

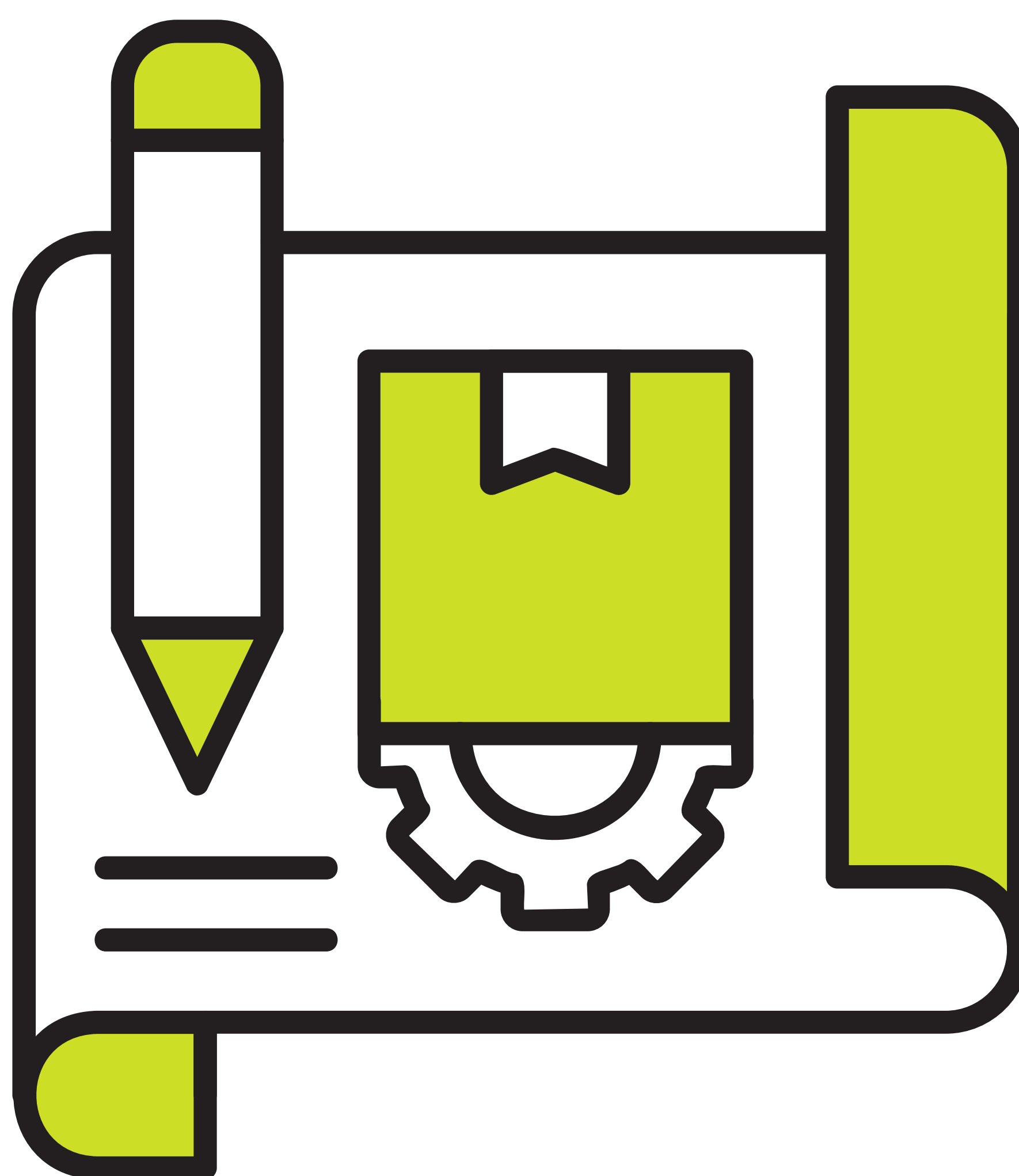


**HoloRuminant**

Understanding microbiomes of the ruminant holobiont

# **HOLORUMINANT PROTOCOL**

## **Protocol for Rumen Fluid Sampling – Esophageal Tube Sampling**



**Authors: Joana Lima,  
Christopher Creevey, Milka  
Popova, Ignas Silinskas  
Version: V1**



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement № 101000213

## Materials needed:

- ◇ Gloves
- ◇ Gag
- ◇ Flexible plastic tube (19 mm diameter)
- ◇ Sterile 15ml transport tube
- ◇ Sterile 1.5 or 2ml tubes
- ◇ Muslin
- ◇ Clean plastic container

Note: Withhold feed from animals for minimum of 2 hours prior to sampling. This is to limit the amount of feed particles that will be present in the fluid sample. The abundance of different microbial groups will alter depending on when the rumen sample is collected.

1. Wear new gloves for each new animal sampled.
2. 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. If so, ensure the head is pointing skyward and the neck extended without forcing to allow passage of the tube. Additionally, a head lift may also be used.
3. Insert gag into mouth of the animal.
4. Insert the stomach tube into the mouth. Do not restrict the animal's lower jaw/tongue movements - facilitates the "swallowing" of the tube i.e. hold the animal by the upper jaw. Do NOT hold the animal by the nose. Ensure the tube is kept in the middle of the mouth / over the tongue to minimise biting. If there is resistance, it may be a sign that the tube is not properly positioned.
5. Use a syringe or a pump to create vacuum and get rumen fluid into the clear tube, through a gentle suction.
6. Remove the tube from the animal, slowly and carefully.
7. Use the pump to pass the rumen digesta from the clear tube to a clean plastic container.
8. Filter the rumen fluid in the container using a muslin layer.
9. Aliquot the sample by pipetting 1 ml of filtered rumen digesta into each of the 2 ml sterile tubes. Fill an extra 15 ml sterile tube with 12 ml of rumen digesta to have as a backup.
10. Label the transport tube, including animal identification number, type of sample, and date.
11. Snap-freeze (for example using liquid nitrogen) or freeze at -80°C.