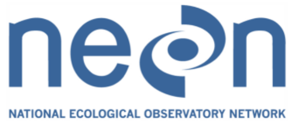

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TOS FIELD AND LAB PROTOCOL: TICK AND TICK-BORNE PATHOGEN SAMPLING

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TABLE OF CONTENTS

1 Description 1
1.1 Purpose 1
1.2 Scope 1
2 Related Documents and Acronyms 1
2.1 Applicable Documents 1
2.2 Reference Documents 1
2.3 Definitions 2
3 Background and Objectives 2
3.1 Background 2
3.2 NEON Science Requirements 2
3.3 NEON Data Products 3
5 Quality Assurance and Control 3
6 Safety 4
7 Personnel Requirements 4
8 Training Requirements 5
9 Field Standard Operating Procedure 5
9.1 Sampling Frequency and Timing 5
9.1.1 Sampling Frequency 5
9.1.2 Criteria for Determining Sampling Dates 6
9.1.3 Sampling Timing Parameters 6
9.2 Equipment and Materials 6
9.3 Preparation 7
9.4 Sample Collection in the Field 7
9.4.1 Drag and walking sampling 7
9.4.2 CO_2 sampling 12
9.5 Sample Preservation 12
9.6 Sample Shipping 13
9.7 Data Handling 13
9.8 Equipment Maintenance, Cleaning and Storage 13
10 Laboratory Standard Operating Procedure 13

DRAFT

10.1 Sample Processing Timing 13
10.2 Equipment and Materials 13
10.3 Preparation 14
10.4 Sample Processing in the Lab 14
10.4.2 Drag and walking sampling 14
10.4.3 CO_2 sampling 14
10.4.4 Off season tick drag 15
10.5 Sample Preservation 15
10.6 Sample Shipping 15
10.7 Data Handling 15
10.8 Equipment Maintenance, Cleaning and Storage 16
Appendix A Sampling Phase Determination 17
Appendix B Tick and Tick-Borne Pathogen Sampling Spatial Sampling Datasheet 18

LIST OF TABLES

Table 1 – Contingent decisions for tick and tick-borne pathogen sampling 4
Table 2 – Field Equipment List 6
Table 3 – Laboratory Equipment List 13

DRAFT

1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of NEON protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices
- site-specific safety practices
- general equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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.000911	NEON Science Design for Vectors and Pathogens
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD [06]	NEON.DOC.001155	NEON Training Plan
AD [07]	NEON.DOC.005003	NEON Level 0 Data Products Catalog
AD [08]	NEON.DOC.014002	FSU Science Requirements
AD [09]	NEON.DOC.001100	Lab Protocols: Ground Beetle and Mosquito Specimen Processing

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

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.014051	Field Audit Plan
RD [05]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.3 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 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 make 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 life cycles of most tick species increases 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 were selected as focal taxa to be monitored within the National Ecological Observatory Network. 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 [04]).

3.2 NEON Science Requirements

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS) (see RD [01] and RD [02] for NEON-associated acronyms and terms). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request (see also AD [08]).

3.3 NEON Data Products

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]) (see also AD [07]).

4 PROTOCOL

Tick and tick-borne pathogen sampling involves the collection of ticks using walking, drag, and CO_2 sampling. Following minimal in-house processing samples will be sent to one or more external facilities where ticks will be identified to species. A subset of identified ticks will be tested to quantify the prevalence of infection by various bacterial pathogens. Some ticks will be set aside for long-term archiving.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD [04]). Additional quality assurance will be performed on data collected via these procedures according to the Data and Data Product Quality Assurance and Control Plan (RD [05]).

Because of the wide range and variance of tick abundance and pathogen prevalence, algorithms that check field data for irregularities may catch some errors but will not be a dependable way to fully quality control data from the field. Familiarity with local conditions and data from past years at each site form the basis for corrective intuition regarding quality issues in data collection. Ultimately, following the protocols exactly at each domain will be required to ensure data quality and this can only be known by conducting “hot checks” where someone who knows the protocols goes out into the field with technicians and observes their data collection. For work done by external laboratories, QA/QC plans will be developed based on pre-existing laboratory protocols modified as needed to meet NEON requirements.

When unexpected field conditions require deviations from the field protocols outlined in this document, implementation guidelines outlined in Table 1 should be followed in the interest of maintaining data quality.

Table 1 – Contingent decisions for tick and tick-borne pathogen sampling

Delay Duration	Action	Adverse Outcome?	Outcome for Data Products
≤ 5 days	If the delay occurs prior to the start of the sampling bout, or during the sampling bout, then resume/continue with normal sampling at the conclusion of the delay. Do not adjust (push back) dates for subsequent sampling bouts and note the duration and cause of the delay	Increases potential for temporal variability in data.	Increases potential for temporal variability in data.
> 5 days	If the delay occurs during the sampling bout, then cancel the impacted bout and repeat the entire bout at the conclusion of the delay. Do not adjust (push back) dates for subsequent sampling bouts and note the duration and cause of the delay. Contact associated TOS staff scientists.	Increases potential for temporal variability in data.	Increases potential for temporal variability in data.

6 SAFETY

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.

Exposure to pathogens transmitted via tick bites is a safety issue associated with fieldwork in general but one that is of particular concern for sampling activities outlined in this protocol since exposure to or handling of a relatively large number of ticks actively seeking a host is likely during implementation of this protocol. 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.

If used insecticide must be applied at least 30 minutes prior to arriving in the field. If using insecticide in spray form do not apply in the vicinity of sampling equipment. After applying insecticide clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment.

7 PERSONNEL REQUIREMENTS

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

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (AD [06]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (AD [05]).

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

9.1.1 Sampling Frequency

There are four distinct sampling options associated with tick and tick-borne pathogen sampling.

1. Spatial sampling: Spatial sampling is conducted during the first year of operations and potentially in subsequent years (Appendix A). A single bout of spatial sampling will involve 15 minutes of drag sampling in up to 20 plots at a site. Three bouts of spatial sampling (one every 3-6 weeks depending on the length of the growing season and pattern of tick phenology at the site) will occur during spring/summer months when the local abundance of nymphal and/or adult-stage ticks is at or near its peak. Plots will be randomly located in vegetation types with greater than 5% cover at a site. The initiation of sampling and inter-bout interval will be specified by the NEON disease ecologist for each domain based on local patterns of seasonal tick phenology.

2. High intensity sampling: A single bout of high intensity sampling will involve a) 160m of drag sampling around the perimeter (fixed path transects) of each of 6 plots at a site and b) a 24 hour deployment of a single CO_2 trap at the center of each plot. Bouts of high intensity sampling will occur at a frequency of once every three weeks. Plots will be randomly located in vegetation types with greater than 5% cover at a site. High intensity sampling will be conducted year-round unless specified collection thresholds that designate the end of high intensity sampling and beginning of off season sampling are crossed.

3. Low intensity sampling: Identical to high intensity sampling except that bouts occur once every six weeks.

4. Off season sampling: Off season sampling is conducted for the purpose of determining the start of high (or low) intensity sampling each year in domains where sampling intensity can be greatly reduced during cold months of the year. Within a domain, the end of high (or low) intensity sampling will occur following three consecutive zero-catch bouts at the indicator site (to be specified by the NEON disease ecologist). This result will initiate weekly off season sampling. A single bout of off season sampling will involve drag and walking sampling (according to the high and low intensity sampling protocol) in each of three sampling plots at the indicator site only. Plots will be randomly located in vegetation types with greater than 5% cover at a site. A bout of off-season sampling will only be performed if the high temperature on the day prior to planned sampling was >0°C and the mean daily high temperature in the

five days prior to planned sampling was >7°C. Off-season sampling will continue until >3 ticks of any lifestage are collected in a single off season bout. This will mark the resumption of high (or low) intensity sampling (walking, drag, and CO_2 sampling at six plots per site) at all sites in the associated domain.

9.1.2 Criteria for Determining Sampling Dates

The initiation and timing of spatial sampling in the first year of operations will depend on local patterns of seasonal tick phenology (i.e., demographic cycles and activity periods). Criteria to determining the nature and timing of sampling in subsequent years is outlined in Appendix A. If spatial sampling is repeated in subsequent years the initiation and timing will be the same as in the first year of operations. In the year in which high or low intensity sampling begins, it will start at the same time as spatial sampling in previous years or as soon thereafter as financial and/or logistic circumstances will allow. Following the start of high or low intensity sampling the frequency and timing of sampling bouts will be consistent through time (i.e., constant inter-bout interval) according to the sampling frequencies detailed above and outlined in Appendix A. Transitions between high/low intensity sampling and off season sampling will be associated with specific tick collection thresholds.

9.1.3 Sampling Timing Parameters

Drag sampling should only be conducted when conditions are dry (e.g., not during or immediately after a rain events or on a morning with heavy dew). Additionally, while drag sampling can be conducted during any time of day, the hottest period of the day (mid to late afternoon) should be avoided if possible. CO_2 sampling should occur under similar conditions as those outlined for drag sampling. CO_2 traps should be set immediately after the completion of drag sampling at a site and retrieved 24 hours later.

9.2 Equipment and Materials

Table 2: Field equipment list

Table 2 – Field Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Handheld GPS		No	No
	Lint rollers and/or duct tape		No	No
	Drag cloth		No	No
	Drag cloth weights		No	No
	Transect tape (recommended)		No	No
	Pin flags		No	No
	Forceps		No	No
	Hand lens		No	No
	Stopwatch		No	No

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Sample vial (10mL centrifuge tube recommended)		No	No
	Printing paper (for datasheets and labels)		No	No
	Pencils (recommended)		No	No
	Clipboards (recommended)		No	No
	Ethanol (90%)		No	No
	Resealable freezer bags		No	No
	CO_2 traps		No	No
	Masking tape		No	No
	Dry ice		No	No
	Gloves for handling dry ice		No	No
	Cardboard cards (recommended)		No	No
	Insulated cooler		No	No
	Reusable ice packs (O°C)		No	No
	Fragrance free laundry detergent		No	No

9.3 Preparation

At least one week prior to a sampling bout:

1. Identify the locations of sampling plots and determine how to access them.

Just prior to heading to the field for sampling:

1. If used insecticide must be applied at least 30 minutes prior to arriving in the field. If using insecticide in spray form do not apply in the vicinity of sampling equipment. After applying insecticide clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment.

9.4 Sample Collection in the Field

9.4.1 Drag and walking sampling

1. Travel to the sampling plot using maps and/or a handheld GPS as necessary. Be sure not to transit through any portion of the plot during this travel.
2. Before you begin drag sampling perform an inspection of your and your partner's clothing, esp. shoes and pants. Use forceps to remove any nymphal and adult ticks (Figure 1) that may have become attached prior to the start of sampling (grab ticks by a leg). Place these ticks into an empty (not containing ethanol) sample vial labeled "release" for release following completion of the sampling.
3. When performing spatial sampling:
   a. You may begin sampling at any spot along the perimeter of or within the plot.
   b. Place a pin flag at the point where you will begin dragging.

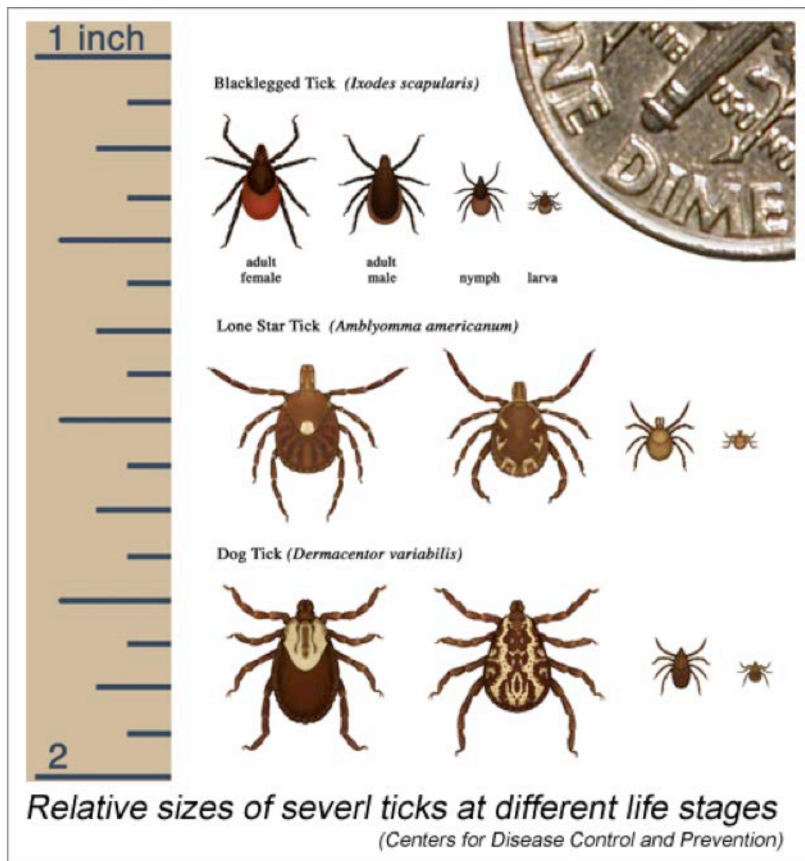


Figure 1

c. You may follow a haphazardly selected path while dragging. This means that the path of the sampling is neither deterministic / fixed (e.g., always walking a straight line with a particular heading) nor random in the strict sense (Figure 2). The path may follow an arbitrary course with three caveats:

i. The path must remain in the vegetation type that the plot is associated with. For example, if the plot is categorized as being in oak woodland vegetation but is close to an adjacent patch of meadow vegetation, be sure to confine sampling to the woodland and not stray into the meadow.
ii. The path should not overlap the path by which you arrived at the plot.
iii. The path should not cross itself at any point during the drag.

d. Each time you change direction while walking the drag path mark your turning point with a pin flag so that you will be able to retrace your steps and measure the total distance covered during the drag at the conclusion of sampling in a plot.

e. Drag sample within the plot for 15 minutes of stopwatch time (actual dragging of the cloth, not including pauses to collect ticks).

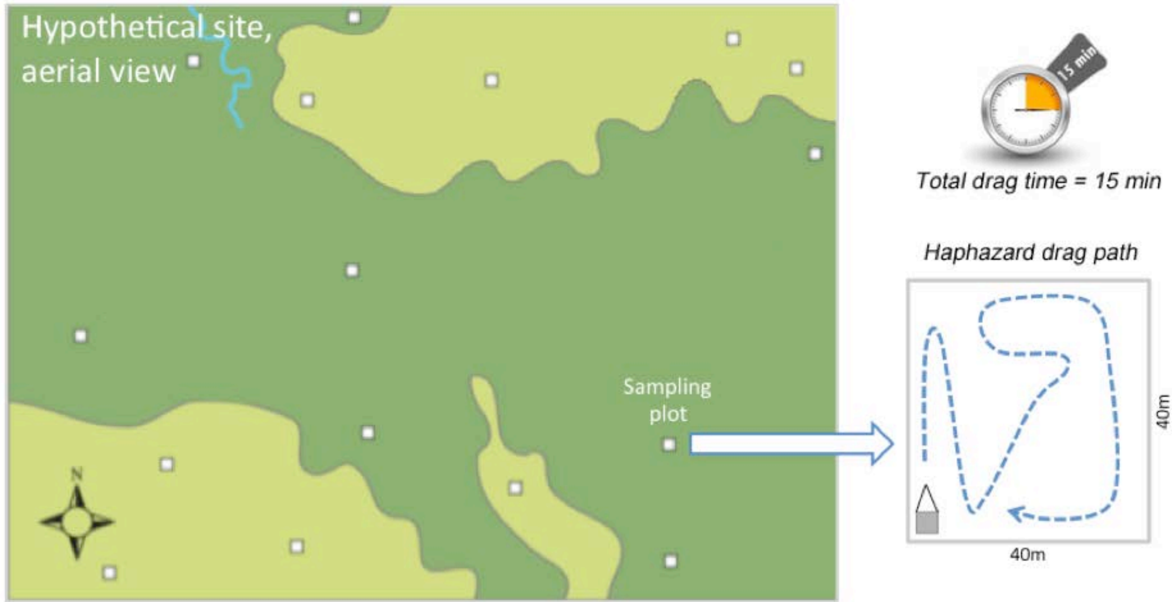


Figure 2

4. When performing high intensity, low intensity, or off season sampling:
   a. You must begin sampling at one of the four corners of the plot.
   b. The drag path must follow the perimeter of the sampling plot in either a clockwise or counterclockwise direction.
   c. The path must follow the shortest straight-line distance between pot corners (these are square plots) (Figure 3). The perimeter of the plot thus becomes a permanent transect for this sampling.

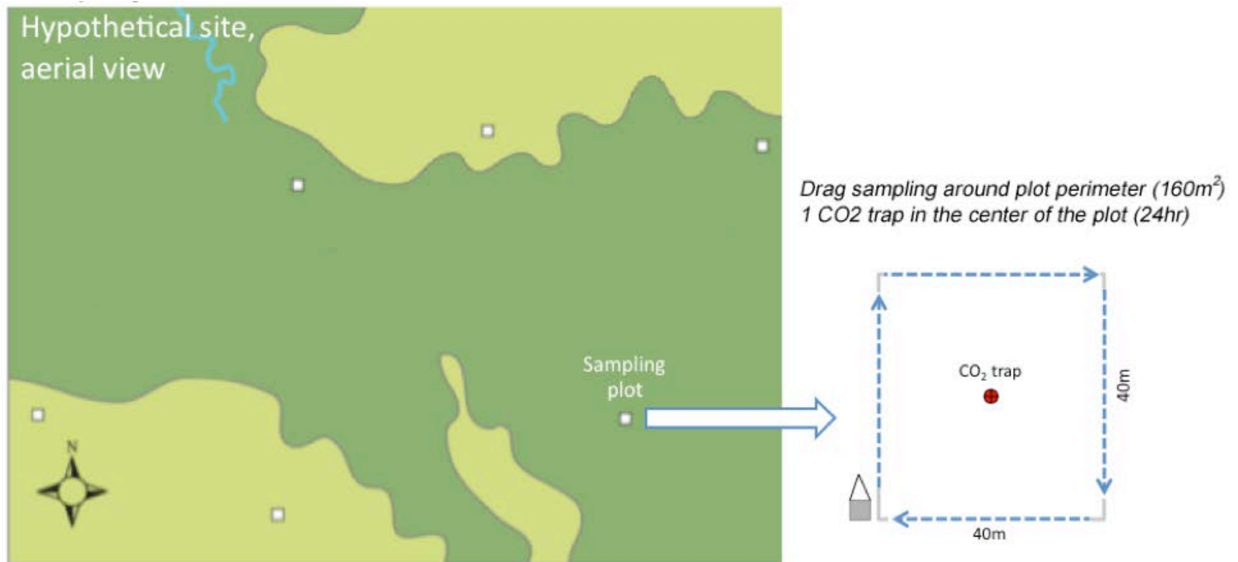


Figure 3

5. Place the drag cloth on the ground, start the stopwatch (if performing spatial sampling) and commence dragging.
   a. One member of the team should pull the cloth. Ensuring that the pace of forward progress is slow and steady (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 slow pace.
   b. Make sure there is plenty of pull cord between your person and the cloth so that the leading edge of the cloth stays as flat as possible on the ground. Too little pull cord will cause the leading edge of the drag cloth to rise up and not contact the ground.
   c. Weights may be attached to the edges of the drag cloth if wind is blowing the cloth up off of the ground.
   d. The other member of the team should walk behind the drag cloth, making sure that the entire cloth stays in contact with the ground (e.g., edges don't fold up) and does not flip over, get bunched up, or become caught on plants or rocks.
6. After dragging for 3-7 meters, stop to count and collect attached ticks. Stop the stopwatch during this period if you are performing spatial sampling.
   a. For ticks on the drag cloth, hold the cloth vertically and scan it in a systematic manner such that you examine the entire cloth on both upper and lower surfaces.
   b. Use the hand lens as necessary to distinguish larval and small nymphal ticks from dirt. Remove any nymphal and adult ticks (Figure 1) with forceps and place them into a sample vial containing 90% ethanol.
   c. Be mindful of the fact that ticks may attempt to crawl onto your hands, arms, or body while you hold the drag cloth.
   d. Additionally, both team members should inspect their bodies for attached ticks. Any attached nymphal or adult ticks should be placed into the sample vial and added to the count of ticks collected.
   e. All of the nymphal and adult ticks collected during sampling at a given plot can be collected into the same sample vial. Additional sample vials can be used if a single vial cannot hold all of the ticks collected during sampling at a given plot.
   f. During spatial sampling the presence of larval ticks should be recorded as a note on the datasheet but these do not need to be counted or collected. Larval ticks only need to be removed from the drag cloth at the conclusion of sampling at a plot (to prevent moving ticks to another sampling plot)
   g. During high intensity, low intensity and off-season sampling collect any larval ticks (from the drag cloth only, not from your clothing/bodies) using masking tape. Affix tape with attached samples to a cardboard card (Figure 4). Larval ticks do not need to be counted in the field but their presence should be recorded as a note on the datasheet.

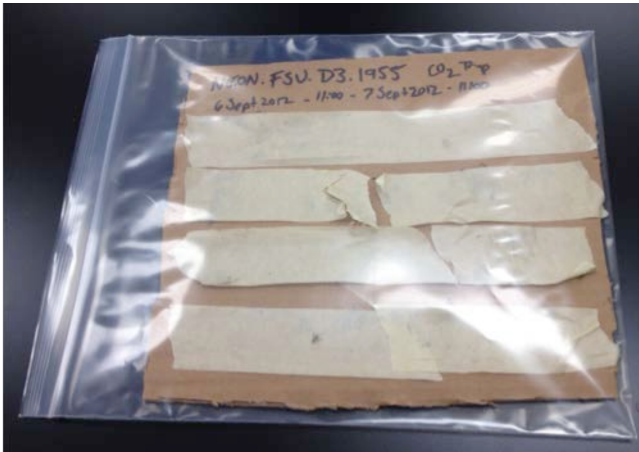


Figure 4

7. Record the number of nymphal and adult ticks collected on the datasheet (Appendix B).
8. Resume dragging as before, restarting the stopwatch when conducting spatial sampling, and stopping again at 3-7m intervals to count and collect attached ticks as described above. Don't forget to stop the stopwatch when counting/collecting ticks during spatial sampling.
9. At the end of dragging (15 minutes for spatial sampling, plot perimeter for high intensity, low intensity and off-season sampling) perform a final collection and count of ticks attached to the drag cloth and technician clothing as described above.
10. Externally label each sample vial. Labels can be pre-printed on adhesive backed paper or written directly onto sample vials using a permanent marker. A label consists of the site (four letter code), plot (three digit code), date (YYYMMDD) followed by a fraction indicating the number of the vial relative to the total number of vials containing ticks collected during a single site/plot/bout combination. As an example, "OSBS00220130802 2/3" would indicate that the labeled vial is the second of three vials containing ticks collected in plot 002 at Ordway Swisher Biological Station on August 2, 2013. Label the body rather than the lid of the vial.
11. Place sealed sample vial(s) into an insulated cooler containing frozen reusable ice packs.
12. Label each cardboard card (with attached larval ticks on masking tape) using the same label format as used for sample vials. Place each card into a resealable freezer bag (Figure 4).
13. Place resealable freezer bag(s) containing cardboard cards into an insulated cooler containing frozen reusable ice packs.
14. For spatial sampling record the total distance dragged during the sampling by measuring the distance between each pin flag along the path walked. This can be done using a variety of methods including with a transect tape (recommended) or a rangefinder, or by pacing if you can accurately quantify distance covered by counting your steps. Remove the pin flags as you go.
15. Record relevant sampling information on the datasheet.
16. As you depart from the plot release ticks in the vial labeled "release" 3 meters outside the perimeter of the plot.

9.4.2 CO_2 sampling

(CO_2 traps are deployed during high and low intensity sampling)

1. Following the completion of drag and walking sampling within a plot (step # in section #) walk to the center of the plot and assemble and deploy a single CO_2 trap (more specific details will be added once trap design is finalized.
2. Return to the plot 24 hours later to retrieve the CO_2 trap and collected ticks. If any ticks are attached to the tap at the base of the CO_2 trap remove the tape and affix it to one or more cardboard cards (Figure 4).
3. Move the trap assembly to a location at least 2 meters away from the spot where the trap was placed and then lay the drag cloth directly over this spot. Gently pat the cloth down so that it touches the ground.
4. Pick up the cloth and collect any attached ticks.
   a. Hold the cloth vertically and scan it in a systematic manner such that you examine the entire cloth on both upper and lower surfaces.
   b. Use the hand lens as necessary to distinguish larval and small nymphal ticks from dirt.
   c. Remove any nymphal and adult ticks (Figure 1) with forceps and place them into a sample vial containing 90% ethanol.
   d. Collect any larval ticks using masking tape and then affix tape with attached samples to a cardboard card as described above.
   e. Be mindful of the fact that ticks may attempt to crawl onto your hands, arms, or body while you hold the drag cloth. Do not collect nymphal and adult ticks from you person(s) when collecting ticks from CO_2 traps.
5. Record the number of nymphal and adult ticks collected from the drag cloth on the datasheet. Larval ticks do not need to be counted in the field.
6. Label each sample vial and cardboard card as described above.
7. Place the sample vial(s) and cardboard card(s) (each in a resealable freezer bag) into an insulated cooler containing frozen reusable ice packs.

9.5 Sample Preservation

1. Nymphal and adult ticks are transferred to sample vials containing 90% ethanol in the field.
2. Cardboard cards with attached masking tape to which ticks are affixed are stored in resealable freezer bags in the field.
3. These vials and freezer bags should be stored in insulated coolers containing frozen reusable ice packs during transit back to the domain lab
4. Once back at the domain lab store vials and freezer bags in a freezer (-20°C) or refrigerator (4°C) if a freezer is not available, until they are sent to an external facility for processing.

9.6 Sample Shipping

N/A

9.7 Data Handling

1. Enter data from datasheets into the database provided by CI within seven days of returning to the lab after a bout of data collection.
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

9.8 Equipment Maintenance, Cleaning and Storage

1. Place the drag cloth in a clothes dryer (set to medium) at a laundromat (recommended) or in an ultralow freezer at the domain lab to kill any larval ticks still attached to the cloth. The duration of exposure to these conditions that is required to kill ticks will likely vary by species but 10-15 minutes of heat or 30 minutes of cold should be sufficient.
   a. When a drier is used ticks should generally be separated from cloths during drying.
   b. Ticks killed by freezing will generally need to be manually removed from cloths.
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 before placing in storage.
3. Clean any other equipment as necessary using dilute fragrance-free laundry detergent and a dish sponge and store in a cool, dry place once dry.

10 LABORATORY STANDARD OPERATING PROCEDURE

10.1 Sample Processing Timing

Process samples within one week of returning to the lab.

10.2 Equipment and Materials

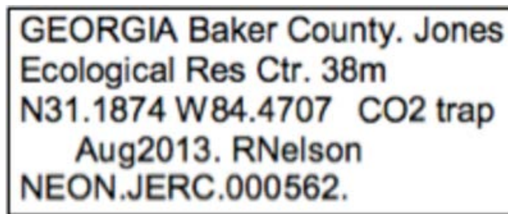
Table 3 – Laboratory Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Forceps		No	No
	Sample vials (10mL centrifuge tubes recommended)		No	No
	Printing paper (for datasheets and labels)		No	No
	Pencils (recommended)		No	No
	Ethanol (90%)		No	No
	Microscope		No	No

10.3 Preparation

1. Prepare locality labels:
   a. Prepare template to print 0.28 in X 0.75 in label
   b. Prepare data for label
   c. Print labels (see Common Insect Lab Protocols (AD [11]) for additional detail on locality labels)

Label Field	Format	Example
State	All capital letters	GEORGIA
County	See example	Baker County
Site Code	Standard abbreviation	Jones Ecological Res Ctr
Elevation	In meters, to the nearest meter	38m
Latitude	In decimal degrees, to 4 decimal places	N31.1874
Longitude	In decimal degrees, to 4 decimal places	W84.4707
Trap Type	See example	Drag sampling or CO_2 trap
Month	Abbreviated to 3 letters	Jun
Year	4 digit year	2013
Collector	Field Operations Manager first initial last name	RNelson
Plot code	NEON.Site Code.Plot Number.	NEON.JERC.000562.



10.4 Sample Processing in the Lab

1. Prepare lab space

10.4.2 Drag and walking sampling

1. Place a locality label into each sample vial containing nymphal and adult ticks. The label material and ink must be compatible with emersion in ethanol.

10.4.3 CO_2 sampling

1. Count and collect ticks.
   a. Use forceps to remove nymphal and adult ticks from the masking tape associated with the CO_2 traps.

b. Count and record the number of nymphal and adult ticks removed from masking tape on the datasheet. These counts should be added to the counts of nymphal and adult ticks collected when the drag cloth was patted down over the spot where the CO_2 trap was deployed.

c. Transfer ticks to a sample vial containing ethanol. Use the same sample vial(s) containing nymphal and adult ticks collected when the drag cloth was patted down over the spot where the CO_2 trap was deployed.

d. Place a locality label into each sample vial containing nymphal and adult ticks collected during CO_2 sampling. The label material and ink must be compatible with emersion in ethanol.

e. Once nymphal and adult ticks have been removed from masking tape, discard the tape and cardboard card(s).

2. Label any new sample vials generated during this processing.
3. Transfer sample vials containing nymphal and adult ticks collected on CO_2 traps into a freezer (-20°C) or refrigerator (4°C) if a freezer is not available.
4. If time permits use a microscope to count the number of larval ticks collected. Record this number on the datasheet.

10.4.4 Off season tick drag

1. Retrieve ticks collected during off-season sampling.
2. Use a microscope to count the number of larval ticks collected. Record this number on the datasheet.

10.5 Sample Preservation

1. Store samples (nymphal and adult ticks in vials, larval ticks on carboard cards) in a freezer (-20°C) or refrigerator (4°C) (if a freezer is not available) until they are sent out for processing at one or more external facilities.
2. Make sure to practice proper sample inventory techniques so that the storage location of a sample can be determined unambiguously at all times.

10.6 Sample Shipping

1. Instructions for sample shipping will be provided by one or more external service providers that will perform taxonomic identification of ticks. All collected sampled (i.e., spatial sampling, high and low intensity sampling, and off season sampling) will be sent out for processing.

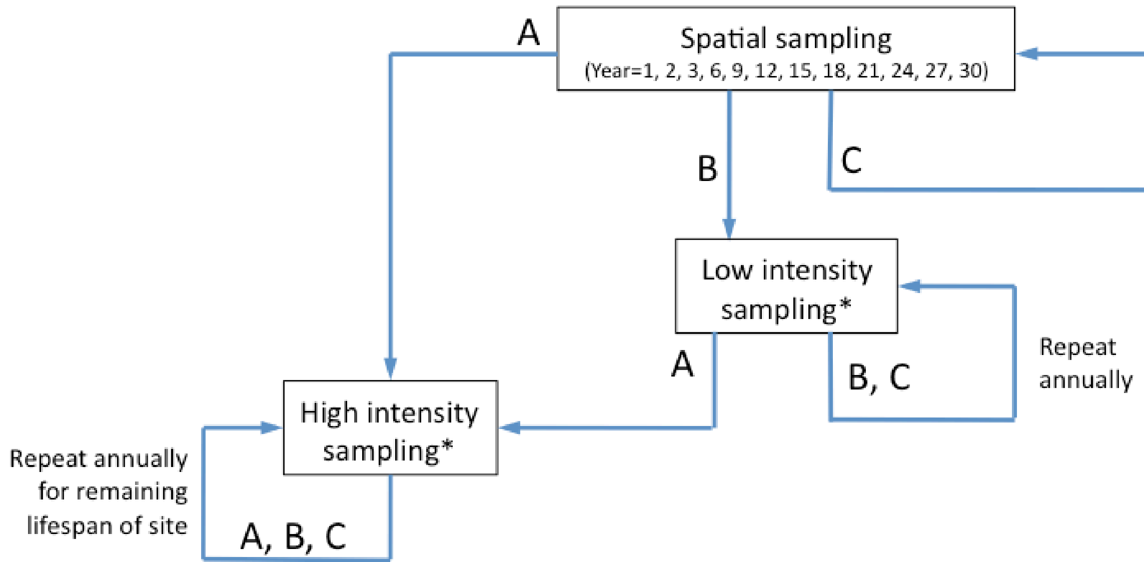
10.7 Data Handling

1. Enter data from datasheets into the database provided by CI within seven days of returning to the lab after a bout of data collection.
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

10.8 Equipment Maintenance, Cleaning and Storage

N/A

APPENDIX A SAMPLING PHASE DETERMINATION



Threshold: ≥6 tick overall or at least two life stages collected during a single bout (there can be multiple bouts during a field season)

Scenarios

A: Threshold reached at the site in question

B: Threshold not reached at the site in question but reached at another site in the same domain

C: Threshold not reached at the site in question or either of the other sites in the same domain

Each continuous arrow path represents a single year (or field season) of sampling unless otherwise noted

*: may transition in/out of off season sampling at some sites

When new sites come online (e.g., relocatables) they start with spatial sampling in the first year of operations. In the second year they assume the lowest intensity sampling plan of the active site(s) in the domain (spatial sampling<low intensity<high intensity) or high intensity tick sampling if the threshold was reached in at least one bout of spatial sampling in the first year.

APPENDIX B TICK AND TICK-BORNE PATHOGEN SAMPLING SPATIAL SAMPLING DATASHEET

NEON tick and tick-borne pathogen sampling, spatial sampling ***Page___of___***

Site:___________________________ Field staff:____

Plot ID	Date of Drag (YYYY/MM/DD)	Drag Start Time (24 HR)	Drag End Time (24 HR)	# nymphal and adult ticks collected	Distance dragged (m)	Label ID
					m	
Notes:						
					m	
Notes:						
					m	
Notes:						

Field	What to fill in
Site	the name of the site (e.g., Ordway, Disney, Jones)
Field staff	the names of staff members performing the field sampling
Plot ID	the unique ID that identifies the distributed plot
Date of Drag	the date when the drag was performed. Use YYYY/MM/DD format
Drag start time	the time when the drag began. Use 24 HR format
Drag end time	the time when the drag was completed. Use 24 hour format
# nymphal and adult ticks collected	the number of nymphal and adult ticks (of all species) collected during the drag
Distance dragged (m)	the total distance covered during the drag (in meters)
Label ID	A label consists of the site (four letter code), plot (three digit code), date (YYYMMDD) followed by a fraction indicating the number of the vial relative to the total number of vials containing ticks collected during a single site/plot/bout combination. As an example, "OSBS00220130802 2/3" would indicate that the labeled vial is the second of three vials containing ticks collected in plot 002 at Ordway Swisher Biological Station on August 2, 2013. Label the body rather than the lid of the vial.
Notes	Can record various details about the sampling bout including environmental conditions and presence/approximate abundance of larval ticks.