

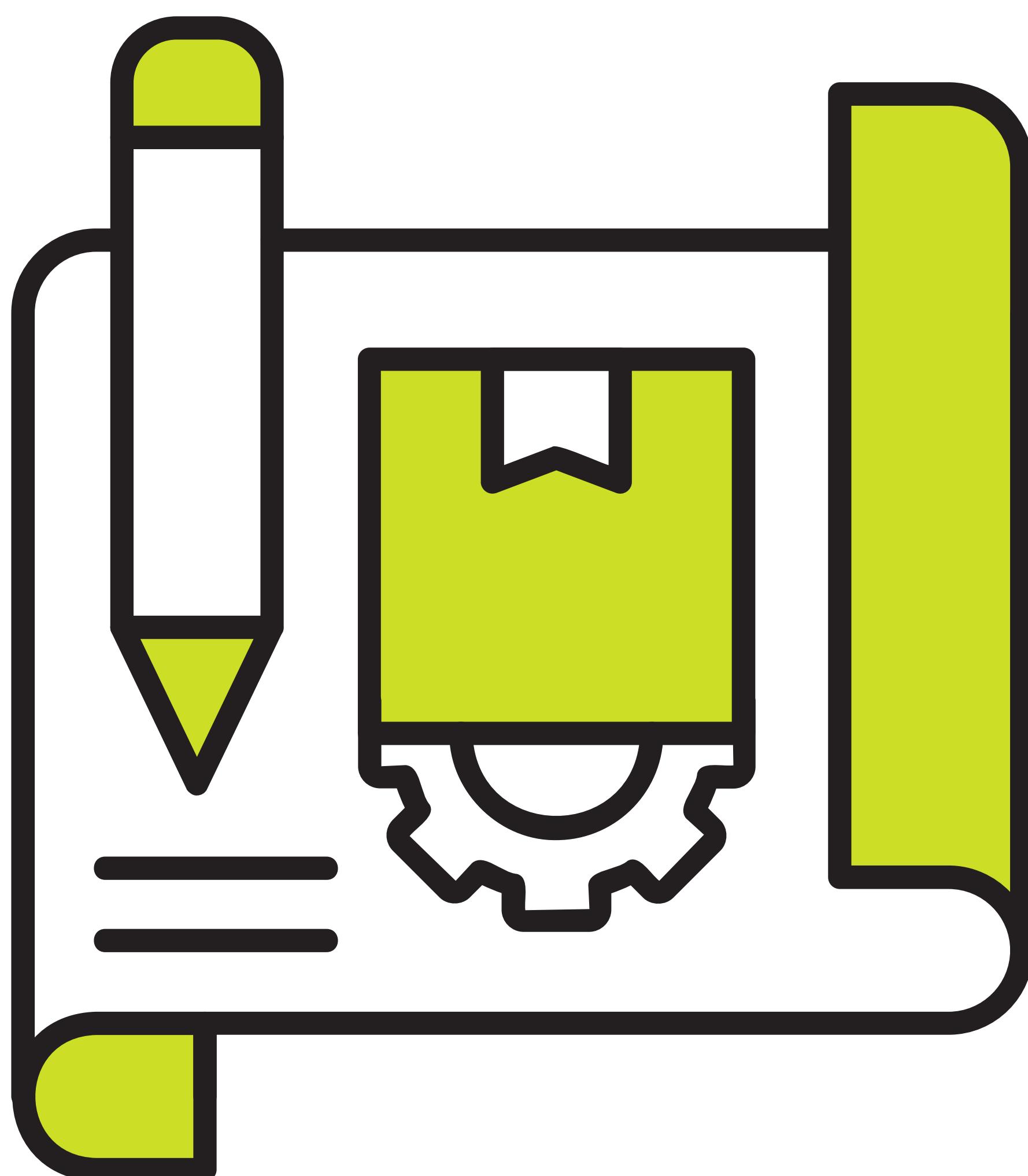


HoloRuminant
Understanding microbiomes of the ruminant holobiont

HOLORUMINANT PROTOCOL

Protocol for

Nasopharyngeal Sampling



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Materials needed:

- Gloves
- Skin disinfectant
- Towels
- Sterile 15ml tubes
- Cotton wool
- Non-cotton sterile wabs

1. Restrain the animal, assuring it is not overly stressed and maintaining a comfortable body position to avoid injury. Depending on animal behaviour, the animal's head may need to be tied.
2. Wear new gloves for each new animal.
3. Wipe off any dirt or bedding with a clean towel.
4. Wipe the outside of the animal's nose using cotton wool impregnated with 70% alcohol or sterile saline solution.
5. Using a new swab per nostril, insert the swab deep (~12cm) into the first nostril carefully to avoid touching the outside nostril. Swabs should be marked prior to sampling so all samples are taken at same nostril depth.
6. Gently rub the swab for 10 seconds using back and forth movements in a gentle circular motion.
7. Put swab into transport tube.
8. Repeat steps 5 and 6 with a new swab for the other nostril. Place into same transport tube.
9. Label the transport tube, including animal identification number, type of sample, and date.

PROTOCOL FOR NASOPHARYNGEAL SAMPLING

10. Tubes containing the swab can be directly frozen at -80 degree C. Alternatively you can add PBS (2ml), vortex at max speed for 30 seconds, remove the swab from tube as you squeeze it against the wall of the transport tube and then freeze the liquid and discard of the swab.