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

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See configuration management system for approval history.

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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	10/01/2014	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.
G	1/29/2016	ECO-02905	Effective starting 2016 field season: Larval collection uses tape only (NEON-247). Changed storage preservative from 95% ethanol to RNA stabilization solution (NEON-350). Ticks found outside the plot may be discarded instead of released. Low-intensity sampling frequency resumes after a year of no ticks (NEON-354). Added instructions to maintain a narrow sampling path (NEON-554). Removed bout from sampleID format. Internal labels should be printed on all-weather copy paper and inserted inside vials. Reduced sizes of collection tubes to 1.5-2 mL (existing supplies may be used until depleted). Distilled redundant information, restructured for clarity.

H	02/17/2017	ECO-04421	Updated template. Effective starting 2017 field season: MODIS-based sampling windows, edited site-specific sampling schedules, added alternate plot establishment protocol (NEON-1514), changed suggested tube numbers (NEON-1744), changed shipping materials to wet ice instead of dry (NEON-2768), specified inclusion of 'blank' preservative in shipment (NEON-1617), clarified instructions in case of delay when plot is partially sampled (NEON-1848). Replaced vialID with sampleID and number of vials. Added language to accommodate mobile data applications. Added plot reallocation section and moved instructions from SOP a into this section. Reformatted SOPs for enhanced readability. Added new guidelines for shipping inventories.
J	02/20/2018	ECO-05256	Adding barcode specific language to the protocol; adjusting date range to a window (NEON-5942)
K	03/18/2019	ECO-05962	• Update Appendix D based on most recent available MODIS and precip data • Update language about switch to low-intensity sampling (>5 ticks captured in the last 365 days, vs. 1 or more ticks captured in the last 365 days) • Remove requirement to count ticks (this will be done by the taxonomist) • Remove requirement to ship ticks on ice packs

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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 (e.g. Eisen et al. 2016, Ogden et al. 2018), 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 characteristics, 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 presence 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[05]).

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]).

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.000727	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000911	NEON Science Design for Vectors and Pathogens
AD[06]	NEON.DOC.004104	NEON Science Data Quality 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.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001583	Datasheets for TOS Protocol and Procedure: Tick and Tick-borne Pathogen Sampling
RD[06]	NEON.DOC.000793	Tick Drag Cloth Assembly Procedure
RD[07]	Available via download of data from NEON portal	NEON Raw Data Ingest Workbook for TOS Tick Abundance, Diversity, and Pathogen-status

2.3 Acronyms

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

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 are sent to one or more external facilities where ticks are enumerated and identified to the lowest taxonomic rank possible (preferably species). A subset of identified ticks are tested to quantify the prevalence of infection by various pathogens. Some ticks are 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.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

At each site, a bout of sampling occurs at six distributed plots. Sampling frequency varies according to whether or not ticks have been detected. At each site, sampling begins with the **low intensity schedule**, which involves one bout every six weeks. Collection of more than five ticks within the last year (i.e., 365 calendar days) of any tick life stage triggers a switch to the **high intensity schedule**, which involves one bout every three weeks. The tick detection datasheet (NEON.DOC.001583) provides a mechanism for sites sampling on the low intensity schedule to assess whether enough ticks have been collected to trigger high intensity collection.

Once high intensity sampling is initiated at a site, it continues for the remainder of the season and all subsequent seasons for as long as ticks are collected at the site. However, if a site is on a high-intensity sampling schedule and no ticks of any life stage are sampled on any plot for a full calendar year, sampling at the site reverts to the low-intensity schedule. High intensity sampling will resume when more than 5 ticks are counted within a year at the site.

4.2 Criteria for Determining Onset and Cessation of Sampling

Bouts of tick and tick-borne pathogen sampling are conducted annually within the site-specific sampling window (Appendix D). The start and end of sampling each season should coincide with key phenological milestones, with sampling beginning within two weeks of the onset of green-up and ending within two weeks of dormancy/senescence. The sampling windows prescribed in Appendix D represent the average timing for these events by site. Sampling should be scheduled to begin within one week of the indicated start date (one week earlier is acceptable) and complete no sooner than one week before the indicated end date. If green-up or senescence at a site differs by more than one month from the listed estimated dates below for two subsequent years, issue a problem ticket to NEON Science.

Temperature threshold: For both the high and low intensity sampling schedules, a bout of sampling will only be performed if the high temperature on two consecutive days prior to planned sampling was >0°C. Obtain meteorological data from the National Weather Service based on sensors located as close as possible to the sampling site.

4.3 Timing for Laboratory Processing and Analysis

Tick samples held in vials containing 95% ethanol and stored at 25°C will retain their integrity as long as the level of ethanol completely covers all specimens. Ideally, samples should be sent to the identification facility within 3 months of collection to enable publication of the data on the NEON data portal prior to the following field season. Domain Support Facilities will follow their domain-specific shipping schedule when sending ticks for identification and enumeration.

4.4 Sampling Timing Contingencies

Before the field season begins, all sampling bouts for the year are scheduled in advance in accordance with the dates listed in Appendix D and sampling intensity of the site (low-intensity vs. high intensity sample schedule). During the season, Field Operations may shift the date of sampling 5 days forward or backward from the planned sampling date if weather or site management would otherwise prevent sampling on the originally scheduled sampling date. This shifting of schedule is at their discretion, but requires that staff are available to perform sampling on the alternate date. If a bout is shifted for this reason, subsequent bouts are expected to be performed on their originally scheduled date.

If **temperature** thresholds (see Section 4.2) are not met and it is not possible to shift the bout +/- 5 days (as described above), you may further delay or cancel the bout according to the directions in Table 1 (high-intensity schedule) or Table 2 (low-intensity schedule). Sampling will occur at the next scheduled bout when temperature thresholds are met.

If the **sampling conditions** below are not met and it is not possible to shift the bout +/- 5 days, you may further delay or cancel the bout according to Table 1 (high-intensity schedule) or Table 2 (low-intensity schedule):

- Sampling must be conducted when the ground is dry. Do not sample if the ground is moist (e.g., heavy morning dew, following a rain event, or in snow).
- If possible, avoid sampling during the hottest part of the day (mid to late afternoon) 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.

Occasionally, inappropriate sampling conditions or low temperatures occur may occur during the **last scheduled bout** of the season and delayed implementation (up to 10 days for high-intensity sites, up to 21 days for low-intensity sites) would result in conducting the bout after the estimated end date provided in Appendix D. The dates in Appendix D are only a scheduling guide; it is permissible to conduct sampling after that date in a delay so long as the temperature and sampling requirements are met when the bout is conducted.

Partial plot sampling or incomplete bouts

A standard and complete tick bout samples 160 m^2 at each of six plots per site and occurs in a *single* day. However, problems may arise during the bout that result in fewer than six plots sampled in a day (e.g., only four of six plots completed) or significantly less than 160 meters sampled at a given plot (i.e., 60 meters sampled at plot CPER_001).

Incomplete bouts due to inclement weather or safety concerns may be completed on a subsequent day and should follow **Table 1** and **Table 2** when determining allowable delays and needed reporting. For example: Ticks are scheduled for sampling on March 4th at SRER; domain staff decide to conduct the bout on March 8th because the weather forecast is rainy earlier in the week. On March 8th, 5 plots are completely sampled, but the last plot cannot be sampled due to weather conditions. The last plot is sampled March 11th (within the 21 day delay window for a low-intensity site; see **Table 2**) and a Service Now incident is made to document the delay (per **Table 2**).

A plot must be sampled more than 80 meters to count as a sampled plot. If less than 80 meters are sampled at a given plot due to inclement weather or safety concerns, the plot may be reattempted on a subsequent day; follow Tables 1 and 2 when determining allowable delays and needed reporting. No data entry should be made for these partial plots and any ticks collected from partial plots should be discarded. For example: Ticks are scheduled for sampling on March 4th at SRER; domain staff decide to conduct the bout on March 5th because the weather forecast is cold earlier in the week. On March 5th, sampling begins at the first plot SRER_001, but is terminated after sampling 40 meters of the transect due to safety concerns. No data entry will be made for this partial plot and any ticks collected at the plot are discarded. On March 6th the team reattempts the bout, successfully conducts the entire 160 m^2 drag for plot SRER_001, and completes normal sampling at the other 5 plots. No Service Now incident is needed because the bout was completed within the 5 day window for the bout.

Table 1. Contingent decisions for high-intensity sampling

Delay/Situation	Action	Outcome for Data Products
Bout +/- 5 days of scheduled date	Ideally, sampling occurs on the day it is scheduled per the master schedule. However, flexibility is allowed only if a bout were otherwise impossible to conduct on the scheduled date (e.g., weather conditions, managed burns, etc.) **No documentation needed** in Service Now.	Slight increases in temporal variability/inconsistency in time series data.
5 days < delay ≤ 10 days of original scheduled date	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects all plots at the site, reattempt the entire bout at the conclusion of the delay. **Submit a problem ticket for delays exceeding five days.** Do not push back dates for subsequent sampling bouts. 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 submit a problem ticket to NEON Science for guidance about sampling at affected plots.	Larger increases in temporal variability/inconsistency in time series data.
Delay > 10 days of original scheduled date	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects 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 does not affect all plots at the site, conduct sampling at the plots that are not affected by the delay and submit a problem ticket to NEON Science for guidance about sampling at affected plots.	
Delays ≤ 10 days that occur at the end of the field season and push the sampling date past the dates listed in Appendix D	At the end of the season, a delay may push a scheduled bout beyond the estimated end dates specified in Appendix D. The bout may still be attempted after the date listed in Appendix D **IF** sampling conditions and temperature requirements are met.	Moderate increases in temporal variability/inconsistency in time series data due to the delay.

Table 2. Contingent decisions for low intensity sampling

Delay/Situation	Action	Outcome for Data Products
Bout +/- 5 days of scheduled date	Ideally, sampling occurs on the day it is scheduled per the master schedule. However, flexibility is allowed only if a bout were otherwise impossible to conduct on the scheduled date (e.g., weather conditions, managed burns, etc.) **No documentation needed** in Service Now or in the data	Slight increases in temporal variability/inconsistency in time series data.
5 days < delay ≤ 21 days of original scheduled date	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects all plots at the site, reattempt the complete bout at the conclusion of the delay. **Submit a problem ticket for delays exceeding five days**. Do not push back dates for subsequent sampling bouts. 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 submit a problem ticket to NEON Science for guidance about sampling at affected plots.	Larger increases in temporal variability/inconsistency in time series data.
Delay > 21 days of original scheduled date	If the delay occurs prior to the start of or during the sampling bout, and the issue(s) causing the delay affects 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 does not affect all plots at the site, conduct sampling at the plots that	

	are not affected by the delay and submit a problem ticket to NEON Science for guidance about sampling at affected plots.	
Delays ≤ 21 days that occur at the end of the field season and push the sampling date past the dates listed in Appendix D	At the end of the season, a delay may push a scheduled bout beyond the estimated end dates specified in Appendix D. The bout may still be attempted after the date listed in Appendix D **IF** sampling conditions and temperature requirements are met.	Moderate increases in temporal variability/inconsistency in time series data due to the delay.

4.5 Criteria for reallocation of sampling within a site

Ideally, sampling will occur at these plots for the lifetime of the observatory (core sites) or the duration of site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that a particular plot be relocated within the site. In general, a plot should be considered for relocation when feasibility of sampling becomes so limited that data quality is significantly reduced.

Sampling limitations can arise when plots can become inappropriately suited to answer meaningful biological questions (i.e., a terrestrial sampling plot becomes permanently flooded). Alternatively, plots may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful. A given tick plot must be sampled at least 50% of the bouts expected for the site over a two-year period. Plots that cannot be sampled on this schedule should be considered for relocation, for which a problem ticket should be submitted. Additionally, if sampling at particular plots requires significantly more time than expected, a problem ticket should be submitted for consideration for relocation.

For the tick sampling program, use the following criteria to evaluate each plot:

1. If permanent obstacles are present over 50% or more of the entire perimeter of the plot, then the plot should be proposed for replacement.
    a. Tick sampling should only be conducted in plots in which you are able to sample for ticks (using dragging, flagging, or a combination, as described in SOP B) over 50% or more of the plot perimeter.
    b. Problems arise when woody vegetation is so tall (i.e., >3-4 ft) 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., >120 min).
    c. Other obstacles include standing/flowing water or wet terrain.
2. If sampling cannot be completed on ≥50% of the planned sampling dates over a two-year period, due to temporally variable obstacles or conditions, then the plot should be proposed for replacement.
    a. In some cases, a 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.

b. Alternatively, large portions of the plot perimeter may be associated with supple, low growing vegetation early in the sampling season that becomes tall/dense/woody later in the season.
c. 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.

3. Representative cover type: When inclined to reject a plot based on obstacles, consider whether the conditions 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. Reallocating plots to one or more other vegetation types at the site may be considered.

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 to 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]. Generally, the incidence of tick-borne diseases in humans is extremely rare and is typically associated with working outside in vegetated areas.

IMPORTANT: Use of insect repellent is highly recommended, but application is left as a personal safety choice. 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-free hand wipes) before handling any sampling equipment. Both permethrin (0.5%) and DEET (up to 40%) are excellent repellents and can be used to treat field clothes well in advance of field sampling (two to four hours

prior). Application of insect repellent *less* than 30 minutes before sampling will reduce tick sampling success and data quality; thus, it is important that use of these products occur before arriving in the field.

When surveying in areas with poison ivy, the use of Technu is recommended after sampling. Staff should also consider wearing gloves to avoid contact with oils that may be on the drag cloth.

This protocol does require the use of chemicals (Ethanol). Safety Data Sheets (SDS) shall be readily available for review whenever chemicals are being transported or used during this activity.

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 freezers, 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

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
VWR	89049940	R	Ice pack, 0°C	Pre-freeze for field preservation of samples	Variable	N
Consumable items						
Ben Meadows Forestry Supplier	010510-1 49247	R	All weather copy paper	Print datasheets, prepare internal vial labels (pre-print or use pencil)	5	N
		S	Waterproof adhesive label or label tape	Prepare external vial labels (pre-print or use permanent marker)	Variable	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Fisher	04355601	R	Ethanol, 190 proof (95%); 55 gallon drum	Weaken packing tape adhesive, to remove larval ticks	Variable	Y
Thomas	C954K61	R D05	Ethanol, 190 proof (95%); 5 gallon carboy	As above. D05 restricted to reduced amounts of ethanol on hand.	Variable	Y

R/S=Required/Suggested

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

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Amazon Grainger	B0006H5B06 1HUG6	R	Cooler, 16 qt	Chill perishable samples in field	1	N
Fisher	08953E	R	Forceps (with flagging or lanyard)	Collect ticks	2	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Forestry Supplier	53430	S	Clipboard	Hold and write on datasheets	1	N
		R	Pencils	Write on datasheets and internal labels	3	N
Amazon Cabela's REI	IK270217 895022	S	GPS receiver, recreational accuracy, e.g. Garmin Etrex20x	Navigate to sampling location	1	N
VWR	89049940	R	Ice pack, 0°C	Chill perishable samples in field	3	N
Forestry Supplier	61082	S	Magnifier hand lens, 2X/5X	Aid in tick identification	1	N
Ben Meadows Forestry Supplier	122732 39945	S	Measuring tape, minimum 50 m	Measure deviations from the drag path	1	N
Amazon Bass Pro	#ILTS34-25 OATS-1	S	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
Consumable Items						

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Fisher Grainger	22363750 3VAL6 5ELT9 6LGZ4	S	Alcohol-free hand wipes	Remove repellent residue	2	N
Arrow Grainger	DUC1265013RL 15F772 26VC84	R	Duct tape	Remove and discard ticks not being archived	1	N
Ben Meadows Forestry Supplier	010510-1 49247	R	All-weather copy paper	Internal labels for sample vials (pre-print or use pencil)	1	N
		S	Waterproof adhesive label or label tape	External labels for sample vials (pre-print or use ethanol-proof permanent marker)	Variable	N
Grainger	4YP37	S	Scissors	Cut labels	1	N
Grainger	15F814 31HJ31 3KHJ7	R	Clear packing or masking tape	Collect larval ticks from cloth	1 roll	N
various		S	Mosquito repellent, up to 40% DEET	Protect personnel from insect bites	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
BioQuip	1154F	R	Permanent marker, archival & ethanol-safe	Write external labels for sample vials	1	N
Grainger	5CNK5	R	Resealable plastic bag, 1 gal, 4 mil	Organize sample tubes	6	N
		S	Rubber band	Organize sample tubes	Variable	N
		S	Survey marking flag, PVC or fiberglass stake	Delineate sampling area	4	N
		S	Resealable plastic bag, 1 gal, 4 mil	Store and transport drag cloths	Variable	N
Fisher	1443222	S	Tubes with caps, 50 mL, or larger as needed	Prepare pre-filled tubes with 95% ethanol for soaking tape with larval ticks	Variable	N
Thomas	1236C14	R	Tubes, Microcentrifuge, 1.5 mL	Prepare pre-filled sample vials with 95% ethanol solution	6	N
Resources						

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
		R	Tablet (mobile data entry) with Tick sampling application downloaded	Record data	1 per team	N
		R	Spare Tablet battery		1	N
RD[06]		R	Field datasheet	Record data	1	N

R/S=Required/Suggested

Table 5. Equipment list – Laboratory processing and analyses

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Grainger	2AJP4	S	Artist paintbrush	Transfer ticks to tubes	2	N
Fisher	08953E	S	Forceps	Transfer ticks to tubes	2	N
Consumable items						
		S	Copy paper, white	Aid in visibility of ticks	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
		S	Waterproof adhesive label or label tape	External vial labels (pre-print or use permanent marker)	Variable	N
Grainger	4TKE5 5GUU1	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
		S	Rubber band	Organize sample tubes	Variable	N
VWR	10025756	R	Tubes, 1.5-2 mL with screw-top cap and O ring	Store ticks in 95% ethanol solution for shipping	12	N
		R	Adhesive barcode labels (Type I)	Labeling sample containers with barcode-readable labels [Note: container curvature will not permit these labels to be used on vials smaller than 10mL]	1 sheet	N
Resources						
		R	Synced data entry tablet	Record data		N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
RD[06]		R	Completed field datasheet	Record data	1	N

R/S=Required/Suggested

Table 6. Equipment list – Shipping

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items						
		R	Cardboard box, UN packing group III	Package specimens for shipment	Variable	N
		S	Plastic bag, 2 mil	Spill containment	2	N
		S	Plastic liner, 2 mil	Spill containment	1	N
		S	Styrofoam sheet	Insulation for samples in shipment boxes	6	N
		R	Up arrow shipping label	Label shipments containing liquids	2	N
Grainger	1HUB7	R	Absorbent pad	Absorb liquid spills during shipment	Variable	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Grainger	30RD13	R	Vermiculite, grade 2	Absorb liquid spills during shipment	Variable	N
Resources						
Printout from Stork Application		R	Shipping manifest	Inventory of specimens being shipped	1	N

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete Field Safety Training as defined in Operations Field Safety and Security Plan (AD[02]) and NEON EHS Safety Policy and Program Manual (AD[01]) and protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

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. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

An experienced two-person team can complete sampling of ticks at a single plot in approximately 30 to 120 minutes (see Figure 1). This entails dragging/flagging around the perimeter of the plot and transferring ticks into one or more sample vials.

Table 7. Estimated time required to complete field sampling and lab standard operating procedures

SOP	Estimated total time	Suggested staff	Total person hours
7SOP A Preparing for sampling	1 hr/bout	2	2 hrs/bout
1.1.1.1.1SOP A Field Sampling	0.5 – 2 hrs/plot	2	1 – 4 hrs/plot
1.1.1.1.1SOP A Laboratory processing and analyses	0.5-1 hrs/bout	1	0.5-1 hrs/bout
1.1.1.1.1SOP A Data entry and verification; QAQC	1 hr/bout	1	1
1.1.1.1.1SOP A Sample shipment	0.5 hr/bout	1	0.5 hr/bout

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 At least one week prior to a sampling bout

1. Identify the locations of sampling plots, determine how to access them, and evaluate current plot conditions according to the criteria in Section 4.5.
2. If available, prepare final sample containers by affixing one adhesive barcode label to each vial and/or Ziploc bag used to contain each sample. Type I barcodes are preferred for medium and large size vials (e.g., 10mL, 50mL) or Ziploc bags. Type IV barcodes are **required** for small size vials (less than 10mL). **Using a Type I barcode on a skinny vial will result in the barcode falling off the vial.**

    a. Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season).
    b. Barcode labels should always be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode *lengthwise* along a vial, not horizontally wrapping around a vial.
    c. If your site generates one vial of ticks per tick transect, affix the barcode label to <u>each vial</u> to be filled with a **unique sample**. If multiple vials are required to contain a sample from one transect, place the barcode on the Ziploc bag that will contain <u>all</u> vials associated with that sample.

        Example: A sample collection from OSBS_005 fills one 10-mL tubes. The single barcode is applied to the sample vial.

        Example: A sample collection from OSBS_005 fills ten 50-mL falcon tubes. The single barcode is applied to the <u>Ziploc container</u> that contains all ten vials, *not* each vial containing 1/10 the sample.

Neither the data entry mechanism nor our database can handle 10 barcodes mapping to the same sample. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to make these up in advance.

Table 8. Barcode types and their application in the tick protocol

Type & format	Use	Barcode Image
Type I Prefix A followed by 11 numbers	Works well on Ziploc bags and 50mL vials	
Type IV Prefix D followed by 11 numbers	Works well on vials less than 10 mL in size	

3. Prepare collection sample vials with both external sampleID label and barcode label affixed. The sampleID (**Figure 1**) is the plotID and the date (YYYYMMDD), separated by a period. As an example, the sampleID "OSBS_002.20130802" would indicate that the labeled vial contains ticks collected in plot 002 at Ordway Swisher Biological Station on August 2, 2013.
    a. External labels: External sampleID labels may be legibly written directly on the vial with ethanol-safe permanent marker or pre-printed on adhesive labels (preferred). Labels should be oriented with the beginning of the sampleID towards the vial opening.
    b. Labeling in the field: If temporary labels are added to vials in the field, be sure that vials have both barcode and external labels before shipping samples.

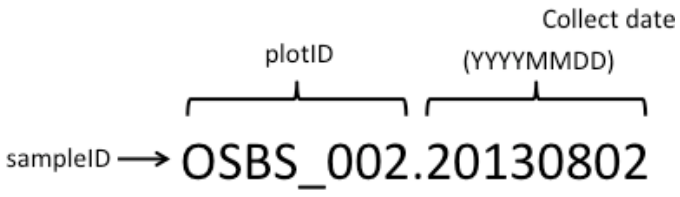


Figure 1. Annotated example of sampleID

4. Print out datasheet(s) on waterproof paper.

5. Be sure reusable ice packs (0°C) are frozen.

A.3 Just prior to field sampling

1. Gather all necessary equipment for field sampling.
2. Fill sample vials ¾ full with 95% ethanol.
3. Recommended: At least thirty minutes prior to sampling, apply insect repellent. 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-free wet wipes) before handling any sampling equipment. Application of repellent less than 30 minutes before sampling will reduce sampling success, so ensure that all application occurs at least 30 minutes ahead of time.
4. Use the checklist (**Appendix C**) to ensure that all required materials are in the field truck prior to sampling.

SOP B Field Sampling

Sample using one or both of the two sampling methods. Drag sampling (SOP B.2) is the preferred method used for tick collection. Flagging (SOP B.3) is used as a substitute for dragging when vegetation is too thick to allow the drag cloth to be pulled along the ground.

B.1 About sampling transects

1. Sample along a fixed path that follows the shortest straight-line distance between plot corners and thus covers the full perimeter of the plot (**Figure 2**). You can sample in either a clockwise or counterclockwise direction. Ideally, you will sample 160 meters (four 40 meter transects).

   a. Always record the total horizontal distance sampled, with a target accuracy of +/-2 meters.

   b. Try to maintain as narrow and consistent a path as possible to minimize trampling surrounding vegetation. Should a path become worn, you may need to drag the cloth beside the path, just inside the plot perimeter.

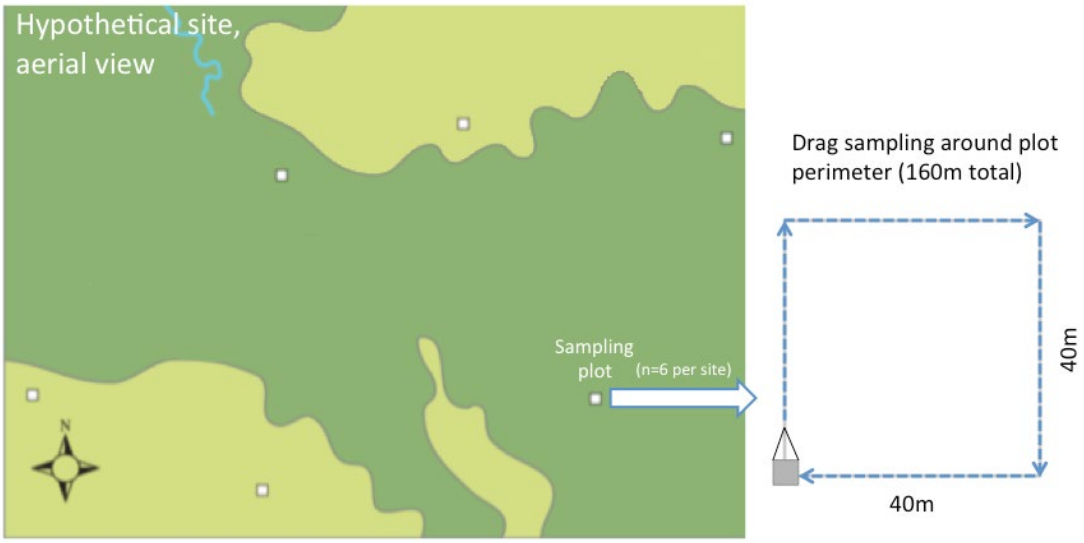
2. If straight-line transects are not possible, choose an alternative path that minimizes detours from the original perimeter, while staying in range of acceptable sampling distance (minimum 80 meters, maximum 180 meters). If the distance covered falls out of the range of accepted sampling distance, issue a problem ticket.

   a. If a large obstacle (e.g., rock, tree, cluster of shrubs) is present along the transect, divert the sampling path as little as possible into the plot. Be sure that all diversions are directed into the plot, rather than outside the plot boundaries.

   b. If the obstacle is too large to divert around, you can sample up to the obstacle, pick up the cloth, make your way through or over the obstacle, 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 crosses the sampling transect.

   c. Flagging landmarks may help maintain consistency across bouts in transect detours.

Figure 2. Schematic of sampling plot and transects.



B.2 Overview of drag sampling method and frequency of checks

1. Place the drag cloth on the ground. One member of the two-person team should pull the cloth while the other walks behind the cloth. 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.
2. Drag the cloth at a pace that is slow and steady.
    a. 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 15 meters at the proper pace.
3. Ensure that the entire cloth stays in contact with the ground or vegetation.
    a. 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.
    b. 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.
4. Stop to collect ticks every 5-10 meters as described in SOP B.5.6.

B.3 Overview of flag sampling method and frequency of checks

1. The flagging cloth is a modified drag cloth: 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. Periodically crouch down and sweep the flag underneath vegetation. While the cloth can be passed over and around vegetation, sampling the ground underneath vegetation will ensure that flagging is most comparable to dragging.
    a. 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.
    b. If the vegetation is low growing and you cannot get the cloth underneath, consider the vegetation an obstacle (see SOP B.1.2).
    c. Note that when flagging, especially underneath vegetation, the cloth will generally wrinkle. This will require estimating the total distance sampled with less precision than when dragging.
4. Stop to collect ticks (SOP B.5.6) every 3-4 sweeps, which should be the equivalent of sampling 3-5 square meters.
    a. This is with greater frequency than with dragging, as sampling in dense vegetation is more likely to dislodge ticks attached to the cloth.

B.4 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.
2. During sampling, it is important to try and keep the cloth in direct physical contact with the ground, vegetation, or overlying leaf litter. When dragging, attempt to make a qualitative assessment of whether the cloth is on or close to these surfaces: is it touching the surface most or all of the time, is it "surfing" up 2-3 inches above the surface as it passes over flexible-stem grasses/forbes, or is it "stilting" 4 or more inches above the surface 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 surface. In particular, flagging is an effective means of getting the sampling cloth underneath shrubs and taller/more dense vegetation.

3. A combination of dragging and flagging may be used if necessary to sample over vegetation types or obstacles. Here are some examples of scenarios that may be encountered:

    - **Low stature grass, 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, supple vegetation (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, woody vegetation (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., ≤3 ft) 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. If plants are >4 ft tall, just sample the ground between and underneath plants (i.e., do not sample trunks or woody stems).

B.5 Tick Collection in the Field

1. Navigate to the SW corner of the tick sampling plot using maps and/or a handheld GPS. Verify the identity of the plot location with plotID listed on the permanent plot marker (created during plot establishment) or with the alternative marker system required by the site host at sites where the site host does not allow permanent plot markers.
    a. Be sure not to transit through any portion of the plot, especially the plot perimeter.
2. Perform an inspection of your and your partner's person after arriving at the plot corner and before you begin sampling. Remove ticks using duct or masking tape and discard.
3. Record bout information (personnel conducting the sampling & protocol version) and location information (especially plotID, date of collection and time that sampling was initiated).

For each sampling interval, conduct steps 4 through 7:

4. Begin drag or flag sampling along the perimeter of the plot.
    a. If necessary, use a compass to orient yourself along the plot perimeter. If the plot corners are not easily visible between intervals, the person following may remain at the plot corner and provide direction until the next plot corner is located. Flagging landmarks may help.
    b. Avoid contact with the cloth. Be aware that ticks may attempt to crawl onto your hands, arms, or body while you inspect the drag cloth.
5. Stop at intervals appropriate to the sampling method and vegetation density to examine the cloth and your person(s) for ticks.
    a. Perform a quick scan over the cloth and your person(s) for adult ticks first, as they tend to drop off more quickly than the other life stages.
    b. Hold cloth at arm height to check for ticks. Do not perform a check for ticks with the cloth on the ground as ticks may crawl off or onto the cloth.
    c. Scan the cloth in a systematic manner such that you examine the entire cloth on both upper and lower surfaces.
    d. Use a hand lens as necessary to distinguish ticks from other arthropods and debris.
6. Collect ticks and transfer them into the appropriately labeled sample vial containing 95% ethanol. Ticks of all life stages collected during a sampling plot/bout combination should be placed together into the same sample vial. Record the barcode identifier of the sample vial in the mobile application.
    a. When handling a tick, use the forceps to pick it up by the leg rather than pinching the body.
    b. Additional sample vials can be used if a single vial cannot hold all of the ticks collected during a sampling plot/bout combination.
    c. If collecting **larvae** with forceps is excessively time-consuming, collect **larvae** with tape. A lint roller may also be used if preferred, follow the same following procedures as for tape.
        i. Remove larval ticks from the drag cloth and your person with packing tape or masking tape. Do not use duct tape for sampling. Collect as many larvae as possible using as little tape as possible. You should strive to find and remove every larval tick from the cloth.
        ii. Completely submerge the tape with ticks in a labeled 50 mL vial (or larger if needed) with 95% ethanol to weaken the tape adhesive. Leave in ethanol for transit back to the lab.
        iii. This method should only be used for larvae.
7. Spend no more than 10-20 seconds checking your person(s) for ticks between intervals. Examine areas around the lower legs and feet especially closely. This inspection may be more thorough if done reciprocally (i.e., each team member inspects the other).

8. Continue sampling at appropriate intervals until the 160 m^2 sampling transect is complete. Inspect your person(s) for ticks and add them to the sampling vial.
9. Data should be entered using the mobile data entry application.
   a. Enter one record for every plot sampled, documenting end time, meters sampled, and barcode for each sample (if assigned).
   b. 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). Large-scale plot disturbances or site management activities (i.e., burning, mowing, etc.) should be documented in the Site Management and Event Reporting application.
10. Place all labeled samples in an insulated cooler with frozen ice packs for transit back to the lab. While ticks in ethanol do not require a cold chain to be maintained, storage of ticks in extremely hot temperatures (>30 C) could compromise sample integrity.
11. After leaving the plot, any ticks found on your person(s) should be removed with duct tape and discarded.

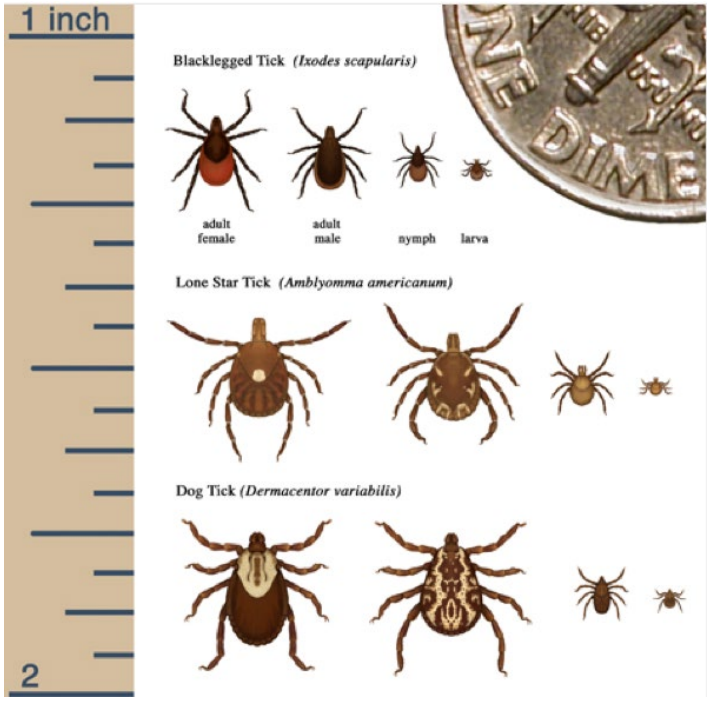


Figure 3. Relative sizes of life stages for selected species (courtesy of the Centers for Disease Control and Prevention)

B.6 Sample storage

When multiple vials are used in the field to contain specimens that correspond to a single sampleID (i.e., one collection date at one plot), they will need to be grouped into a single container (i.e., Ziploc bag, a larger vial). This grouping must be done before samples are stored.

1. If these specimens can be consolidated into one vial, transfer all ticks from the same plot and date of collection into a vial that is labelled with a barcode and the appropriate sampleID. Update the electronic record with the corrected sample barcode and indicate that the number of vials per sampleID is 1. OR
2. If specimens corresponding to the same sampleID cannot be consolidated into one vial, then remove any pre-existing barcode labels from the vials, place these vials into a single bag, and externally label the bag with the sampleID and barcode. If available, you may use a bag that has been pre-labelled with barcode from SOP A. Update the electronic record with the corrected sample barcode and revise the number of vials per sampleID, if necessary.

Upon returning to the lab, repackage any samples as described above and store them at 25°C. Process tick samples within one month of collection. Store samples from the same site/bout combination together (e.g., using a rubber band and/or placing within a resealable bag).

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 work surface 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. Ticks of all life stages from the same plot and bout may be combined in the same vial.
2. If tape was used in the field, use forceps to carefully remove ticks remaining on the tape.
3. Transfer ticks into the appropriate sampling vial(s).
    a. A paintbrush may be useful for blotting, and handling larvae gently to avoid crushing them. All larvae need be transferred to the sample vial.
4. Make sure all vials are properly labeled. See SOP A for label format instructions. Ensure that there is only one barcode per vial or set of vials associated with a given sampleID. See SOP B for how to appropriately repackage a sample when multiple vials are associated with one sampleID. Make any needed updates to the electronic record to reflect changes in the numbers of samples or barcode associated with the sample.
5. Send labeled vials of ticks (do not send tape) to the external identification facility.

C.3 Equipment maintenance, cleaning, and storage

1. Staff are encouraged to use gloves when working with a cloth that touched a *Toxicodendron* species in the field (e.g., Poison Ivy, Poison Oak).
2. Place the drag cloth in an ultralow (-80°C) freezer for at least 30 minutes to kill any larval ticks attached to the cloth.
3. Remove seeds stuck to the drag cloth to prevent introduction to other plots and sites, either by hand or with duct tape.
4. If the drag cloth is dirty, wash it using fragrance-free laundry detergent, using bleach if necessary, 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.

5. Clean any other equipment as necessary using dilute fragrance-free laundry detergent, dry and store in a cool, dry place.

SOP D Data Entry and Verification

The importance of thorough, accurate data entry cannot be overstated; the value of field efforts are only manifested once the data are properly entered for delivery to NEON's end users. Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

Although data entry via mobile application is preferred, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Be sure to enter data for all plots within a bout even if collected on a different schedule than originally planned. If an entire bout is missed, no data need to be entered, but a problem ticket should be issued.

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data regarding observations and samples are accurate and complete. This protocol requires that certain QA checks be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before a sampling window closes. Incomplete data and/or sample sets cannot be supplemented by subsequent sampling efforts if the sampling window has closed. Invalid meta-data(e.g. collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location. Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess duplicative data to maintain data ***validity*** and ***integrity***.

All QA measures needed for this protocol are described in the Data Management Protocol (RD[08]).

Sample Labels & Identifiers

By default each sample or subsample produced by this protocol is assigned a human-readable sample identifier which contains information about the location and date of the collected sample. Each sample may also be associated with a scannable barcode, which will not contain information specific to sample provenance, but will reduce transcription errors associated with writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, it is encouraged to make these up in advance. Use the appropriate barcode label type with each container (i.e., small size labels for vials less than 10mL, etc).

Barcodes are scanned into the mobile application when the sample is placed into the container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. If multiple vials or containers are required to contain a sample from one trap, place the barcode on the outer container that will hold all vials associated with just that sample (i.e., if a collection fills ten 50-mL falcon tubes, the single barcode is applied to the outer Ziploc container not each vial containing 1/10 the sample; the database cannot handle 10 barcodes mapping to the same sample).

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.

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 and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

E.1 Supplies/Containers

Use corrugated cardboard boxes lined with insulation. Alternatively, a pre-insulated container (i.e. polystyrene) may be used (request return from the recipient if cost-reasonable).

Double-bag the tubes containing samples using minimum 2-mil watertight plastic bags with absorbent liner inserted into the outer bag, and close securely. Fill all void space with bubble wrap to absorb any spills and prevent movement.

E.2 Conditions

Samples should be stored in vials containing 95% ethanol, at 25°C post-processing until shipped to an external facility. Samples should be shipped so that they are in transit for no more than 3 days.

E.3 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 and sorted at the external facility.

E.4 Return of Materials or Containers

Be sure to include instructions to external facilities on how to return reusable materials. CLA can provide details.

E.5 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping manifest AND a corresponding electronic version of the manifest. 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 using the Stork Shipment Verification Tool. The electronic manifest should be emailed to the taxonomic ID facility as soon as possible after a batch of samples has been shipped.

- Navigate to the "Shipping Information for External Facilities" document on CLA's NEON intranet site. Check whether there are items such as permits or cover letters required to include in the shipment. *Check this document often as instructions are subject to change*.

- Print out required documents (if needed) to include in shipment box.
- Prepare a shipping inventory detailing the contents of the shipment, using the Shipment Creation and Shipment Review applications. Include a printed copy of the inventory in the shipment box (downloaded from the Stork Shipment Verification Tool).
- Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.

E.6 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. 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, remove with duct tape, and discard.

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

STEP 3 – Drag cloth SLOWLY for 5-10 meters.

WALK SLOWLY!

STEP 4 – Stop and inspect drag cloth. Collect ticks into vial containing 95% ethanol.

STEP 5 – Verify that vial has the correct sampleID and scan the barcode into the mobile application.

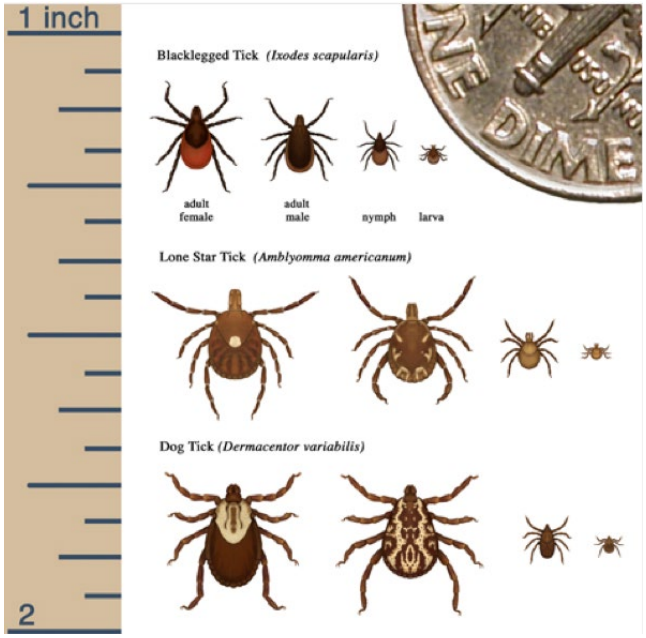
STEP 6 – Collect larval ticks with clear packing tape if necessary. Store in 95% ethanol for transport back to lab (ticks collected on tape only).

STEP 7 – Repeat drag and collection cycle until you have sampled the entire perimeter of the plot (i.e. returned to the plot corner where you began your sampling).

STEP 8 – Store specimen vials in cooler with ice packs.

STEP 9 – After leaving the plot, check yourself for ticks, remove with duct tape, and discard.

Tick Life Stages



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 Sampling Datasheet.
- ☐ Bring a synced and charged tablet that has the Tick application loaded.
- ☐ Upload sample coordinates to GPS and obtain maps.
- ☐ Bring all supplies and extras.
- ☐ Check your pace. Can you accurately pace 5-10 meters?

PERSONAL SAFETY: PROTECT YOURSELF BY…

- ☐ Wearing appropriate clothing.
- ☐ Tucking pant legs into socks.
- ☐ Using tape to seal gaps.
- ☐ Applying insect repellent: at least 30 min before going into field; away from sampling equipment.

You are collecting live ticks.

If you choose to 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.
- ☐ Associate the barcode identifier with the electronic record.
- ☐ Include ticks on your clothes in your specimen vial.
- ☐ Store tick samples in cooler with ice packs.

BEFORE LEAVING DRAG SITE, CHECK THAT…

- ☐ Field portion of datasheet or electronic record 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.

AT THE END OF THE DAY, LIMIT YOUR EXPOSURE TO TICKS BY…

- ☐ Putting your field clothes in a dryer to kill ticks, or if not possible, stowing them in a plastic bag to contain ticks.
- ☐ Checking yourself for ticks.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in **Table 10** below are based on MODIS (Moderate Resolution Imaging Spectroradiometer) EVI phenology data from NASA records from the most recently available decade (2005-2014). The season is bounded by increasing green-up as the start date and the mid-point between decreasing greenness and minimum greenness as the end date. If sites experience two peak greens, the start date is based on the first cycle of greening and the end date is based on the second cycle. Estimates for the start and stop dates of sampling are provided for each site. For convenience, this table also provides: whether more than 5 ticks were detected ('Y') or not ('N') at each site and the expected number of bouts based the estimated seasonal duration and presence of ticks. Locations that have not yet been sampled may utilize the high intensity or low intensity sampling schedule depending on tick captures during the season.

These dates are estimates and local conditions may vary. If the listed start date passes and no observable green-up has occurred, then the start of the sampling season may be delayed until green-up is observed such that the estimated number of bouts occur. Completion of the sampling season should also coincide with the observation of senescence in the field; if senescence occurs prior to the end date listed in the table below, the sampling season may be terminated before the estimated end date.

However, if green-up or senescence at a site differs by a more than one month from the listed estimated dates below (either earlier or later), issue a problem ticket to NEON Science.

Note: MODIS data are of limited utility for tropical sites (e.g., D04). For these locations, a six-month window of sampling has been selected based on patterns of precipitation at the site.

Table 10. Estimated sampling dates based on historical 'green-up' dates

Domain	Site	Start	End	More than 5 Ticks Collected in 2018	Expected Number of Bouts
1	BART	29-Apr	16-Sep	N	3
1	HARV	23-Apr	20-Sep	Y	7
2	BLAN	22-Mar	19-Sep	Y	9
2	SCBI	28-Mar	27-Sep	Y	9
2	SERC	18-Mar	1-Oct	Y	9
3	DSNY	5-Mar	17-Sep	N	5
3	JERC	24-Mar	17-Sep	N	4
3	OSBS	6-Mar	19-Sep	Y	9
4	GUAN*	14-Apr	13-Oct	N	4
4	LAJA*	14-Apr	13-Oct	N	4

Domain	Site	Start	End	More than 5 Ticks Collected in 2018	Expected Number of Bouts
5	STEI	30-Apr	10-Sep	Y	6
5	TREE	28-Apr	12-Sep	Y	7
5	UNDE	1-May	9-Sep	N	3
6	KONA	4-Apr	16-Sep	N	4
6	KONZ	3-Apr	17-Sep	Y	8
6	UKFS	23-Mar	28-Sep	Y	9
7	GRSM	3-Apr	20-Sep	N	4
7	MLBS	18-Apr	23-Sep	N	4
7	ORNL	18-Mar	23-Sep	Y	9
8	LENO	10-Mar	26-Sep	Y	10
8	DELA	2-Mar	16-Sep	Y	9
8	TALL	17-Mar	24-Sep	Y	9
9	DCFS	1-May	4-Sep	Y	6
9	NOGP	19-Apr	2-Sep	Y	6
9	WOOD	6-May	6-Sep	Y	6
10	CPER	30-Mar	12-Oct	N	5
10	RMNP	10-May	7-Sep	N	3
10	STER	28-Mar	9-Aug	N	3
11	CLBJ	28-Feb	6-Oct	N	5
11	OAES	10-Mar	25-Nov	Y	12
12	YELL	6-May	15-Aug	N	2
13	MOAB	16-Mar	8-Oct	N	5
13	NIWO	31-May	2-Sep	N	2
14	JORN	22-Mar	10-Oct	N	5
14	SRER	2-Mar	10-Oct	N	5
15	ONAQ	18-Mar	29-Jul	N	3
16	ABBY	19-Apr	6-Sep	Y	7
16	WREF	22-Apr	8-Sep	N	3
17	SJER	8-Oct	6-May	N	5
17	SOAP	31-Mar	10-Sep	N	4

Domain	Site	Start	End	More than 5 Ticks Collected in 2018	Expected Number of Bouts
17	TEAK	5-May	3-Sep	-	3 or 6
18	BARR	27-Jun	4-Aug	N	1
18	TOOL	7-Jun	11-Aug	N	2
19	BONA	14-May	17-Aug	N	2
19	HEAL	19-May	18-Aug	N	2
19	DEJU	13-May	19-Aug	N	2

* sites where precipitation data were used in lieu of MODIS data

APPENDIX E SITE-SPECIFIC INFORMATION

The following alternative plot establishment guidelines have been approved for use at the following sites:

E.1 GUAN

Guidelines for alternative tick sampling plot establishment

Field Operations staff will identify a target six plots (transect paths) within the TOS sampling boundary where tick sampling is feasible, in accordance with the following guidelines:

1. Required: A minimum area of 80 m2 (with an ideal target of 160 m2) can be sampled using either the flagging or dragging method. If necessary, the area may consist of multiple segments, each preferably within 10 meters of another segment.
2. Strongly preferred: The majority (50% or more) of the area is located within:
   - 300 m from the center of a mammal plot,
   - 300 m from the center of a bird plot, OR
   - 300 m from the center of a Distributed Base Plot

Ideally the area will be in range of all three plot types, but the order reflects priority (mammals are first preference). Ideally both this criterion AND the Distance from other TOS plots criterion (Table 11) will be met, but if this is not possible, this criterion takes higher priority.

3. Preferred: Does not cross roads or frequently-used paths to other plots. If no other options are available, plots may be located linearly along the edge of a dirt road or path, where vegetation or leaf litter can be sampled.

For each sampling area identified, Field Operations staff will provide to Science staff:

- A line (vector file) of the sampling path (preferred), OR
- Start, end, and change-of-direction points from which the sampling path can be approximated with linear segments

Other than these changes in plot establishment, the tick sampling process remains the same. This includes the fixed nature of these locations until sampling is no longer possible (issue a problem ticket) or instructed otherwise.

Table 11. Standard vs. Alternative Tick Plot Location Criteria

Parameter	Standard	Alternative
Plot Size	160m transects around a 40m by 40m plot	160m transects of any shape
Maximum distance from roads	None	No change
Minimum distance from Paved Roads	Avoid high traffic areas	No change
Minimum distance from Dirt Roads	Avoid high traffic areas	Adherence to standard preferred, but not required
Distance from Buildings	Avoid high traffic areas	No change
Distance from oil pads	NA	No change
Distance from other TOS plots	Edge of plot is 150m from tower, phenology, and mammal plots. Centroid of the plots is 150m from a base plot. Can be within a bird grid, not on bird points. 50 m from a mosquito point.	Adherence to standard preferred, but not required
Distance from same type of plot	500m, centroid to centroid	No change
Vegetation characteristics	40m by 40m plot matches NLCD definition	Minimum-area polygon matches NLCD definition
Stratification	Proportional to NLCD dominant vegetation types	No change
Collocation needs	Collocated with target distributed base plots	Collocated with mammal, bird, or distributed base plots

Parameter	Standard	Alternative
Placement Method	Follow the M_Order list of accepted base plots. A random azimuth is determined at a distance of 150m from the base plot centroid. This distance can be shifted +/- 15m. If the first azimuth does not work 2 more random directions are tried before moving onto the next M_Order. If the target number of tick plots is still not met after going through all target base plots then grids can be placed subjectively 150m +/- 15m from the target base plot, starting at the beginning of the M_Order list again.	Locate subjectively where sampling is possible, preferably within 300 m from the center of a mammal, bird, or distributed base plot.
Target number of plots	6	No change
Contingency # of plots	0-100%	No change
Streams	A stream cannot bisect the transect	Avoid placing plot segments more than 10 m apart
Post Processing Accuracy (m)	2	No change
Comments	Effort is made to avoid having tick plots be in between the road and another TOS plot	Avoid having tick plots intersect frequently-used paths