



TITLE: Routine Sterility Testing for GF mice Colony

SOP Category: Gnotobiotic

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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 routine testing in the Gnotobiotics Core. This SOP applies to all trained gnotobiotic staff at the Rutgers University facilities.

OBJECTIVE:

The objective is to describe the routine monitoring of germ-free (GF) animals housed in IVC cages and flexible-film isolators to ensure rodents remain germ-free. Routine testing will be performed every time the cage or isolator is open.

DEFINITIONS:

- **Fluid Thioglycollate Medium (FTG)** - this broth media is used to grow aerobic and anaerobic bacteria.
- **Sabouraud Dextrose (SD)** - this media is used to grow fungi and yeast organisms incubated at room temperature.

PROCEDURES:

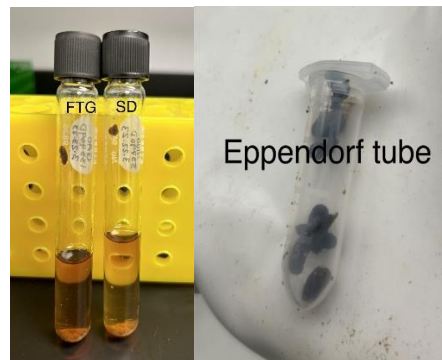
Collecting samples in the isolator

1. If animals are present, 2 fecal pellets are collected from 1 or 2 random cages and are placed into each tube of culture media FTG and SD. If requested by the investigator at least 3 or more fecal pellets are collected into a single Eppendorf tube for analysis by the investigator.

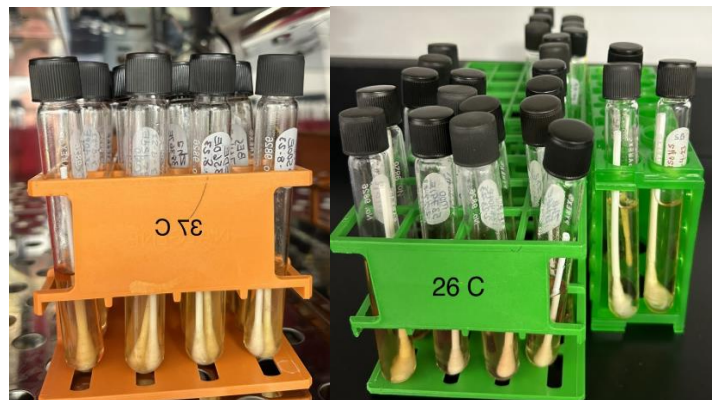
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2. If no animals are present, isolator surfaces are swabbed. Ensure the swabs are moistened with sterile water prior to swabbing isolator surfaces. One swab is placed in culture media FTG and the other swab in SD.
3. The tubes are tightly closed after the samples have been added while they are still in the isolator.
4. The microbiological samples are placed in the incubator. The FTG samples are incubated at 37°C, SD samples are incubated under 26°C or at room temperature.



5. The FTG samples must be kept under incubation at 37°C and SD samples must be kept at room temperature for 14 days. If we see some growth or abnormal appearance, the samples will be sent to an external diagnostic laboratory.

Collecting samples in the IVC Cages

1. During the cage change procedure, the GF mouse is scruffed and 2-3 fresh fecal pellets are collected directly from the animal's anus and placed directly into each culture media tube FTG, SD and Eppendorf tube. Fecal pellets are collected in Eppendorf tube for analysis by the investigator.
2. The FTG and SD tubes are tightly sealed after the samples have been added, under the BSC. Ensure that the Eppendorf tube is properly sealed.

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3. The microbiological samples are placed in the incubator. The FTG samples are incubated at 37°C and SD samples are incubated at room temperature. The Eppendorf tube will be labeled and stored in the –80°C freezer.
4. The samples must be incubated for 14 days, as specified in “Collecting Samples in the Isolator” step #5.

How to Label Samples

1. All the samples will be labelled with the appropriate information, including date of 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

How to process controls

1. Every 14 days there should be a new set of positive and negative controls.
2. For the negative Control use one FTG and one SD Tubes and put the date when the samples are placed in the incubator. Label the tube as Negative control.
3. For the positive controls cut a *Bacillus* spore strip into 2 sections and place into the FTG and SD tubes. If spore strips are not available, an alternative is to use SPF mouse feces as a positive control. Put the date when the samples are placed in the incubator. Label the tube as Positive control.

Recording Results

1. All media must be checked at the 24-hour, 72-hour, 7-day and 14-day timepoints and recorded.
2. Record all the samples into the online in-house testing log with the collection date and date samples were incubated with final results.
<https://rutgersconnect.sharepoint.com/:x:/r/sites/ored/cmr/newark/Test/Gnotobiotics/IDEXX%20%26%20Colony%20Surveillance%20Reports/In-House%20Culture%20Results%20%26%20Record%20for%20Colony%20Surveillance.xlsx?d=wc288f72e71f04cc8a45c83333e03db6f&csf=1&web=1&e=w0xG9Z>

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