

SNPRC Immunology Core Laboratory

Collection of vaginal samples with an ophthalmic sponge

1. Tare the weighing balance and ensure balance has been calibrated within the past year.
2. Remove one sponge (Merocel eye-wick spears, Fisher Scientific # NC0093269) from the box, wear gloves at all times when handling sponges.
3. Place the sponge into an appropriately labeled 5mL cryovial (Nunc cat#5000-0050 or similar), labeled with a unique animal identifier.
4. Mark the exterior of one cryovial with the same color permanent marker used to label the sponge shaft. NOTE: ALWAYS REPLACE SAME SPONGE TO THE SAME VIAL
5. Weigh the dry sponge + labeled cryovial and document the weight (pre-weight) on the Tracking Sheet.
6. The vet tech will collect specimen using the pre-weighed sponges according to the following procedure.
7. Cut ~ 1 inch off the distal end of a transfer pipettes (Fisher Scientific # 13-711-20 or similar); these will serve as extension/holder devices for the sponges.
8. Attach the sponge, via the stick, to the transfer pipette and ensure that it is secure.
9. With animal placed in left lateral recumbent position slowly open the vagina and insert the sponge.
10. Record the sampling time (time of sponge insertion), and after approximately 2 minutes slowly remove the sponge from the vaginal canal.
11. Disengage the sponge and plastic stick from the plastic holder and place the sponges back into the original weighed cryovial (by matching the color code of the sponge to the tube) and ensure that the cap is fully tightened.
12. Transport the cryovials so that they can be weighed using the same balance that was used in the preparation of the sponges. Weigh the sponge + labeled tube and document the weight (post-weight) on the ICL tracking sheet.
13. Cryovials must be transported on ice to the laboratory to allow storage within 2 hours of collection.
14. Log into study notebook and place vials in a $\leq -70^{\circ}\text{C}$ freezer for storage.
15. Record the time that the sample is introduced to the freezer on the Tracking Sheet.
16. For elution of vaginal secretions from the sponges use UltraFree Durapore-PVDF Centrifugal Filter Units (Millipore, cat. no. UFC30DV00). Pre-wet filter membrane with 50 μl

of extraction buffer (PBS, 0.1 % Tween, 1 X protease inhibitors) and incubate 10 minutes with shaking at 1000 rpm, room temperature. Spin tubes for 5 minutes at 10,000 g.

17. Eliminate buffer from the filtrate collection tube using vacuum and sterile tip.
18. Place the sponge in 250 μ l of extraction buffer in the filter cup of the UltraFree Durapore-PVDF Centrifugal Filter Unit.
19. Incubate tubes for 60 minutes with shaking (1000 rpm) at 4°C. Note: cut the sponge shaft with scissors while the sponge is resting in the upper chamber of the tube.
20. Spin Durapore tube at high speed (10,000 x g for 10 minutes) at 4°C.
21. Estimate the volume in the filtrate collection tube, aliquot the eluted buffer in 2 tubes, and placed at -80°C storage.
22. Because the volume of sample collected could not be standardized, all cytokine values will be corrected using a previously reported dilution factor $\{[(x - y) + 0.2625]/(x - y)\}$, where **x** is the weight of sponge with sample (in grams), **y** is the weight of the dry sponge, and 0.2625 g is the weight of 250 μ l of buffer added for elution}.

Extraction Buffer

1X PBS pH 7.4

0.05% Tween 20

1X Halt Protease Inhibitor Cocktail (Thermo Fisher 87785)