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We use this protocol and it's working

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REDI-NET T-1A ACTIVE VERTEBRATE TICK FIELD SAMPLING

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Remote Emerging Disease Intelligence - NETwork (REDI-NET)

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DISCLAIMER

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ABSTRACT

To outline steps for properly collecting tick samples actively collected from cattle to evaluate the risk of zoonotic disease transmission by the detection of pathogens from invertebrate DNA (iDNA).

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GUIDELINES

OBJECTIVE

To document the field processes for collecting ticks from non-human vertebrate animals.

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 of the protocol for the correct active tick sampling at CONUS/OCONUS sites to evaluate and predict the risk of zoonotic disease transmission.

RESONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component 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
	Rubber boots	Personal protective equipment	Locally sourced
	Falcon tubes	15 ml or 50 ml for sample storage	Locally sourced
	Forceps	For sample collection	Locally sourced
	Bleach wipes	For material cleaning	Locally sourced
	Timer	For sampling timing	Locally sourced
	Squeeze chute	For cattle restraint	Locally sourced
	Ice/Cooler	For transport of samples	Locally sourced
	Tablet	For data entry and picture of sampling site	Locally sourced
	Writing utensils	Pencil/pens, marker pens	Locally sourced

SAFETY WARNINGS

NOTE: Cattle are restrained in a squeeze chute for all procedures.

- **Never** stand in front of the chute until the animal has been completely restrained.
- **Always** be aware of the location of the gate hinge when standing next to the chute as it is opening and closing.
- **Always** make sure that all drop-down bars and fold-out panels are closed before the chute is opened.
- **Always** communicate verbally with the team operating the squeeze chute when you are finished checking the animal so that they know when it is safe to open the chute.
- **Always** be aware of the fact that the animal can still move somewhat inside the chute. Be aware of where your hands and arms are, and be able to remove them from the chute quickly.
- **If you ever feel uncomfortable checking an animal** (animal is too worked up or aggressive in the chute), or you are unable to safely access part of the animal in the chute, **skip it**.

BEFORE START INSTRUCTIONS

NOTE: Obey all safety procedures in this SOP.
Cattle are restrained in a squeeze chute for all procedures.

- **Never** stand in front of the chute until the animal has been completely restrained.
- **Always** be aware of the location of the gate hinge when standing next to the chute as it is opening and closing.
- **Always** make sure that all drop-down bars and fold-out panels are closed before the chute is opened.
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- **If you ever feel uncomfortable checking an animal** (animal is too worked up or aggressive in the chute), or you are unable to safely access part of the animal in the chute, **skip it**.

SAMPLING TEAMS

1 This SOP assumes that the animals' owners/handlers/veterinarians are on site to corral cattle, move them through the chute, and operate the chute. The three minute search time assumes at least three study personnel are available for tick collection. If only two individuals are present, increase search time to five minutes.

2 One person should be assigned to search the head/neck/dewlap region, one person should be assigned to search the legs/belly region, and one person should be assigned to search under the tail and the udder.

Note

It is suggested not to use the tablet for data collection unless a dedicated collection individual is available among the study personnel as your hands are likely to get very dirty and could contaminate the tablet.

3 Record the ambient temperature outside the barn or enclosure if working indoors.

SAMPLE COLLECTION

4 Once an animal is completely restrained in the squeeze chute, label a collection tube with the date, collection site, and animal's ear tag number.

5 Set a timer for three minutes. (See above note in Step 1.)

6 Lower the rear drop-down bars to access the tail. Lower the lower fold-out panels to access the legs. If necessary, the front swing arm can be used to pin the animal's head to one side.

Note

These are **heavy**. Use caution when opening and closing parts of the chute.

7 Moving your hands over the animal, feel for bumps on the skin. Ticks will be round or flat, smooth, and somewhat moveable, compared to scabs, burs, and warts.

8 When you feel a tick, grasp it close to the skin at the mouthparts either using your fingers or forceps. Pull forcefully straight out to remove the tick.

9 Place the tick into the labeled tube. No more than 10 live ticks should be placed in a vial. All stages should be collected. Place a blade of grass in vial before capping. Close the tube to ensure that no ticks escape.

10 At the end of the three minute search period, close the drop-down bars and fold-out panels and verbally confirm with the chute operator that you are done.

Note

If you are having trouble closing something, make sure you let the operator know not to release the animal until all panels are closed and secured.

11 For each animal, indicate whether ticks were collected from that individual ('Yes'), whether the animal was checked, but no ticks were found ('No Ticks Present'), or whether that animal was not checked for ticks ('No') on the data collection sheet.

12 Repeat this process until at least 20% of the animals have been sampled. Ideally, all animals will be sampled, but occasionally this will not be possible due to time or personnel restraints.

13 Transfer collection tubes to a cooler on ice for transport back to the lab.

TICK STORAGE

14 If ticks **do not** need to be kept alive for research purposes:

14.1 Place collection tubes into a -20C freezer for at least five hours, and up to 24 hours to kill ticks.

14.2 Ticks should then be stored at -80C until they are identified to species and processed for TNA extraction. (Follow the REDI-NET T-3 Tick Storage.)

15 If ticks are to be kept alive for research purposes:

15.1 Place collection tubes into a refrigerator (4C). It is not recommended that ticks remain in the refrigerator for more than two days prior to processing. (Follow the REDI-NET T-3 Tick Storage.)

16 Proceed with sample testing following the REDI-NET SOP T-2 Tick Processing.