RUM_020 Rumen Sampling of Cattle via a Rumen Fistula (Expiry: August 2028)

I. OBJECTIVE

This Standard Operating Procedure (SOP) describes the procedure of rumen sampling of cattle via a rumen fistula. Specific details related to the placement and maintenance of rumen fistulas is covered in separate documents.

II. DEFINITIONS

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments." (as per, Australian Code for the Care and Use of Animals for Scientific Purposes, 2013)

III. COMMENTS / RECOMMENDATIONS

- This procedure is only to be performed by investigators when they are trained and deemed competent
- Supervisors are responsible for ensuring that all investigators authorised to use this SOP have achieved an acceptable level of understanding and competence in the procedure.
- Other than the routine handling associated stress, sampling from, and maintenance of a rumen fistula in cattle should not result in any notable impacts to animal wellbeing. The following precautions, however, must be taken:
  - BE GENTLE INSIDE THE ANIMAL – when sampling you are working within the animal's innate protective structures (ribs, hide, etc.) so great care must be taken to avoid causing any damage to delicate organs and structures, e.g., the liver, kidneys and rumen wall.
  - DO NOT LEAVE THE BUNG OUT FOR EXTENDED PERIODS – exposing the rumen to the ambient environment for extended periods will cause heat loss and will have deleterious impacts to the rumen's anaerobic microflora.
- The following safety precautions need to be considered:

Potential Hazard	Control measures
The animal may cough at any time causing rumen contents to be expelled with great force	Use of overalls or a protective apron.
Rumen content contains many bacteria and protozoa. If splashed on the face or eyes	Wear safety glasses. Ensure that area is flushed with copious quantities of cold running water. If irritation results, seek medical advice.
Skin burn	Take care if handling buckets of hot water to avoid scalding. Hot water should be <100^{o}C and not hot enough to burn.
Smelly skin	Contact between rumen fluid and the skin will result in persistent unpleasant odours. Ensure that skin is protected by wearing gloves, and if contact is made, wash as soon as possible with warm soapy water.
Injuries from close contact with animals can occur	Ensure that animals are well restrained using the cattle crush. Be careful not to allow any body parts to be trapped between the animal and railing or other immovable objects when you are sampling, if the animal moves unexpectedly.
Digesta contamination	Protective clothing appropriate to shield from digesta contamination should be worn at all times
Animal kicks or stand on human feet.	Care should be taken that the animals do not kick out at or stand on the toes of people working in this area. Wear steel toe boots.

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

Risk of contracting zoonoses, e.g. from urine (e.g., leptospirosis) or faeces (e.g., salmonellosis)	The cattle may defaecate or urinate on the administrator. This creates a risk of contracting several zoonoses spread in urine (e.g., leptospirosis) or faeces (e.g., salmonellosis). Even though it is routine to vaccinate UQ cattle against leptospirosis, vaccine efficacy is never 100%. Wash off with hose water and wipe with a clean towel. Wear protective clothing (overalls, apron and boots) and a hat to prevent hair contamination.
Falling on the ground	Ensure that there is a safe non-slippery platform area at the side of the race on which to stand. Avoid: - standing on a fence rail which will often be slippery, and allows animals to kick your toes - leaning far out over the animals so that if they move you fall into the race.

IV. SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Animal handling should be as safe as possible using species specific approaches
- Appropriate risk assessments should be in place for working with cattle in a crush
- Operators should be advised about protection against Q fever, or tested for immunity and vaccinated if not immune

V. TRAINING CONSIDERATIONS

- All unsupervised procedures must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training of staff and students can be undertaken on live animals under supervision.
- Animal temperament should be assessed as suitable prior to commencement.
- Students must receive prior instruction on the relevant aspects of cattle anatomy and physiology. If possible, they should practice techniques with abattoir-derived organs and/or appropriate models before using live animals.
- An acceptable level of competence in trainees can only be achieved by providing supervised training with live animals.

VI. EQUIPMENT

- Personal protective equipment (PPE); recommended inclusions:
    - overalls, boots (preferably steel-capped), protective waterproof apron, safety glasses, hat, at least one shoulder length glove, disposable gloves (wrist length),
- 10 litre bucket of hot water (<100^{o}C) if needed for holding the bung (typically only required for very new or very old bungs)
- Water for cleaning the animal (via a hose or alternatively warm water in a bucket)
- Sample containers – as appropriate,
- Thermos flask and/or chilly-bin,
  if the sample is required to be kept at body temperature (thermos flask) or chilled (chilly bin);
- Purpose-built suction tube that is fenestrated on one end and suction pump assembly (cheesecloth or sieve alternatively) for use if the research protocol requires the liquid phase to be separated.

VII. PROCEDURE

Restraint:

Animals should be loosely packed within the platform of a herringbone, a cattle race, cattle stall or cattle crush, such that the animal is appropriately restrained while standing and there is unimpeded access to the left-hand side of the animal.

Procedure (right-handed manual sampling):

1. Apply PPE.
    a. the shoulder-length glove is applied to the right arm (the 'sampling' arm)
    b. a disposable wrist-length glove should be applied to the right hand, over the top of the shoulder length glove (to protect the shoulder-length glove), as well as the left hand
2. Fill the bucket to approximately ¾ with hot water (as noted above)
3. Remove the rumen fistula's bung:
    a. Grasp the outer edge of the cannula with the left hand and push the bung (centre piece) into the rumen with the right hand. This can be facilitated by peeling the edge of the bung inwards.
    b. Turn the bung on its side and withdraw it from the rumen.
    c. Wash the bung with water and then place it into the bucket of hot water. This will clean the bung and help to soften it, making later replacement easier.
4. Remove the sample
    a. Use the suction tube and pump if collecting liquid only, otherwise use your gloved hand. As the rumen contents tend to be stratified, many small handfuls from different areas will give a more representative sample than one single large handful.
    b. Be sure to sample from each area - dorsal, middle, ventral, cranial and caudal areas.
    c. Place the sample into the appropriate sample container. Ensure the sample container is appropriately labelled.
    d. If only the liquid phase is required, put multiple handfuls collected from the various areas into the piece of cheesecloth and squeeze out the fluid into a suitable container. The solid phase, i.e., grass, can then be replaced through the cannula back into the rumen.
5. If a large amount of liquid has been removed from the rumen (e.g. 10-12L in a 700-800kg animal), replace roughly the same amount with warm water.
6. Replace the softened bung:
    a. Clean around the inside of the cannula to remove grass from its surface.
    b. Place the waist of the bung over the ridge at the bottom of the cannula with the flat side innermost.
    c. Fold the inner edge in all the way around and push in.
    d. Check that the outer edge is evenly bedded and that there are no folds or tucks. The bung must fit snugly otherwise leakage of gas and/or liquid may occur.
7. Post-sample cleaning:
    a. Wash the area around the cannula including the animal's underside with liberal amounts of water to remove any residual rumen fluid. This is important to reduce fly attraction and the risk of "fly-worry", fly-strike or superficial dermatitis.

VIII. REFERENCE INFORMATION

This SOP deserves notable reference and acknowledgement of work performed by Dr Garry Waghorn – DairyNZ, Hamilton; Alex V Chaves - The University of Sydney; Dr Janice McCauley, University of Technology Sydney. Also Dr Sarah Meale and Dr Joe Olm who provided review and updates in 2025.

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Outcome
1.0	PCA	July 2022	Approved
2.0	PCA	August 2025	Approved