

SOP-### Sentinel-Free Soiled Bedding Testing		Page 1 of 2
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PURPOSE:

The intent of this SOP is to define the process for assessing the pathogen status of rodent colonies by the Sentinel-Free Soiled Bedding (SFSB) environmental health monitoring method.

RESPONSIBILITIES:

It is the responsibility of the husbandry staff, lab staff or technician to ensure compliance with the steps outlined in this SOP.

BACKGROUND:

Sentinel-Free Soiled Bedding (SFSB) and Exhaust Dust Testing (EDT) are two PCR-based Environmental Health Monitoring (EHM) testing approaches to monitor the health status of rodent colonies without using sentinel animals. Environmental Health Monitoring methods are highly appealing because they align with the 3R's in replacing the use of sentinel rodents for this purpose, but also have the added advantages of improved pathogen detection and, as demonstrated with EDT, having an overall decreased health monitoring program cost. While EDT is applicable only with racks having an open airflow design, SFSB provides a universal EHM approach for use with any rodent housing type. This process involves the transfer of soiled bedding from colony cages into a central testing cage that lacks animals but contains an IDEXX **REPLACE™** matrix. Over the course of the health monitoring period, the **REPLACE™** matrix will bind pathogens, or their components, allowing detection by PCR testing and reveal health status of the colony rodents.

RELATED DOCUMENTS:

(Add existing related husbandry SOPs here)

SAFETY INSTRUCTIONS:

Wear required PPE for all procedures.

MATERIALS:

Disposable lab gloves
Sterilized clean empty cage with lid for soiled bedding cage (SBC)
Standard lab labeling tape (i.e., Fisher Scientific Cat # 15-901-10Q)
REPLACE™ environmental health monitoring matrix
SFSB Laminated cage card
2 oz. disposable sample cup
50 mL sterile conical tube
Sharpie® pen
Dry-erase pen

PROCEDURES:

1. Create Soiled Bedding Cage (SBC) as follows:
 - 1.1. Don appropriate PPE.
 - 1.2. Prepare change station/biosafety cabinet accord to husbandry facility SOP.
 - 1.3. Obtain empty cage with lid and place in change station/cabinet.
 - 1.4. Correctly label laminated cage card with rack number/side being monitored and place on card holder on SBC.

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- 1.5. Open REPLACE™ environmental health monitoring matrix package over SBC and drop filter into cage.
2. Fill SBC with dirty bedding as follows:
 - 2.1. From each soiled colony cage, scoop 2 oz. of soiled bedding from the latrine area and add to the SBC.
3. Close and agitate SBC:
 - 3.1. Once all dirty colony cages have been sampled, place the lid securely on the SBC.
 - 3.2. Agitate the cage by shaking in an elliptical motion (similar to cooking stir-fry) for 15-30 seconds.
4. Place SBC on designated rack location or in designated location in animal housing room. Cage will not be agitated again until the next cage change period or final matrix collection (refer to steps 5 and 6).
 - 4.1. If the SBC cage will be maintained in a sealed, static condition until the next cage change, remove the bedding to prevent high humidity that could lead to sample degradation.
5. At subsequent cage changes throughout the testing time period:
 - 5.1. Obtain the SBC that is monitoring the appropriate rack.
 - 5.2. Agitate the cage by shaking in a stir-fry motion for 15-30 seconds.
 - 5.3. Obtain a new sterilized empty cage.
 - 5.4. With gloved hands, transfer the REPLACE™ matrix from the previous SBC to the new sterilized empty cage.
6. Repeat steps 2-5 throughout the quarter or designated testing time period.
7. When the quarter or designated testing time period is completed, submit filter as follows:
 - 7.1. Obtain 50 ml conical tube.
 - 7.2. Appropriately label tube with room/rack ID.
 - 7.3. Place SBC into change station/biosafety cabinet.
 - 7.4. Perform agitation as previous for 15-30 seconds.
 - 7.5. Remove REPLACE™ matrix from SBC and place, curl lengthwise with white fleece side facing inward and place into 50 ml conical tube.
 - 7.6. Submit tube containing the filter for testing.

REFERENCES:

1. Include any references or “None”.

APPENDICES:

- A. List any appendices that have been added or “None”. (Appendices should start on the next page.)