Shock Center Protocol

Protocol: Fecal Sample Collection

Date: 2/10/17
Originator: J. Neal

Note:

Following is the JAX SOP for Fecal Sample Collection

1. Fecal samples for Calico Phenotyping Protocol are performed with the following modifications from the JAX SOP:
    a. Samples are "fresh" samples collected in 1.8ml cryovials
    b. Prior to sample collection, cryovials are loaded with 1ml of RNAlater
    c. Once collected, sample is disarranged with 6 inch wooden applicator to allow RNAlater to penetrate sample.
    d. After collection is complete, samples are stored in REVCO -80 freezer

1. PURPOSE AND SCOPE

Describes the procedure for collecting fecal samples; applies to trained personnel and CLAM Technicians.

2. MATERIALS

- 500-microliter Eppendorf tubes
- 70% ethanol
- Associated documents:
- Cleaning cloth
- Forceps
- Media tubes
- PPE per area requirements
- Process NPD working solution
- Wescodyne working solution

3. DEFINITIONS AND ACRONYMS

- **CLAM:** Clinical Laboratory Animal Medicine
- CMQ: Comparative Medicine & Quality
- **NPDWS:** Process NPD Working Solution
- **PCR:** Polymerase Chain Reaction
- **WWS:** Wescodyne Working Solution

4. ANIMAL WELFARE KEY POINTS

Gently push down on the mouse's hips while arching the tail to collect the sample. Do not push down on the hips forcefully.

5. SAFETY KEY POINTS

5.1 Follow all safety precautions related to use of NPDWS and WWS. Refer to and before preparing and/or working with either of these chemicals.

5.2 Do not spray 70% ethanol on energized air flow tables.

6. QUALITY KEY POINTS

6.1 Fecal pellets may be collected fresh directly from the animals. For some applications, they may also be collected from the top of the bedding; check with CMQ staff as to which organisms can be sampled for using this collection method.

6.2 Do not let the fecal pellets touch the table surface. If a pellet does touch the table, discard into a dirty mouse box and collect another sample.

6.3 For culture, collect the pellets into 5 mL culture tubes containing the appropriate growth media

6.4 If collecting samples in media (i.e., not for PCR), use duplicate dot labels on the tops of the media tubes that correspond with the dot label numbers on the cage card and paperwork.

6.5 For PCR testing, collect the pellets into 500 µL Eppendorf tubes

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6.6 Numbering Eppendorf tubes on both the side and on the top helps laboratory personnel read the numbers.

6.7 Follow all preparation and use instructions regarding NPDWS and WWS per and

7. PROCEDURE

7.1 Collection of fecal samples for PCR or in media (for culture):

7.1.1 Thoroughly disinfect gloved hands with 70% ethanol.

7.1.2 Open the cage.

7.1.3 Open the Eppendorf or media tube with the number that corresponds to the sample sheet and the dot. **NOTE:** If using a media tube, place the tube lid upside-down on the table, out of the way.

7.1.4 Collection of fresh fecal samples in Eppendorf tubes for PCR:

7.1.4-A Pick up a mouse at the base of the tail with a pair of forceps.

7.1.4-B Place mouse on flat surface, holding the mouse by the tail. With two fingers, gently press down on the hips while arching the tail. The fecal pellet, if present, should come out. If not, use the Eppendorf tube to gently apply upward pressure to the perianal area. If a fecal pellet is present, it should come out; if not, put the mouse down and either try another mouse from the cage or wait a few seconds before trying again. Catch the fecal pellet in the Eppendorf tube.

NOTE: Do not let the fecal pellets touch the table surface. If a pellet does touch the table, discard into a dirty mouse box and collect another sample.

7.1.4-C Place the animal back into the home cage.

7.1.4-D Close the cage cover and the Eppendorf tube.

7.1.4-E Place the tube into the rack.

7.1.4-F Complete

7.1.5 Collection of pellets from the cage bottom for culture or PCR:

7.1.5-A Prepare a container with WWS. Place two clean forceps into the solution.

7.1.5-B Dry off one forceps, and then pick up a fecal pellet from the top of the bedding.

7.1.5-C If you are sampling for cultures, collect two fresh pellets in succession and place them both in one tube. If you are sampling for PCR, place one pellet in the centrifuge tube.

7.1.5-D Place the used forceps into the WWS Solution. Use the other pair for the next sample.

7.1.5-E Complete