T-1 TICK FIELD SAMPLING V.1

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Protocol status: Working
We use this protocol and it's working

DISCLAIMER

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ABSTRACT

This protocol describes tick field sampling.

GUIDELINES

OBJECTIVE

To clearly document the correct process for effective standardized field collections of ticks, and recommended personal protection measures.

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 field tick collections in the vicinity of watering holes by dragging.

RESPONSIBLE PERSON

Principal Investigator, Study Coordinator, Entomology Component Lead, Managers

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Note

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

MAINTENANCE OF EQUIPMENT

BEFORE EACH COLLECTION

1. Clean forceps with 70%-ethanol.
2. Freeze and clean ice-packs.
3. Clean cool-boxes
4. Fully charge all equipment (e.g., GPS unit, tablets/phone). Make sure the tablet has enough free-space for field sampling pictures.

AFTER EACH COLLECTION

1. Clean all equipment thoroughly between sampling sites, including boots, cooler box (inside and outside), etc.
2. Tick drags should be sealed into trash bags for transport and cleaned *without detergents* (only use tap water and then air-dry) before the next collection.
3. Lint roller sheets should be disposed of in the waste bag after all ticks have been removed for testing.
4. Store sterile equipment separate from used equipment and samples.

QUALITY CONTROL

This SOP is reviewed by the applicable supervisor annually or as required in order to maintain its relevance.

MATERIALS

EQUIPMENT AND MATERIALS

Note

NOTE: If product number is listed, please ensure use of this or equivalent product.

	A	B	C
	Equipment / Material	**Description**	**Mfg / Product #**

	A	B	C
	Tick Drags	(~1m) made out of white brushed cotton denim, flannel, or utility cloth with dowel and rope attached; see Appendix 2 for detailed description and instructions	Locally Sourced
	Writing utensils	Pen / Pencils, Marker Pens	Locally Sourced
	Vials (2 options)	▪ Snap Cap: 2.5 Drams (10/16" x 1 7/16") ▪ O-Ring: 8 ml SC tube 16.5x57 FB PP O-Ring cap	▪ Snap Cap: UNSPSC: 41000000 ▪ O-Ring: Sarsedt Product# 60.542.007
	Fine-tip forceps	Stainless metal or plastic, for removing ticks from cloth	Carolina.com; # 624734
	Lint roller	For removing ticks from tick drags	Locally sourced
	Cooler and cool packs	For cooling ticks during transport	Locally Sourced
	Microcentrifuge tubes 1.5 ml, RNAse, pyrogenic free	For individual tick sorting	ThermoFisher, AM12450 equivalent
	Wet towels	For cooling ticks during transport	Locally Sourced
	Ziplock bags	For transporting vials containing ticks	Locally Sourced
	Roll of flagging tape	For transect flagging	Uline; S-6089FP
	Hand counters / tally	For tick counting	Amazon; AMZ011Counter
	GPS Unit	WGS 84 and precision of 5 decimal degrees	Locally Sourced
	Tick collection form	REDI-NET DCS T-1 Tick collection form	REDI-NET Data Portal
	Tablet	For data entry and picture of sampling site	Based on specs provided by CRC

Lint roller.

SAFETY WARNINGS

RISKS AND PERSONAL PROTECTION

BEFORE COLLECTION

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**. This creates a barrier to prevent ticks from contacting skin, and the light color increases the likelihood of finding a tick on clothing and removing it before it can attach and feed. **Be sure that clothing is sturdy and can withstand long thorns**.
3. Spray boots and pants with permethrin (i.e. Permanone®, Sawyer®). **This should be done prior to removing the drag from the collection kit, and ideally before arriving at the collection site**. Apply DEET to skin only. **DO NOT get insect repellant on the tick drag. DO NOT stand near the drag when applying bug spray – it will deter ticks and result in inaccurate estimates**.

AFTER COLLECTION

1. EVERY 100 meters (5 drags), **check yourself for ticks**. Any ticks collected from your clothing or body can be placed in a vial and counted in the transect .
2. EVERY time you complete a sampling site **check yourself for ticks**.
3. EVERY night **check yourself for ticks**. Especially where clothing is tight against skin, e.g., waistband, and your hair/head.
4. If a tick is found attached, remove using instructions in Appendix 2.

TICK TRANSECT SELECTION

1 Find two suitable sampling locations at least 10-20 m away from the water body representing different ecological systems, if possible.

SAMPLING TEAMS

2

Field sampling of iDNA (tick) 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 these 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.

TICK TRANSECT SELECTION

3 Determine two 20m x 20m grid blocks for tick collections. Grid blocks should not be overlapping a location sampled the previous month's collection trip. Each block-transect is composed of 5 ticks drags/passes.

4 The two grid-transects should be separated at least by 30 meters at the nearest points, when possible. Both transects should be in areas animals pass through to get to the watering hole.

TICK DRAGGING

5 Tick drags should not get very wet when sampling. Wait until the dew has burned off in the morning, and do not sample if it is raining.

6 For each transect, 5 tick-drags/passes should be performed; each tick-dragging being done in opposite directions (e.g., if the first drag of the first transect is oriented from North to South, the second drag should be performed from South to North, and so on).

7 Drag sampling should be performed by one person.

8 After each pass within the transect, turn the drag over and remove any ticks on the drag using tweezers to remove all ticks and place them in the appropriately labeled collection vial. No more than 10 live ticks should be placed in vial. All stages should be collected. Teams of two people can remove ticks from the drags (Figure 2).

Figure 2: Tick removal from the drag.

9 Place a blade of grass in the vial before capping (Figure 3).

Figure 3: Ticks in labeled vial after drag collection.

10 After collecting ticks from the drag from each pass, attention should be taken to remove any foreign object on the drags.

11 Place all labeled snap-cap vials from a completed transect into a Ziploc bag and into a cooler with wet towels in bottom with frozen water bottles, in order to slow them down.

12 Continue to the next transect and continue sampling ticks as in steps 1-10.

13 DO NOT mix ticks from different transects.

14 If needed, use a lint roller to remove debris, burrs or the remaining ticks prior to leaving the site.

15 The lint roller paper should be placed in a ziplock bag (labeled with a barcode) for transport back to the laboratory.

TICK STORAGE

16 Keep freshly collected ticks alive, keeping on a 4 °C cold chain (cooler) until return to the laboratory. Ticks are not recommended to be stored at 4 °C for long periods of time.

17 Once at the laboratory, if samples can not be processed on the same day of collection, all adult ticks (within field vials or on lint roller papers) must be individually sorted into appropriately labeled 1.5mL centrifuge tubes and should be stored at -80 °C. Immature ticks remaining on lint roller papers should also be stored at -80 °C until ready for testing. At -80 °C, ticks can be stored for 2 months or longer.

18 If -80 °C storage is unavailable, ticks should be stored at -20 °C until processing. Ticks can be stored at -20 °C for up to 2 months.

APPENDIX 1. TICK DRAG CONSTRUCTION -- TICK DRAG SUPPLIE..

19 One piece of white heavy-duty fabric with a soft nap, cut to approximately 1 square meter (39.5” x 39.5”). We used a waterproof white rubber sheeting (utility cloth) from JoAnn fabric and craft store (item #1491315), which is normally used to sew crib liners. White brushed bull denim could also be used (this should have a softer nap than regular bull denim).

20 Two smaller pieces of the same fabric cut to 39.5” x 4” and 39.5” x 3”. These will be sewn to the top and bottom of the flag to hold the dowel and the washers.

21 2 eye hook screws for each end of the dowel, ours are size #12.

22 3 large flat washers to weight the back of the flag, 1-3/4” diameter, 1/8” thick.

23 1 length of rope, 9 ft long.

24 1 48” dowel, ¾” in diameter.

25 Cotton thread and sewing machine for stitching flags together.

Figure 4: Ticks drag construction.

Figure 5: Details of the ticks drag construction.

APPENDIX 2. HOW TO REMOVE A TICK ATTACHED TO SKIN

26 Use fine-tipped forceps to grasp the tick as close to the skin's surface as possible.

27 Pull upward with steady, even pressure. Don't twist or jerk the tick; this can cause the mouth-parts to break off and remain in the skin. If this happens, remove the mouth-parts with forceps. If you are unable to remove the mouth easily with clean forceps, leave it alone and let the skin heal.

28 After removing the tick, thoroughly clean the bite area and your hands with rubbing alcohol, an iodine scrub, or soap and water.

29 Store the tick individually in ethanol and note the estimated duration of time the tick was attached. If desired, these ticks can be sent with those collected from the sampling areas for pathogen testing.

30 Seek medical attention if a rash or eschar appears around the site of attachment, and monitor for other symptoms including fever, fatigue, malaise, and any other abnormal symptoms. Be sure to inform the physician that you have been conducting tick research, and that a tick-borne disease is possibly responsible for symptoms.