal cultures) and 16S RNA PCR
    2. OR comparable comprehensive germ-free panel (i.e. Idexx – complete germ-free profile, Charles River –surveillance plus PRIA).
    3. For recently re-derived animals or facilities with recent contamination events from originating room, Rutgers may request additional testing such as parasitology, viral screening, and microbiology such as direct testing of the animals being shipped.
    4. Recent contamination is defined as occurring within 6 months.
    5. 3 year-gnotobiotic facility contamination history.
4. SC will review and upload all documents to an archived folder.
5. The documents are then submitted and reviewed by the Shipping Veterinarian for approval.
6. The SC will be in contact with the gnotobiotic manager to coordinate testing and shipping once import has been approved according to the *CMR SOP # 8.02 External Rodent Imports*. Any positive results, GF import may be rejected, or additional samples may be collected for confirmation testing.
7. GF shipment delivery dates should be scheduled to arrive on Monday or Tuesdays. No GF shipments will be scheduled to arrive during triannual testing periods (January, May, and September).

## **PROCEDURES:**

### **Labeling Samples**

1. All imported cages will be labelled with the appropriate information including room number and date of fecal collection:
  - a. Isolator Samples:
    - i. The first 3 letters of the PI's last name
    - ii. Isolator #
    - iii. For example: Iso 1-Moo Month/Day/Year
    - iv. For example an isolator with 2 PIs: Iso 1 Moo-Bes Month/Day/Year
  - b. IVC Sample:
    - i. The first 3 letters of the PI's last name
    - ii. Cage number and the date of collection
    - iii. For example: Moo123456 - Month/Day/Year

## **Sterility Testing of GF mice from Approved Vendor**

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1. Upon arrival GF animals will be unpacked from GF shipper cages into the GF breeding room in MSB A685D for testing & quarantine according to the *CMR SOP # 4.28 Unpacking Germ-free Mice Imports*.
2. Animals from approved vendors will be tested at one time point before release from quarantine to the PI.
  - a. Test on day of arrival.
3. During the first time point (day 0), 1-3 fresh fecal pellets will be obtained from each cage, during the transfer from shipper to sealed positive pressure (SPP) cages.
  - a. At least one fecal pellet will be used for molecular testing which will be sent to an external diagnostic lab (IDEXX – Bioanalytics or Molecular Resource Facility is preferred) and recharged back to PI via iLab.
4. Once negative results are obtained by fecal 16S rRNA PCR to verify GF status, the PI will be notified and the animals will be released to the investigators for use. (~1 week turnaround time).

### **Sterility Testing of GF mice from Non-Approved Vendor**

1. Upon arrival GF animals will be unpacked from GF shipper cages into the GF breeding room in MSB A685D for testing & quarantine according to the *CMR SOP # 4.28 Unpacking Germ-free Mice Imports*.
2. Animals from non-approved vendors will be tested at 2 time points before release from quarantine to the PI.
  - a. Test on day of arrival (day 0)
  - b. Test at least 72 hours post-arrival (day 3).
6. During the first time point (day 0), 1-3 fresh fecal pellets will be obtained from each cage, during the transfer from shipper to SPP cages.
  - b. At least one fecal pellet will be used for molecular testing which will be sent to an external diagnostic lab (IDEXX – Bioanalytical or Molecular Resource Facility is preferred).
  - c. Once *two* negative results are obtained by fecal 16S rRNA PCR to verify GF status, the PI will be notified and the animals will be released to the investigators for use. (~1-2 weeks).