TOS PROTOCOL AND PROCEDURE: TICK AND TICK-BORNE PATHOGEN SAMPLING

PREPARED BY	ORGANIZATION	DATE
Yuri Springer	FSU	12/05/2014

APPROVALS	ORGANIZATION	APPROVAL DATE
Mike Stewart	PSE	03/11/2015
Dave Tazik	SCI	01/12/2015

RELEASED BY	ORGANIZATION	RELEASE DATE
Judy Salazar	CM	03/17/2015

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by NEON, Inc. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	05/16/2011	ECO-00151	Draft protocol
A_DRAFT	10/03/2011	ECO-00280	Updated draft after 2011 field season
B_DRAFT	07/12/2012	ECO-00497	Updated draft for 2012 field season
C_DRAFT	01/10/2014	ECO-01139	Updated draft for 2013 field season
D	03/19/2014	ECO-01669	Production release, template change, and other changes as detailed in Appendix C (only in rev D)
E	December 2014	ECO-02321	Migration to new protocol template Contingent decisions updated to inform responses to site and plot level delays that are acute (FOPS-1228, FOPS-1582, FOPS-1629, FOPS-1241, FOPS-1171, FOPS-1018) and delays that may be more chronic and require consideration of dropping/replacing one or more sampling plots (FOPS-1568, FOPS-1365, FOPS-1224) SOP A: format for internal sample vial labels changed (locality label format no longer used) SOP B: Text added to clarify what was formerly the “>50% draggable” rule and more clearly define when to use dragging versus flagging (FOPS-1188, FOPS-1183, and FOPS-1170). This text also provides more explanation of the efficacy of dragging vs. flagging in tall grass or where understory vegetation prevents the cloth from touching the ground (in response to FOPS-1167, FOPS-838). The text further explains how to modify sampling when “difficult veg” (including water) is encountered along the sampling path (addresses FOPS-1227). The total distance that can be sampled as each plot has been modified

			accordingly, and new figures are included here. Ticks of all three life stages can now be stored and shipped in the same sample vial(s) (versus previously, larvae were separate from adults/nymphs). Text has been added to clarify how frequently larvae should be rinsed from the reusable lint rollers during sampling in a plot. Text was added in response to FOPS-1566 (when to use masking tape vs. sticky buddy methods to collect larval ticks) indicating that reusable lint rollers should be used to collect larval ticks unless NEON HQ science staff have approved use of masking tape method. VialID format has been modified slightly to create unique identifiers for each sample vial. In response to FOPS-1478, siteID has been added to the datasheet. SOP C: Text was added in response to FOPS-1566 (when to use masking tape vs. sticky buddy methods to collect larval ticks) indicating that reusable lint rollers should be used to collect larval ticks unless NEON HQ science staff have approved use of masking tape method. VialID format has been modified slightly to create unique identifiers for each sample (here, ticks on masking tape attached to cardboard cards) SOP D: Ticks of all three life stages can now be stored and shipped in the same sample vial(s) (versus previously, larvae were separate from adults/nymphs) (addressed FOPS-1574). Information on and format of lab on internal vial label has been changed (was locality label, now vialID) SOP E: format of shipping manifest has been adjusted with the addition of fields and changes to the name and format of some existing fields
F	03/17/2015	ECO-02564	Update of tick TOS protocol based on 2014 field experience and budget analysis. Details of the changes are located in the change record.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

Ticks transmit numerous pathogens of wildlife, livestock, and humans, including the etiological agent of Lyme disease (*Borrelia burgdorferi*), the most frequently reported vector-borne disease of humans in the United States. Among arthropod vectors, ticks are particularly sensitive to meteorological conditions and associated physiological constraints, making it highly likely that the demography and biogeography of many tick species, and the pathogens they transmit, will be affected by climate change.

Further, the multi-host lifecycles of most tick species increase their ecological connectivity and sensitivity to community-level perturbations that may arise from changes in human land- and resource-use practices. Based on these epidemiological and ecological characteristic ticks and tick-borne pathogens will be sampled within the National Ecological Observatory Network (NEON). The objectives of sampling are to quantify spatio-temporal changes in the abundance of ticks at NEON sites and in the prevalence of infection by associated tick-borne pathogens. Rationale for the sampling protocol provided in this document can be found in the NEON Science Design for Vectors and Pathogens (AD[06]).

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

N/A

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000911	NEON Science Design for Vectors and Pathogens
AD[07]	NEON.DOC.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001100	TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing
RD[06]	NEON.DOC.001583	Datasheets for TOS Protocol and Procedure: Tick and Tick-borne Pathogen Sampling
RD[07]	NEON.DOC.000793	Tick Drag Cloth Assembly Procedure

2.3 Acronyms

All acronyms used in this document are defined in RD[01].

2.4 Definitions

N/A

3 METHOD

Tick and tick-borne pathogen sampling involves the collection of ticks using drag and/or flag sampling. Following minimal in-house processing, samples will be sent to one or more external facilities where ticks will be identified to lowest taxonomic rank (preferably species). A subset of identified ticks will be tested to quantify the prevalence of infection by various prokaryotic pathogens. Some ticks will be set aside for long-term archiving.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[07]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[08]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Bouts of tick and tick-borne pathogen sampling will be conducted annually from March through December. At each site, sampling occurs at six distributed plots that are iteratively resampled for every sampling bout. Two sampling plans, each with a different sampling bout (aka event) frequency, are possible.

At each site, sampling begins with the **low intensity plan**, which involves one bout every six weeks. Collection of one or more ticks triggers a switch to the **high intensity plan**, which involves one bout every three weeks. Once high intensity sampling is initiated at a site it continues for the remainder of the season and all subsequent seasons irrespective of per-bout sampling success.

4.2 Criteria for Determining Onset and Cessation of Sampling

The first bout of tick and tick-borne sampling each year will occur as soon as the temperature thresholds are met. Sampling windows will be based around temperature thresholds for all sites (see Appendix D for estimated dates); at most northern temperate sites, the sampling window will be from March through December.Subsequent events will be scheduled according to the

fixed sampling frequency: every three weeks for high intensity sampling, every six weeks for low intensity sampling.

4.3 Timing for Laboratory Processing and Analysis

Tick samples held in vials containing 95% ethanol and stored at -20C (or 4C) will retain their integrity for DNA barcoding up to ten years following collection (Shokralla et al. 2010), however, the preference is to conduct laboratory processing within 3 months of collection in order to enable publication of the data on the portal prior to the following field season.

4.4 Sampling Timing Contingencies

For both the high and low intensity sampling plans, a bout of sampling will only be performed if the high temperature two days prior to planned sampling was >0°C and the mean high temperature in the five days prior to planned sampling was >7°C. Obtain this information from a publically-available source of meteorological data based on sensors located as close as possible to the sampling site. Eventually this information will be available as data streaming from the NEON tower.

Drag and/or flag sampling should only be conducted when conditions are dry (e.g., not during or immediately after a rain event or on a morning with heavy dew). Additionally, while sampling can be conducted during any time of day, the hottest period of the day (mid to late afternoon) should be avoided if possible.

When unexpected conditions require deviations from the field protocols outlined in this document, implementation guidelines outlined in the tables below should be followed.

General contingent decisions that apply to tick sampling include:

- Sampling must be conducted when the ground is dry. Do not sample if the ground is moist (e.g., heavy morning dew or following a rain event).
- If possible, avoid sampling during the hottest part of the day on days for which the high temperature is at or near the annual high temperature for the site.
- Sampling may be delayed in high wind conditions (in excess of 20 mph) where winds disrupt appropriate execution of tick sample protocols.

Table 1. Contingent decisions for high-intensity sampling

Delay/Situation	Action	Outcome for Data Products
Delay ≤ 2 days	If the delay occurs prior to the start of the sampling bout, and the issue(s) causing the delay affect all plots at the site, reattempt the complete bout at the conclusion of the delay. Submit a problem ticket. If the issue(s) causing the delay do not affect all plots at the site, submit a problem ticket in JIRA for guidance. If the delay occurs during the sampling bout, and the issue(s) causing the delay affect all plots at the site, resume and complete the bout at the conclusion of the delay. If the issue(s) causing the delay does not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and resume and complete sampling at the affected plots at the conclusion of the delay. In either case, note the duration and cause of the delay in the notes section of the datasheet. Do not push back dates for subsequent sampling events.	Increases potential for temporal variability/inconsistency in time series data.
2 days < delay ≤ 10 days	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affected all plots at the site, reattempt the complete bout at the conclusion of the delay. Submit a problem ticket. Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay do not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket in JIRA for guidance about sampling at affected plots. In either case, note the duration and cause of the delay in the notes section of the datasheet.	
Delay > 10 days	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affect all plots at the site, cancel the sampling bout and submit a problem ticket. Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay do not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket in JIRA for guidance about sampling at affected plots. In either case, note the duration and cause of the delay in the notes section of the datasheet.	

Table 2. Contingent decisions for low intensity sampling

Delay/Situation	Action	Outcome for Data Products
Delay ≤ 7 days	If the delay occurs prior to the start of the sampling bout, and the issue(s) causing the delay affect all plots at the site, reattempt the complete bout at the conclusion of the delay. Submit a problem ticket. If the issue(s) causing the delay do not affect all plots at the site, submit a problem ticket in JIRA for guidance. If the delay occurs during the sampling bout, and the issue(s) causing the delay affect all plots at the site, resume and complete the bout at the conclusion of the delay. If the issue(s) causing the delay do not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and resume and complete sampling at the affected plots at the conclusion of the delay. In either case, note the duration and cause of the delay in the notes section of the datasheet. Do not push back dates for subsequent sampling events.	Increases potential for temporal variability/inconsistency in time series data.
7 days < delay ≤ 21 days	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affected all plots at the site, reattempt the complete bout at the conclusion of the delay. Submit a problem ticket. Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay do not	

	affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket in JIRA for guidance about sampling at affected plots.	
Delay > 21 days	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affect all plots at the site, cancel the sampling bout and submit a problem ticket. Do not push back dates for subsequent sampling bouts. If the issue(s) causing the delay do not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket in JIRA for guidance about sampling at affected plots.	

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Field personnel are collecting biting arthropods, but there is no increased risk of infection by zoonotic pathogens during implementation of this protocol than in general fieldwork. We recommend that field personnel wear light-colored clothing when implementing this protocol to improve visibility of ticks on clothing prior and following sampling. Follow guidelines provided the Operations Field Safety and Security Plan (AD [02]) to prevent tick bites and take appropriate action if an embedded tick is found. Personnel working with ticks should familiarize themselves with the Zoonotic Diseases section of AD [02]. The incidence of these diseases in humans is extremely rare, with the exception of Lyme disease in certain regions of the country, and is typically associated with working outside in vegetated areas.

IMPORTANT: If used, insect repellent must be applied at least 30 minutes prior to arriving in the field. If applying insect repellent in spray form DO NOT apply in the vicinity of sampling equipment. After applying insect repellent, clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment. Both permethrin (0.5%) and DEET (up to 30%) are excellent repellents and can be used to treat field clothes well in advance of field sampling (two to four hours prior).

6 PERSONNEL RESOURCES

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc. Quantities specified represent ideal scenarios for a team of two conducting a sampling bout (sampling at 6 plots at a site). Staff may wish to bring extra equipment to account for contingencies.

Table 3 Preparation for field sampling

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Beverage cooler	Store/transport water to be used in larval tick collection	1	N
MX105087	R	Ice pack, 0° C	Pre-freeze for field preservation of samples	Variable	N
Consumable items					
	S	Adhesive label, ethanol-safe or label tape	Print vial labels	Variable	N
MX103942	R	All weather copy paper	Print datasheets	5	N
MX100213	S	Ethanol, 190 proof (95%)	Pre-fill sample vials	Variable	Y
	R	Water	Prepare cooler	Variable	N

R/S=Required/Suggested

Table 4. Equipment list – Field sampling a single bout, team of two

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	R	Beverage cooler with water	Store/transport water to be used in larval tick collection	1	N
	R	Cooler, 16qt	Chill perishable samples in field	1	N
	R	Forceps	Collect nymphal and adult ticks	2	N
	R	Funnel	Collect larval ticks from lint roller	2	N
MX100703	S	GPS receiver, recreational accuracy	Navigate to sampling location	1	N
MX105087	R	Ice pack, 0° C	Chill perishable samples in field	3	N
	R	Low lint hand towel	Dry reusable lint roller	1	N
MX104829	S	Magnifier hand lens, 2X/5X	Aid in tick identification	1	N
MX104369	S	Measuring tape, minimum 50 m	Measure deviations from the drag path	1	N
	R	Reusable lint roller (Sticky Buddy)	Collect larval ticks	1	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
323580000		Sinker weights for tick drag cloth assembly	Weigh drag cloth to maintain contact with ground	5	N
EB03180000	R	Tick drag cloth assembly	Collect ticks	2	N
MX100308	R	Unitary wash bottle	Store/transport water and ethanol used during collection of larval ticks	2	N
Consumable items					
MX100714	S	Alcohol wipe	Remove repellent residue	2	N
	S	Cardboard, cut to fit in resealable plastic bag	Contain ticks on masking tape, use only after attempting collection with lint roller and reporting problem with preferred method	12	N
MX102000	R	Duct tape	Remove larval ticks from clothing	1	N
MX100213	R	Ethanol, 190 proof (95%)	Rinse larval ticks	Variable	Y
MX100634	S	Label tape, ethanol-safe	Label sample vials	1	N
	S	Masking tape	Collect ticks from cloth, use only after attempting collection with lint roller and reporting problem with preferred method	1 roll	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100710	S	Mosquito repellent, 30-50% DEET	Protect personnel from insect bites	1	N
	R	Paper coffee filter	Collect larval ticks from lint roller	12	N
MX104422	R	Permanent marker, archival ethanol-safe	Label sample vials	1	N
	S	Permanent marker, fine tip	Label cardboard	2	N
MX100592	R	Resealable plastic bag, 1 gal, 4 mil	Contain larval ticks, organize tubes	6	N
	S	Rubberband	Organize sample tubes	Variable	N
	S	Survey marking flag, PVC or fiberglass stake	Delineate sampling area	4	N
MX103241 MX103242	R	Tube, 10 mL with cap	Prepare pre-filled sample vials	6	N
MX103241 MX103242	R	Tube, 10 mL with cap empty and prefilled with ethanol	Contain and preserve ticks	6	N
Resources					
RD[06]	R	Field datasheet	Record data		N

R/S=Required/Suggested

Table 5. Equipment list – Laboratory processing and analyses

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX104751	S	Artist paintbrush	Count and transfer larval ticks to tubes with adult and nymphal ticks	2	N
	S	Forceps	Count and transfer larval ticks to tubes with adult and nymphal ticks	2	N
	R	Unitary wash bottle	Rinse larval ticks	1	N
Consumable items					
	S	Copy paper, white	Aid in visibility of ticks	1	N
MX100213	R	Ethanol, 190 proof (95%)	Rinse larval ticks	Variable	Y
MX103249	R	Label paper, ethanol-safe	Label samples inside vial	1	N
MX100634	S	Label tape, ethanol safe	Label sample vials	1	N
MX100635	R	Liquid laundry detergent, fragrance free	Wash tick drag cloth	1	N
	S	Resealable plastic bag, 1 gal, 4 mil	Organize sample tubes	Variable	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	S	Rubberband	Organize sample tubes	Variable	N
MX103241 MX103242	R	Tube, 10 mL with cap with field samples	Contain ticks	12	N
Resources					
RD[06]	R	Completed field datasheet	Record data		N

R/S=Required/Suggested

Table 6. Equipment list – Shipping

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100358	R	Ice pack	Chill specimens during shipment	Variable	N
Consumable items					
	R	Cardboard box, UN packing group II	Package hazardous materials for shipment	Variable	N
	R	Class 3 flammable liquid label	Label shipments containing ethanol	1	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Limited quantity shipping label	Label shipments containing hazardous materials	1	N
	S	Plastic bag, 2 mil	Spill containment	2	N
	S	Plastic liner, 2 mil	Spill containment	1	N
	S	Styrofoam sheet		6	N
	R	Up arrow shipping label	Label shipments containing liquids	2	N
	R	Vermiculite, grade 2	Absorb liquid spills during shipment	Variable	N
Resources					
	R	Shipping manifest	Inventory of specimens being shipped	1	N

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

6.3 Specialized Skills

Prior experience collecting ticks or conducting entomological fieldwork is desirable but not required. Personnel should have good fine manual coordination for handling individual specimens.

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

An experienced two-person team will require approximately 30-120 minutes to complete sampling of ticks at a single plot. This entails dragging/flagging around the perimeter of the plot and transferring all adult and nymphal ticks into one or more sample vials, and transferring all larval ticks into coffee filters (filter paper).

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 At least one week prior to a sampling bout

1. Identify the locations of sampling plots and determine how to access them.
2. Prepare external sample vial labels on ethanol-safe adhesive labels. Each vial should be labeled with its vialID, the format of which is described in SOP B. Note that while labeling can also be done by writing directly on vial with an ethanol-safe marker, use of pre-printed labels is recommended.
3. Print out datasheet(s) on waterproof paper.
4. Be sure reusable ice packs (0°C) are frozen.

A.2 Just prior to heading to the field for sampling

1. Gather all necessary equipment for field sampling.
2. Fill sample vials with 95% ethanol.
3. Fill beverage cooler with de-ionized water for rinsing lint roller.
4. If used, insect repellent must be applied at least 30 minutes prior to arriving in the field. If using insect repellent in spray form, do not apply in the vicinity of sampling equipment. After applying insect repellent clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment.

Field Sampling

This SOP begins with a brief overview of the basic steps involved in two sampling methods that can be used to collect ticks. Following this are specific instructions on how to carry out sampling during a bout of tick and tick-borne pathogen sampling. Drag sampling is the preferred method used for tick collection. Flagging is used as a substitute for dragging when vegetation is too thick to allow the drag cloth to be pulled along the ground.

A.3 Overview of drag sampling

1. Place the drag cloth on the ground
2. One member of the two-person team should pull the cloth while the other walks behind the cloth.

    - The team member pulling the cloth must ensure that the pace of forward progress is slow and steady. Qualitatively, this pace is equivalent to a leisurely stroll (think wedding procession). Slowly counting “1 Mississippi” for each step forward is a good approximation of appropriate cadence. When measured on a grass soccer field it took ~50 seconds to drag 15m at the proper pace.
    - The team member pulling the cloth must also ensure that the entire cloth stays in contact with the ground or vegetation. When pulling the cloth, make sure there is enough pull cord between your person (the individual pulling the cloth) and the cloth so that the leading edge of the cloth stays as flat as possible on the ground. Too little pull cord between your person and the cloth will cause the leading edge of the drag cloth to rise up and not contact the ground.
    - Weights may be attached to the edges of the cloth as necessary if conditions are windy. Note that the weights are not intended to hold the cloth down in the absence of wind. Under calm conditions, the downward pull of gravity on the cloth is acceptable to keep the cloth in contact with the ground.
    - The team member walking behind the cloth must ensure that the cloth does not flip over, get bunched up, or become caught on plants or rocks while being pulled along the ground.

3. After dragging for 5-10m, stop to count and collect ticks.
    a. Inspect the drag cloth. Scan it in a systematic manner such that you examine the entire cloth on both upper and lower surfaces. Use a hand lens as necessary to distinguish larval and nymphal ticks from dirt.
    b. Inspect your person(s). Examine your body, especially areas around the lower legs and feet. This inspection may be more thorough if done reciprocally (i.e., each team member inspects the other).
    c. Remove any nymphal and adult ticks attached to the drag cloth or your persons using forceps (Figure 2), and place the ticks into a properly labeled vial containing 95% ethanol

(see A.6 for additional information on collecting and counting ticks). When handling a tick, use the forceps to grab it by a leg rather than by its body.

d. Remove larval ticks from both the drag cloth and your person with a reusable lint roller. You should strive to remove every larval tick from the cloth, but it may be difficult to remove every individual from your person(s). As such, spend only 5-10 second maximum per check removing larval ticks from your person(s). See instructions on how to handle larval ticks in sections A.6 andC.2.
    A. NOTE – One extra sample vial (hereafter the 'larval sample vial' should be available for larval ticks that become dislodged from collection equipment in the field, and that can easily be transferred to a sample vial. This vial will also be used to hold larval ticks that are later counted and processed in the lab.
e. Be mindful that ticks may attempt to crawl onto your hands, arms, or body while you inspect the drag cloth.

4. Place the drag cloth back on the ground and resume sampling, checking the cloth and your persons and counting/collecting ticks every 5-10m.
5. Continue this iterative process of sampling and collecting ticks until the designated sampling distance has been covered.

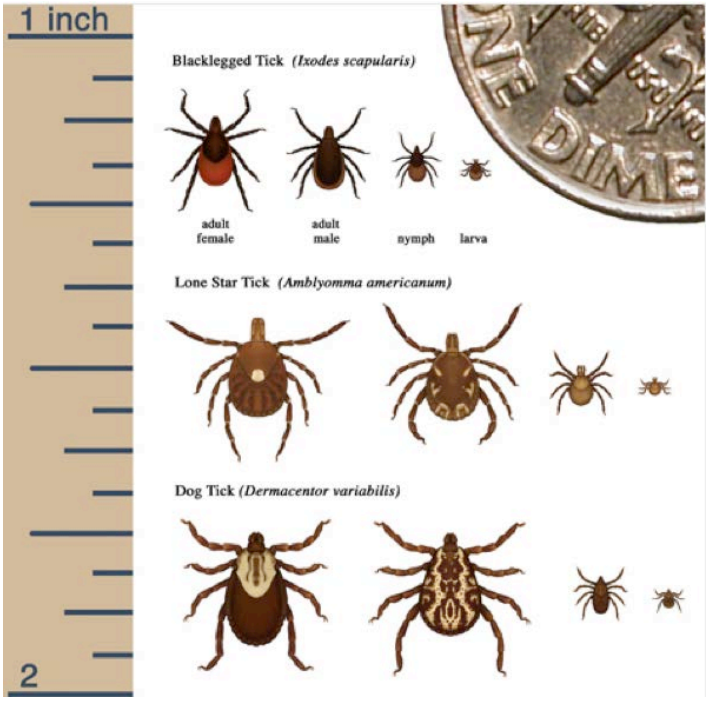


Figure 1. Relative sizes of different tick life stages (courtesy of the Centers for Disease Control and Prevention).

A.4 Overview of flag sampling

1. The flag used in flagging is a modified drag cloth. To make a flag, unclip the drag cloth pull cord and any attached weights from the drag cloth.
2. To sample, hold the drag cloth by one end of the wooden dowel. Gently "wave" the flag, guiding it over a sampled area. This movement and manner of holding the cloth allow greater precision to move it over/around/beneath vegetation.
3. While the cloth can be passed over and around vegetation, sampling the ground underneath vegetation will ensure that flagging is most comparable to dragging. To accomplish this, periodically crouch down and insert the flag underneath vegetation.
4. Do not attempt to flag spiny/thorny vegetation (e.g., brambles, cacti) as this will damage the cloth. Instead, drag underneath this vegetation if you can avoid catching the cloth on spines/thorns. Alternatively, if the vegetation is low growing and you cannot get the cloth underneath, consider the vegetation an obstacle and either drag around it or stop sampling, walk around the obstacle, return to the drag path on the other side of the obstacle, and continue sampling there.
5. Periodically remove ticks from the cloth and your persons as described for dragging. This should be done with greater frequency than with dragging as sampling in dense vegetation is more likely to dislodge ticks attached to the cloth. It is recommended that you check the cloth every 3-4 sweeps, which should be the equivalent of sampling 3-5m^2.
6. Note that when flagging, especially underneath vegetation, the cloth will generally not remain flat and completely in contact with the sampled surface over its entire area (i.e., it will get wrinkled). This will require estimating the total distance sampled with less precision that when dragging.

A.5 Plot validation/acceptance, and when to use drag sampling versus flagging

1. Drag sampling is the preferred sampling method since it allows the area sampled to be more accurately quantified. This is important for estimating tick density. As such, attempt to sample using the drag method whenever possible.
2. During sampling, it is important to try and keep the cloth in direct physical contact with the ground or overlying leaf litter. When dragging, attempt to make a qualitative assessment of whether the cloth is on or close to the ground/leaf litter: is it touching the ground most or all of the time, is it "surfing" up 2-3 inches above the ground as it passes over flexible-stem grasses/forbes, or is it "stilting" 4 or more inches above the ground as it passes over rigid-stemmed shrubs? The first scenario is ideal for dragging, the second is acceptable for dragging, and the third scenario is one in which flagging should be used to keep the cloth closer to the ground. In particular, flagging is an effective means of getting the sampling cloth underneath shrubs and taller/more dense vegetation.

3. Tick sampling should only be conducted in plots in which you are able to sample for ticks (using dragging, flagging, or a combination of both) over ≥50% of the plot perimeter. Problems arise when woody vegetation is so tall (i.e., >3-4ft) and/or dense that a drag cloth cannot easily be pulled over or around the base of plants and flagging in between plants becomes exceedingly time consuming (i.e., >120min). This "difficult vegetation" also includes standing/flowing water, flooded terrain or wet vegetation as neither is amenable to sampling by dragging or flagging.
4. If difficult vegetation is present over the entire perimeter of the plot, then the plot should be rejected. If difficult vegetation, large rocks, and/or water is patchy over the perimeter of the plot and present over <50% of the total plot perimeter, then it is acceptable to conduct tick sampling (using dragging, flagging, or some combination of the two) over the portion(s) of the perimeter that are amenable to sampling. Always record the total horizontal distance covered during sampling in a plot. Here are some examples of scenarios that may be encountered:
    - Low stature grass or herbs, or leaf litter: you can sample using dragging or flagging, but the former is preferred since it allows for more accurate quantification of total distance covered during sampling.
    - Medium to tall grass or herbs, vegetation supple (not woody/rigid): the cloth might not be in physical contact with the ground, but it can be easily pulled (dragging) or waved/passed (flagging) over the top of the vegetation. Because the vegetation is supple, the weight of the cloth will allow it to be pulled down into the vegetation and closer to the ground by gravity. This can be further accentuated with flagging as the cloth can be pushing down into the vegetation by holding the dowel lower to the ground. In this scenario, you can sample by dragging or flagging, but the latter may be preferred when the vegetation is tall because the cloth can be pushed down to a greater degree than by gravity alone.
    - Medium to tall shrubs, vegetation woody (non-supple) and patchy: if the vegetation (e.g., woody shrubs) is present at low density such that the drag cloth can be pulled between plants, then use dragging to sample the ground underneath the shrubs. If vegetation density is higher and the drag cloth cannot be pulled between plants, use flagging to sample this interstitial area. If the vegetation is not tall (i.e., ≤3ft) you can additionally sample the sides and tops of shrubs using flagging.
    - Tall woody (non-supple) vegetation: As with low/medium stature woody vegetation, drag or flag the ground between plants if density is low enough to allow space. Assuming plants are >4ft tall, stick to sampling the ground between and underneath plants (i.e., do not sample trunks or woody stems).

5. When difficult vegetation, large rocks, or water is patchy in space: Often there will be one or more patches of difficult vegetation along the perimeter of a plot. If a plot has been accepted for sampling, there are essentially two options when a patch of dense vegetation that cannot be sampled is encountered along the plot perimeter. First, you can divert the sampling path around the patch of difficult vegetation provided that this diversion is not too lengthy (>20 m). Divert

the sampling path into the plot and follow the shortest path possible to get around the patch of difficult vegetation. Continue sampling along this diversion path. Issue a problem ticket in JIRA if the total diversion distance in a plot is >20m. Second, if the patch of difficult vegetation, large rocks, or wateris too large to divert around you can sample up to the patch of difficult vegetation, then stop sampling, make your way through or over the patch, and begin sampling again on the other side. For example, if a narrow creek runs down the middle of the plot, you can simply step over or cross the creek where it bisects the plot perimeter. Both options are equally acceptable, but efforts should be made by the technician to choose the alternative path with the least amount of detour from the original perimeter.

6. When difficult vegetation is patchy in time: In some cases a given plot may be acceptable for sampling for a proportion of the sampling season and unacceptable for the remainder of the season. For example, large portions of a plot perimeter may be wet early in the sampling season but dry out later. Alternatively, large portions of the plot perimeter may be associated with supple, low growing vegetation early in the sampling season that becomes difficult vegetation (e.g., tall/dense/woody) later in the season. You will need to use local knowledge to estimate these proportions, and one or two field seasons may be required to quantify them with confidence for questionable plots. Over the long term, each accepted plot needs to be amenable to sampling on $\geq$50% of the planned sampling dates.
7. Plot versus vegetation type: When you are evaluating a particular plot for acceptance/rejection, and you encounter difficult vegetation that makes you lean towards the rejection, consider whether the vegetation conditions upon which this decision is based are unique to this plot or are typical of plots within this vegetation type. If the former is likely, then rejecting the plot and evaluating alternative plots in the same vegetation type is advisable. Alternatively, if all of the plots in the vegetation type are likely to be characterized by these features (e.g., all of the woody wetland plots are too wet, or the plant density in all of the shrub plots is too high), then issue a problem ticket in JIRA. We may consider dropping plots in this vegetation type and reallocating them to one or more other vegetation types at the site.

A.6 Tick Collection in the Field

1. Use maps and/or a handheld GPS as necessary to navigate to one corner of the plot. Be sure not to transit through any portion of the plot, especially the plot perimeter.
2. After arriving at the plot corner and before you begin sampling, perform an inspection of your and your partner's person. Remove any attached nymphal and adult ticks and place them into an empty sample vial labeled "release" for release following completion of the sampling. Remove any larval ticks using duct tape.
3. Determine whether to use drag or flag sampling at the plot (see B.3).

4. During sampling, you must travel a fixed path that follows the shortest straight-line distance between plot corners and thus describing the full perimeter of the plot (Figure 3). You can sample in either a clockwise or counterclockwise direction.
5. If a large obstacle (e.g., rock, tree, cluster of shrubs) is present along the path, sample around the obstacle by diverting the sampling path into the plot. Use the shortest distance possible to get around the obstacle. If the total increase in the length of the drag path in a plot caused by such diversions exceeds 20m, submit a problem ticket in JIRA. Keep track of the distance so that it can be recorded on the datasheet as 80-180m with a target accuracy of +/-2m.

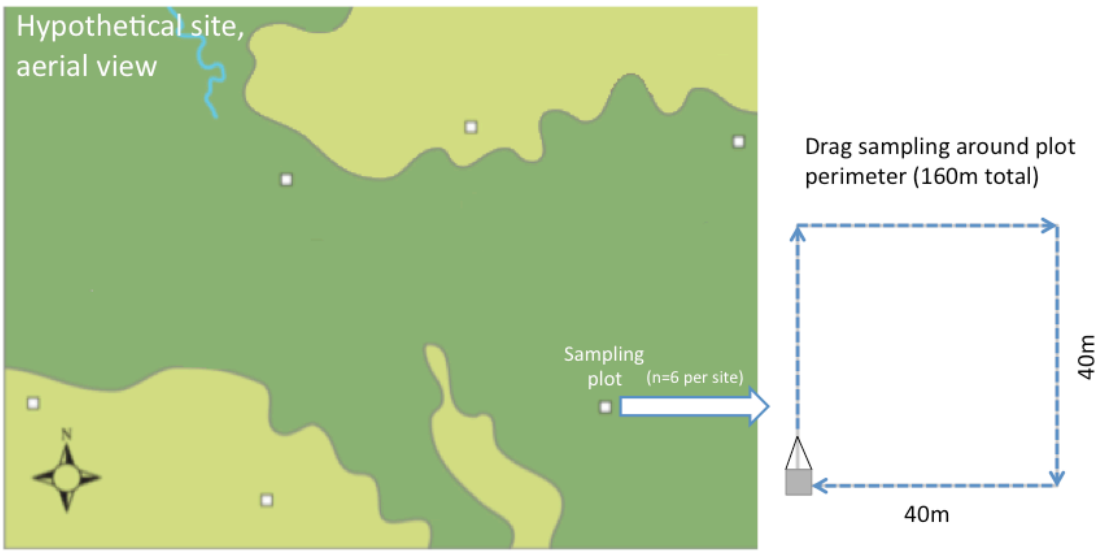


Figure 2. Schematic of high- and low-intensity sampling

6. Begin drag or flag sampling. Stop every 5-10m to examine the drag or flag cloth and your persons for ticks, and place collected ticks into a properly labeled vial containing 95% ethanol. You may want to check more frequently when dragging/flagging over dense vegetation that could dislodge ticks from the cloth.

    a. Count and collect all nymphal and adult ticks
        i. Collect nymphal and adult ticks using forceps and transfer them into a sample vial containing 95% ethanol. All adult and nymphal ticks collected during a sampling plot/bout combination can be placed together into the same sample vial. Additional sample vials can be used if a single vial cannot hold all of the ticks collected during a sampling plot/bout combination.
        ii. Record the number adult and nymphal ticks in each sample vial, on the Datasheet for Field and Lab Protocol: Tick and Tick-borne Pathogen Sampling (RD[06]). It is preferred that these counts of are recorded in the field datasheet during the sampling event, in

the field. If time does not allow for this, however, counts of adult and nymphal ticks per vial may be done in the lab.

b. Collect larval ticks

i. Gently roll a reusable lint roller over larval ticks on the drag/flag cloth and/or your persons.

ii. Remove larval ticks from the reusable lint roller; hold the roller over a double paper coffee filter (one filter nested within another) set within a funnel, and rinse the roller with de-ionized water from a wash bottle. This should wash larval ticks off of the roller and down into the coffee filter.

A. Note that you only need to rinse larval ticks off of the lint roller once, at the conclusion of sampling within a plot. It is not necessary to rinse the lint roller after each time that it is used to remove larval ticks from the cloth during sampling in a plot.

B. If nymphal and/or adult ticks are accidentally collected with the lint roller you can either remove these individuals from the roller using forceps and place them in an adult/nymph sample vial or wash them into the filter paper with the larvae and transfer them to an adult/nymph sample vial when larvae are counted in the lab (be sure to record counts of these adults and nymphs on the datasheet).

iii. Once larval ticks are rinsed into the filter, spray the larvae with 95% ethanol from a wash bottle to kill them.

iv. Fold the doubled coffee filters flat and roll/fold the upper lip down to seal in the larvae. Roll the filters so that larvae not killed by the ethanol spray cannot crawl out, but be mindful not to crush the specimens. If possible, wash all of the samples to the bottom of the conical filter and fold near the top of the filter. This will reduce the chance that specimens will be crushed in creases when the filter paper is folded.

v. Place the doubled coffee filters into a resealable plastic bag.

vi. Larval ticks can be counted upon return to the lab.

vii. Dry the reusable lint roller with a hand towel to reuse.

A. Note: Collection of larval ticks using reusable lint rollers is the preferred method and should be used whenever possible. Issue a problem ticket in JIRA if local environmental conditions make use of this method difficult or impossible. Do not collect larval ticks using SOP C without first obtaining permission from relevant science staff at NEON headquarters.

h. While larval ticks should be removed from the drag or flag cloth and your persons, most attention should be focused on the cloth. Do not spend more than 10-20 seconds examining your persons for larval ticks as they will likely be difficult to see on clothing.

7. Once all ticks have been collected from cloth, continue sampling along the perimeter of the plot. Stop again at 5-10m intervals to examine the drag or flag cloth and your persons and collect ticks. Place any nymphal and adult ticks into the same sample vial(s) used above. Wash

any larval ticks into the same doubled coffee filter used above, or use a new doubled filter as necessary or if you think specimens will be better preserved or captured through the use of a new filter. Store all doubled filters in the same resealable plastic bag.

8. Sample along the perimeter of the plot until you return to the corner of the plot where you began sampling. In total you should have covered 80-180m (target is 160m, essentially four 40m transects). If more than 180m was covered due to diversions around obstructions in the drag path, note this on the datasheet in the notes section and submit a problem ticket in JIRA.
9. Label samples. The external label format (vialID) consists of the sampleID and a vial number (the two-digit number of the vial relative to the total number of vials containing ticks collected during a single site/plot/bout combination, 01 or higher) (**Figure 3**). The sampleID includes the plotID, the date (YYYYMMD) and the bout number. As an example, the vialID "OSBS_002.20130802.3.02" would indicate that the labeled vial is the second vial containing ticks collected in plot 002 at Ordway Swisher Biological Station on August 2, 2013 (the third sampling bout of the year at that site).
   a.

Figure 3. Structure of vialID

10. Place all labeled sample vials generated during sampling in a plot inside the resealable plastic bag containing larval ticks collected in that plot.
11. Place the plastic bag into an insulated cooler containing frozen ice packs for transit back to the lab.
12. Fill in the field datasheet in RD[06], with one record for every vial of ticks collected. Be sure to also record any notes regarding unusual field conditions that may have affected sampling results. (e.g., cows walking through plot during sampling).
13. As you depart from the plot release ticks in the vial labeled "release" at least three meters outside the perimeter of the plot.
14. Larval ticks will be counted in the lab, and these counts recorded in the larvalprocessing datasheet of RD[06] (see SOP C for additional information).

A.7 Sample preservation

Upon returning to the lab, immediately transfer plastic bags containing ticks into a refrigerator (4°C) until specimens can be processed. When storing samples, take steps to keep samples from the same site/bout combination together (e.g., using a rubber band and/or placing within a resealable bag).

A.8 Refreshing the sampling kit

1. Print out new sample labels and datasheets as necessary.
2. Obtain new supplies of consumable equipment (e.g., sample vials, ethanol)

A.9 Equipment maintenance, cleaning, and storage

1. Place the drag cloth into an ultralow freezer at the lab to kill any larval ticks attached to the cloth. The duration of exposure to kill ticks will likely vary by species but 30 minutes should be sufficient.
2. If the drag cloth is dirty, wash it using fragrance-free laundry detergent and hang it to dry. If a laundry drier is used select a medium heat setting to prevent the drag cloth from shrinking. Always make sure the drag cloth is completely dry and in good condition (i.e., same size as at the beginning of the season, free of holes) before placing in storage.
3. Clean any other equipment as necessary using dilute fragrance-free laundry detergent, dry and store in a cool, dry place.

SOP B Collection of Larval Ticks Using Masking Tape

This SOP can be implemented if collection of larval ticks using the reusable lint roller method described in SOP B Field Sampling does not work effectively. Note that collection of larval ticks using reusable lint rollers is the preferred method and should be used whenever possible. Issue a problem ticket if local environmental conditions make use of this method difficult or impossible. Do not use this SOP without first obtaining permission from relevant science staff at NEON HQ.

B.1 Before going to the field

Prepare cardboard cards by cutting them to a size that is as large as possible to still fit within 1 gal. resealable plastic bags.

B.2 Overview of sampling

1. Collect larval ticks using masking tape:
2. While larval ticks should be removed from the drag or flag cloth and your persons, most attention should be focused on the cloth. Do not spend more than 10-20 seconds examining your persons for larval ticks as they will likely be difficult to see on clothing.
3. Remove a strip of tape with length that does not exceed the width of the cardboard cards.
4. Touch the tape to the drag cloth to remove larval ticks. Do not collect any larval ticks on either of the two ends (1-2 inches) of the strip.
5. When the strip of tape begins to lose its adhesive properties or has many attached ticks, attach it to a cardboard card. Fold the two ends of the strip (which should still be sticky) under so that they touch/stick to the cardboard card. The non-adhesive side of the tape should be facing away from the card, and the ticks should be sandwiched between the tape and cardboard (Figure 5). Multiple strips of tape can be attached to a single card, but each card should only be associated with larval ticks from a single sampling plot for a particular bout.

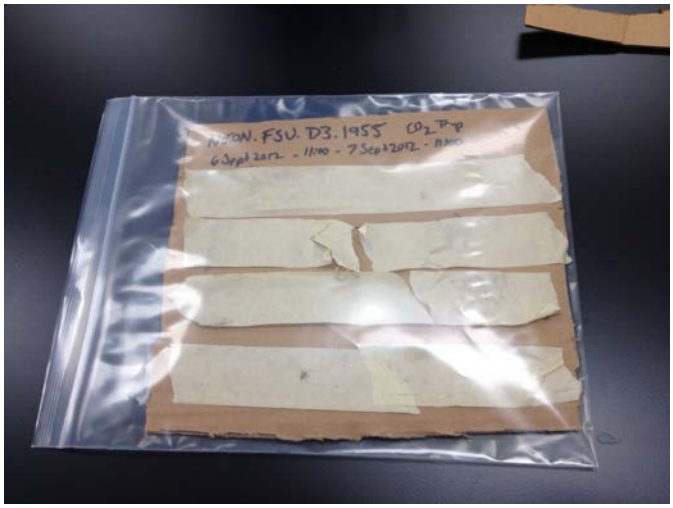


Figure 4. Cardboard card with larval ticks attached to masking tape

6. Use a permanent marker to label the cardboard card(s). Use the same vialID format as for sample vials containing adult and nymphal ticks and count each cardboard card as the equivalent of a vial (see Fig. 4). In the notes section on the datasheet record the number of vials and cards generated during sampling for that bout in each plot.

7. Place each labeled cardboard card with attached larval ticks into a resealable plastic bag.
8. Place the sealed resealable plastic bag(s) into an insulated cooler containing frozen reusable ice packs for transit back to the lab.

B.3 Sample processing in the lab

1. Count larval ticks attached to masking tape and record on the datasheet. Do not attempt to remove larval ticks from the tape.
2. Send resealable plastic bags containing cardboard cards with attached larval ticks to the external ID facility.

SOP C Laboratory Processing and Analyses

C.1 Preparation

1. Clear space on a lab bench where tick samples can be sorted. It may be helpful to cover the benchtop space where you are working with white paper so that any ticks that may be accidentally lost during sample processing and transfer can be easily located.
2. Gather all necessary equipment for laboratory processing and analyses.

C.2 Sample processing in the lab

1. Remove a resealable bag from the refrigerator and place it on the lab bench.
2. Open the bag and remove any sample vials.
3. Apply 95% ethanol from a wash bottle to the doubled coffee filter(s) as necessary to kill any surviving larval ticks.
4. Count larval ticks enclosed in filter paper and transfer them into the larval sample vial created in step A.3. If using forceps, handle larvae gently and try to avoid crushing them during transfer. A paintbrush may be more suitable.
5. Record the larval sample vial ID in the 'sourceVialID' field of the larvalprocessing datasheet, and record the number of larvae in the vial (including those transferred there after being rinsed from the filter as well as any that were placed into the vial in the field).
    a. NOTE - *If* there are too many larvae on the filter to fit into the larval sample vial created in step A.3, create a new record in the larvalprocessing datasheet. Record the larval sample vial ID (from the field) in the 'sourceVialID' field (this helps with sample tracking), the ID of the newly created vial in the 'vialID' field, and the number of larval ticks in the new vial in the 'Larvae count' field. Be sure that you don't repeat a pre-existing vialID (e.g., if OSBS_002.20130802.3.02 was created in the field, this vialID may not be re-used)
6. Make sure all vials are properly labeled. Labels should be printed on ethanol safe paper using ink that will not run when exposed to ethanol. See SOP B for label format instructions.
7. Fill in any remaining blank fields on the datasheet.

C.3 Sample preservation

1. After counting and transferring larvae into one or more sample vials, transfer all sample vials containing ticks into a freezer (-20°C) or refrigerator (4°C, if a freezer is not available).
2. To facilitate sample sorting and tracking it is recommended that all sample vials associated with a single sampling bout/site combination be grouped (e.g., bind them together with a rubber band or place them in a resealable plastic bag.

C.4 Equipment maintenance, cleaning, and storage

1. Clean all laboratory equipment following use as necessary according to the manufacturer's instructions.
2. Store all laboratory equipment in a cool, dry location when not in use.

SOP D Data Entry and Verification

Field data collected on paper datasheets should be digitally transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

1. Scan datasheets and save in PDF file format (file location TBD as of Rev E of this document)
2. Save paper copy of datasheets (location TBD as of Rev E of this document)

SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site (https://neoninc.sharepoint.com/sites/cla/SitePages/Home.aspx) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

E.1 Handling Hazardous Material

Ethanol is a Class 3 regulated material, and must be shipped according to CFR 49 Subchapter C, Hazardous Materials Regulations. Ethanol must be packaged using UN packing group II compliant materials. The maximum amount of ethanol in ground shipments must be less than 1L in each inner container and 30 kg in entire package (limited quantity exception). Refer to Chemical Hygiene Plan and Biosafety Manual (AD[03]) for additional requirements on commercial shipment of hazardous or dangerous materials.

E.2 Supplies/Containers

Use corrugated cardboard boxes which meet UN packing group II requirements. Add Styrofoam along the walls of the box as insulation. Freeze the ice packs and precool the shipping container, if possible.

Double-bag the tubes containing samples using minimum 2-mil watertight plastic bags and line the inside of the shipping container with a minimum 2-mil plastic liner and absorbent material.

Arrange products inside the insulated container, allowing space for ice packs. Place a sufficient number of ice packs on top of and around the samples. Fill all void space with grade 2 vermiculite to absorb any spills and prevent movement. Close the liner bag securely.

Complete packaging and labeling for liquid Limited Quantity Hazardous Materials shipment.

E.3 Conditions

Samples should be stored in vials containing 95% ethanol. Sample vials should ideally be stored at -20°C (4°C acceptable) until shipped to an external facility. Samples should be shipped 1-day freight with dry ice to maintain temperatures below 4° C during shipping. Avoid shipment on days that will require transit on a weekend or over a holiday period.

E.4 Grouping/Splitting Samples

All samples collected during each bout must be shipped together. Sample vials containing samples collected as part of the same bout can be taped or rubber-banded together, or placed in a separate bag, to allow them to be easily inventoried/sorted at the external facility.

E.5 Return of Materials or Containers

Be sure to include instructions to external facilities on how to return reusable materials (e.g., ice packs). CLA can provide details.

E.6 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping manifest AND a corresponding electronic version of the manifest (excel file) emailed to the taxonomic ID facility. The shipping manifest is the Tick_shipping_datasheet tab of RD[06]. Place the hard copy shipping manifest in resealable plastic bag on top of Styrofoam, and send electronic copy to the CLA contact **and** the receiving laboratory.

The hard-copy manifest (Figure 5) lists every sample vial in the identifier field (for which the entry will be vialID).. The other required information on the shipping manifest, sentTo (name of the facility to which the specimens are being sent), sentDate, and recordedBy, must only be entered once per shipping manifest.

NEON Tick and Tick -borne Pathogen: Shipping

sentTo: Taxonomic ID Facility A	sentDate: 10/02/2013	recordedBy: nrobinson@neoninc.org
identifier *(vialID - for vials of ticks)*	remarks	
HARV_001.20130802.1.01		
HARV_001.20130802.1.02		
HARV_001.20130919.2.01		
HARV_001.20130919.2.02		
HARV_001.20130919.2.03		

Figure 5. Example shipping manifest for the taxonomic ID facility

The electronic manifest should be emailed to the taxonomic ID facility as soon as possible after a batch of samples has been shipped.

E.7 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

Shokralla, S., G. A. C. Singer, and M. Hajibabaei. 2010. Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. BioTechniques 48:233–234.

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 7. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001583	Datasheets for TOS Protocol and Procedure: Tick and Tick-Borne Pathogen Sampling

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

Quick Reference: Collecting Tick Specimens

STEP 1 – Check yourself for ticks and place any ticks in "release" sample vial.

STEP 2 – Start sampling at one corner of the plot.

WALK SLOWLY!

STEP 3 – Walk with drag cloth for 5-10m.

STEP 4 – Stop and inspect drag cloth. Collect and count adult and nymphal ticks and store in vial with 95% ethanol

STEP 5 – Collect larval ticks using a reusable lint roller. Store inside coffee filters in resealable freezer bags.

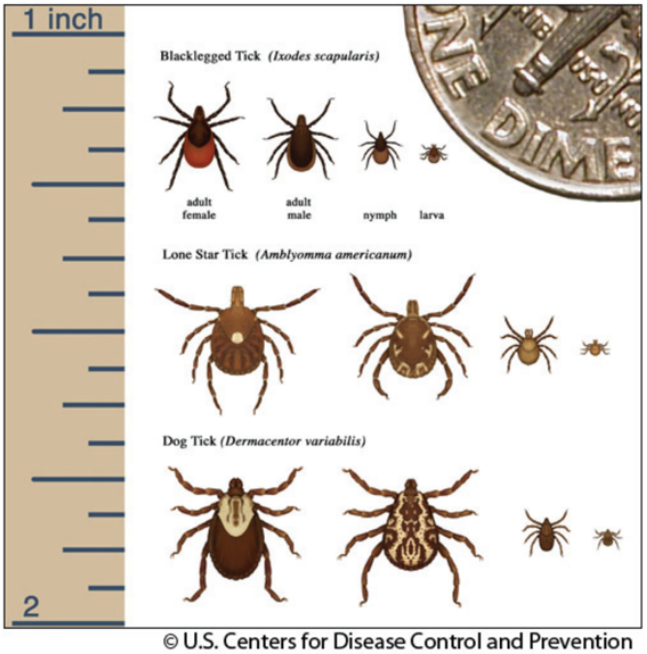
STEP 6 – Repeat 5-10 m drag and collect cycle until you have sampled the entire perimeter of the plot (i.e., returned to the plot corner where you began your sampling).

STEP 7 – Label specimen vial.

STEP 8 – Place tick specimens in cooler with ice packs.

STEP 9 – Release ticks in vial labeled "release" once 3 m outside plot boundary.

Tick Life Stages



© U.S. Centers for Disease Control and Prevention

APPENDIX C REMINDERS

Getting Ready for Sampling

Equipment: Be sure to…

- ☑ Inspect drag cloth for tears and ticks.
- ☑ Check that binder clips are attached to dowel.
- ☑ Print Tick and Tick-Borne Pathogen Sampling Data Sheet.
- ☑ Upload sample coordinates to GPS and obtain maps.
- ☑ Bring all supplies and extras.
- ☑ Check your pace. Can you accurately pace 5-10 m?

Personal safety: Protect yourself by…

- ☑ Wearing appropriate clothing.
- ☑ Tucking pant legs into socks.
- ☑ Using tape to seal gaps.
- ☑ Applying insect repellent ½ hour before going into field and away from sampling equipment.

You are collecting live ticks.

If you use insect repellent, apply it at least 30 minutes PRIOR to heading to field site.

Wash hands thoroughly with soap and water after applying insect repellent to avoid transferring repellent to sampling equipment.

Collecting Quality Tick Data

Dragging: Remember to…

- ☑ Check yourself for ticks BEFORE you start dragging.
- ☑ Sample only under dry conditions.
- ☑ Keep drag cloth relatively flat on ground.
- ☑ SLOW DOWN! Your pace is probably too fast.
- ☑ Remain on a path that traces the shortest (straight line) distance between plot corners.
- ☑ Include ticks attached to your clothes (from the waist down) in your count/specimen vial.
- ☑ Label vials and store in cooler with ice packs.

Before leaving drag site: Check that…

- ☑ Data Sheet is complete.
- ☑ All ticks have been removed from drag cloth and your person(s).
- ☑ Drag cloth is stowed in plastic bag for transport to next site.

When you are 3 m outside plot perimeter: Remember to…

- ☑ Release ticks from the "release" vial.

At the end of the day: Limit your exposure to ticks by…

- ☑ Putting your field clothes and the drag cloth in a dryer to kill ticks or, if not possible stowing them in a plastic bag to contain movement of ticks.
- ☑ Check yourself for ticks.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are based on historic records and are estimates for the start and stop dates of sampling. It is essential that domain staff monitor real-time conditions to determine when to start and stop sampling, as described in Section 4 of this protocol. This information will be updated as new field data is available

..

Table 8. Estimated sampling dates based on historical temperature thresholds

Domain	Site	Approx. Start Date	Approx. End Date
01	HARV	March 15	December 5
	BART	March 20	November 19
02	SCBI	March 1	December 25
03	OSBS	March 1	December 31
	DSNY	March 1	December 31
	JERC	March 1	December 31
05	UNDE	April 4	November 3
07	ORNL	March 1	December 31
08	TALL	March 1	December 31
09	WOOD	March 31	November 4
10	CPER	March 1	December 6
10	STER	March 1	December 6
15	ONAQ	March 1	November 27

APPENDIX E SITE-SPECIFIC INFORMATION

N/A