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REDI-NET F-1 FECES FIELD SAMPLING V.1

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Remote Emerging Disea...

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We use this protocol and it's working

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Disclaimer

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Abstract

To outline steps for properly collecting fecal samples from pastures to evaluate the risk of zoonotic disease transmission by the detection of pathogens from vertebrate DNA (vDNA).

Guidelines

OBJECTIVE

To document the field processes for collecting non-human animal feces.

SUMMARY/SCOPE

The overarching aim of the REDI-NET is to develop a collaborative laboratory network between domestic and international partnering institutions to address disease surveillance needs in order to effectively detect, predict and contain potentially emergent zoonosis. This SOP provides guidance on the collection of blood-sucking leeches, to be used as sentinels for xenosurveillance of pathogens in wild and domestic animals frequenting the watering holes.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Field Team Lead, Managers

Note

All study procedures must be conducted in compliance with national and local policies for prevention and control of COVID-19 infection.

Materials

	A	B	C
	Equipment / Material	Description	Mfg / Product #
	Nitrile gloves	Disposal, non-latex gloves	Locally sourced
	PVC fecal samplers	1 ¼" lightweight PVC pipe, 5" long	Locally sourced
	Metal spatulas	For scraping samples into collection bag	Locally sourced
	Whirl Pak bags	532 mL capacity (?), write-on bags, sterile	Uline, S-19793 (500/ctn)
	Sealable plastic bag	For sample storage; Zip-lock slider	Locally sourced
	Water	For use in a rinse container	Locally sourced
	90% ethanol	100% (molecular biology grade)	Locally sourced
	Ice/Cooler	For transport of samples	Locally sourced
	Tablet	For data entry and picture of sampling site (sample?)	Locally sourced
	Writing utensils	Pencil/pens, marker pens	Locally sourced

Safety warnings

1. Know the risks associated with the study site and sampling location and take appropriate personal safety precautions and/or wear appropriate equipment.
2. Wear light-colored long pants and a long sleeve shirt. Wear rubber boots or hiking shoes and white socks, and tuck pant legs into socks. Be sure that clothing is sturdy and can withstand long thorns.
3. Always use caution when entering pastures with cattle and when working in the vicinity of animals.
4. Use the buddy-system whenever possible.
5. Don't forget to close and lock any gates that you open.

Before start

1. Know the risks associated with the study site and sampling location and take appropriate personal safety precautions and/or wear appropriate equipment.
2. Wear light-colored long pants and a long sleeve shirt. Wear rubber boots or hiking shoes and white socks, and tuck pant legs into socks. Be sure that clothing is sturdy and can withstand long thorns.
3. Always use caution when entering pastures with cattle and when working in the vicinity of animals.
4. Use the buddy-system whenever possible.
5. Don’t forget to close and lock any gates that you open.

SAMPLING TEAMS

1

Note

Always use caution when entering pastures with cattle and when working in the vicinity of animals. Use the buddy-system whenever possible. Don't forget to close and lock any gates that you open.

Field sampling of vDNA feces samples **involves two people.** One person serves as the 'sampler' and the other person serves as a 'helper'. The helper can look up details in the instructions when needed, keep track of samples, handle objects that are contamination risks, serve as a second set of eyes for potential contamination, and ensure safety of the sampler in potentially hazardous field conditions.

SAMPLE SITE SELECTION

2 Locate a fecal sample and estimate its age in days and score its consistency.

2.1 Age Estimate

	A	B
	Days	Description
	0	Fresh, steaming, saw it come from the animal
	0-1	Wet surface
	1-3	Dry, crusty exterior but wet interior
	3-10	Dry exterior, but interior may still have some moisture
	>10	Completely dry

2.2 Consistency Score - Cattle (see photos in Appendix 1)

	A	B
	Score	Description
	1	Soupy, pat has no shape (i.e. diarrhea)
	2	Cake batter, pat <1" tick, does not stack, no consistent form
	3	Thick pancake batter, does not stack but does have consistent form
	4	Peanut butter, does not stack but deep with consistent form
	5	Dry, stacking >2" high

2.3 Consistency Score - Sheep (see photos in Appendix 2)

	A	B
	Score	Description
	1	Soupy, no pelleting at all, forms a wet pat with no shape (i.e. diarrhea)
	2	Peanut butter, no pelleting, but may have a distinct shape
	3	Large clumps, but no distinct pelleting
	4	Pellets present, but wet and clump together
	5	Dry, distinct, individual pellets

3 Label a new Whirl-Pak bag including the collection date, collection site, sequentially numbered bag used, age estimate and consistency score.

SAMPLE COLLECTION

4 At each fecal sample, the helper opens the labeled Whirl Pak bag for the sampler. The helper will open and close the bags to prevent potential contamination of samples.

5 Use the PVC sampler to take a core sample from the center of the pat.

Note

If the pat is extremely wet or extremely dry, you may need to use the spatula to scoop the sample or keep the sample in the PVC.

6 Use the spatula to scrape the sample into the labeled Whirl Pak bag and roll the bag shut to seal it.

7 Place the sample in the cooler on ice for storage.

8 Rinse the PVC sampler and spatula in water to remove feces and then rinse in ethanol before sampling the next pat.

9 When done sampling for the day, both rinse containers can be dumped out onto the ground.

FECES STORAGE

10 Keep freshly collected samples on a cold chain (ice cooler) until returning to the laboratory. Fecal samples are not recommended to be stored for long periods of time at room temp.

11 Samples are placed into 1 gallon Ziploc bags and stored at -80 °C indefinitely. If -80 °C is not available, fecal samples should be processed using **REDI-NET SOP F-2 Feces Processing**, as soon as possible.

12 Wash both empty rinse containers with 10% bleach solution and rinse thoroughly.

13 Wash PVC samplers and spatulas with 10% bleach solution and rinse.

APPENDIX 1: CATTLE MANURE CONSISTENCY SCORING

14 Feces is scored on a 1 to 5 basis, with a score of 1 being very fluid and 5 being extremely dry and segmented. The next few paragraphs will detail each score and associated diet

quality. Reference photographs have been included with approximate levels of dietary protein and energy (TDN) listed.

A feces **score of 1 is of cream soup consistency**. It can indicate a sick animal or a highly digestible ration that contains excess protein, carbohydrates or minerals, and low fiber. The addition of hay will slow down the rate of passage and thicken the feces.

Feces that will **score a 2** doesn't stack; the pat is usually less than 1 inch thick and will lack consistent form. This feces has the consistency of **cake batter**. Excess protein, carbohydrates and low fiber characterize the diets that produce this feces. Rate of passage is very high, and adding hay to this diet will slow it down to allow for more absorption in the intestinal tract.

Feces **score 3** is ideal and will typically start to take on a normal pat form. The consistency will be similar to **thick pancake batter**. It will exhibit a slight divot in the middle. The pat will be deeper than a score 2 pat, but will not stack. This diet is not lacking nutritionally, yet is not in excess for the cow and her physiological stage.

Score 4 feces is thick and starting to become somewhat deeper, yet is not stacking. The consistency of the feces will be equivalent to **peanut butter**. This feces indicates a lack of degradable rumen protein, excess low quality fiber or not enough carbohydrates in the diet. Supplementation of additional protein with high rumen-degradable protein can increase total diet digestibility. Cottonseed meal and soybean meal are excellent sources of this type of protein.

The highest and least desirable **score is 5**. This feces is firm and stacks over 2 inches in height. It will also have **clearly defined segments and is very dry**. This feces indicates the cow is eating a poor quality forage diet that is inadequate for protein and carbohydrates, and high in low quality fiber. Rate of passage has slowed down to the point that excess water has been reabsorbed in the intestines. The rancher will need to consider additional supplementation to meet the cow's protein and energy requirements. Cattle have to be in good health for feces scoring to be accurate. Fecal scoring is a valuable tool to determine the quality of nutrition the cow has recently consumed and can be used effectively to adjust supplementation to prevent loss of body condition.

Score 2: >20% CP; >68% TDN of diet

Score 3: 12-15% CP; 62-70% TDN of diet

Score 5: <6% CP; <55% TDN of diet

APPENDIX 2 - SHEEP MANURE CONSISTENCY SCORING

15 **Assessing fecal consistency score for sheep and goats**
Leo Jambre, 2022 https://wormboss.com.au/tests-tools/assessing-faecal-consistency-score/

The consistency of faeces can bias the results of a worm egg count. The wetter the faeces, the lower will be the worm egg count relative to drier faeces, because the higher water content increases the faecal volume and dilutes the worm eggs. As a result, scouring animals will have artificially lower worm egg counts – that is, without the scouring, their WECs would be considerably higher.
The faeces can be scored for faecal consistency and then the worm egg count can be multiplied by the adjustment factor for that score to gain an adjusted WEC—the WEC that would be likely if the faeces were in a drier pellet-like state.
Very watery faeces (scouring, Score 3.5) may indicate a severe infection with scour worms. However, some animals may scour like this for short periods (generally only days to a week) when they are exposed to just a small number of scour worm larvae. This is called hypersensitivity scouring, and generally occurs once an animal has developed resistance to the worms, but has not been exposed to worm larvae for some months.
Animals may also scour for reasons unrelated to worms, the most common being a diet of lush green feed or the protozoa infection, coccidiosis.
The following images show varying faecal consistencies each with a score, as well as a multiplication factor.
Score the faeces before a WEC is carried out. Once the result is at hand, multiply it by the score, which is the adjustment factor, to gain an adjusted WEC (which is about

what the WEC would be if the faecal pellets were discrete.
If you have a group of samples being tested together, either as a bulk count or as individuals, but with an average count calculated, give an average score for the group and then multiply the bulk or average result by the adjustment figure that matches the average score for the group of faeces.

Score 1

Score 2

Score 3

Score 4

Score 5

Protocol references

REDI-NET Overview Summary