NEON Protocol:
Draft NEON FSU Field and Lab Protocol for Ops D3 2012: Tick-borne disease sampling

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A_DRAFT	10/03/2011	ECO-00280	INITIAL DRAFT RELEASE
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1 INTRODUCTION

1.1 Purpose

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. It serves as a change-controlled version of an observatory protocol and is the version used for external review by subject-matter experts. It provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change-controlled document, not through field manuals or training materials.

1.2 Scope

1.3 Acknowledgements

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

2.2 Acronyms

3 BACKGROUND AND OBJECTIVES

3.1 Background

The protocol detailed herein provides instructions for the collection of samples that will be used to quantify spatiotemporal variation in the prevalence of various tick-borne diseases and/or the tick species that transmit them.

Objectives: many organisms have highly specific habitat requirements or associations that warrant spatially focused / targeted sampling. Ticks may be good examples of this as their spatial distributions are often highly clustered. A strategy for selecting sampling plots is required to avoid needless sampling in areas where ticks do not occur or are found in very low numbers. The original sampling strategy involved contracting with local experts to identify these tick habitats at a site and then locating NEON sampling plots within these areas. This approach has a number of problems. The use of local experts could introduce bias into the sampling plan and violates assumptions of the randomized sampling design on which FSU terrestrial sampling is predicated. Further, at the observatory scale, there will likely be sites for which local expertise is not available or reliable. These problems point to the need for an alternative strategy that can be more systematically implemented.

One such option is to oversample sites as part of site characterization, attempting to collect ticks in multiple plots distributed across all of the dominant vegetation types, and let resulting site-specific data on the presence/absence/abundance of ticks delineate local tick habitat associations and drive subsequent plot selection decisions. This approach involves sampling numerous plots and could therefore be time consuming, and one goal for the summer is to assess the magnitude of these logistic challenges. The tentative plan is to identify a set of multiple validated plots in each of the major vegetation types at each site and have NEON technicians perform drag sampling within them quantify spatial variation in tick abundance. A second goal is to prototype the CO_2 trapping method, especially trap design, for future deployment planning.

3.2 Science Requirements

Currently in revision

3.3 Data Products

Currently in revision

Table 1 A summary of field and related lab measurements and the associated NEON Data Products

Measurement	Data Product

4 PROTOCOL

The protocol detailed herein provides instruction for sampling to be conducted at all three sites in Domain 3 during spring and summer of 2012 as part of site characterization activities and methods development. For a number of reasons this sampling is a prototype activity and as such this protocol, including associated objectives/priorities, equipment list, and methods, is still under revision and considered to be in ***draft*** form.

5 QUALITY ASSURANCE AND QUALITY CONTROL

6 DECISION TREE

7 SAFETY

Safety issues specific to this activity include exposure to pathogens transmitted via tick bites while working in wildland areas. The following steps can be taken to reduce the risk of tick bites and exposure to / infection by associated pathogens:

1. Wear appropriate clothing: light colored garments will make it easier to see ticks crawling on your body. Long pants are strongly recommended and pant legs can be tucked underneath socks to provide additional protection. In cases of exposure to very high numbers of ticks, wearing rubber wading/rain boots, tucking pant legs inside boots, and sealing the juncture with duct tape can provide extra protection. Tyvek pants may be used although comfort could be an issue in hot and/or humid environments. A long-sleeved shirt with relatively tight/snug cuffs around the wrists is also recommended.

2. Wear insecticide: insecticide, preferably containing DEET, may be applied prior to heading to the field for sampling. Insecticide should be applied to clothing and around ankles, wrists, waistband, and neck. Be aware that applying insecticide directly to skin may produce an allergic reaction in some individuals. Clothing pretreated with insecticide may also be worn.

   NOTE: extreme care must be taken to avoid contaminating sampling equipment with insecticide as this could bias collection results by repelling ticks. Application of insecticide, particularly in spray form, should be done outside in a well-ventilated area and away from people and sampling equipment. Apply insecticide at least 30 minutes before arriving in the field. Wash or clean hands thoroughly after applying insecticide to avoid transferring it to sampling equipment during handling. Any sampling equipment that accidentally comes in contact with insecticide should be carefully cleaned with perfume-free soap.

3. Perform tick checks: conduct a careful visual inspection of your body following every day of sampling in areas where ticks are common. Once back at home or the a field station strip down and check your body for ticks, paying particular attention to parts of the body where ticks are inclined to hide or bite: in hair, under arms, between legs, behind knees, and along the edges of tight fitting clothing (e.g., waistband). Tick checks can and should be done in pairs to the extent that personal modesty will permit. If performing a tick check alone use a mirror if one is available. Remember that ticks may be present on field clothing, which should also be inspected. If possible, clean field clothing in a drier set on high before washing as the hot, dry air will kill most ticks. Placing the drag cloths used for sampling in a -80C freezer for 2 hours will also kill ticks. Changing into a fresh pair of clothing after sampling, and enclosing clothing worn during sampling in a sealed plastic back for transport back to the lab, home, or laundry facility can reduce exposure to and spread of ticks.

4. Shower: washing with soap and water shortly after returning from the field can effectively remove ticks that have not yet become imbedded.

5. Properly remove imbedded ticks: once imbedded, a tick's feeding bout can often last for multiple days, and for some tick-borne diseases this multi-day exposure is required for transmission of pathogens. As such, prompt removal of imbedded ticks can significantly reduce the risk of pathogen transmission. Use forceps to grasp an imbedded tick as close to the surface of your skin as possible. Pull upward with a steady, even pressure and avoid twisting or jerking the tick as this can cause its head or mouthparts to break off and remain imbedded in the skin. If this happens, attempt to remove the mouthparts with forceps. After removing the tick, thoroughly clean the bite area and your hands with rubbing alcohol, an iodine scrub, or soap and water. Save any ticks that you have removed as they may be useful for later medical identification and testing. You can simply affix the tick(s) to a piece of paper with tape. Make sure to note the date you first noticed the biting tick(s), the date(s) of removal, and if possible, information on where you likely picked the tick(s) up. Do not use matches or other hot objects to try and remove ticks by burning them off, and do not attempt to smother ticks with Vaseline or other oily substances.

6. Communicate with the domain manager: if you have been bitten by a tick you may have been exposed to a tick-borne pathogen and should notify NEON EH&S and the domain manager. Watch for the onset of general symptoms of tick-borne diseases that may develop over the next 3-30 days. These include fever and chills, muscle and/or joint pain, fatigue, headaches, and rashes of various sizes/patterns (e.g., large, circular, "bulls eye" shapes or small, flat, pink, non-itchy spots on wrists, forearms, or ankles). Promptly seek care from a physician should you experience any of these symptoms and be sure to clearly convey that you have been exposed to a tick-borne pathogen. If you were bitten by a tick and have preserved it as described above, make the specimen available to the physician.

8 PERSONNEL REQUIREMENTS

Willingness to work with ticks and potentially pathogenic biological agents is required.

9 TRAINING REQUIREMENTS

The following training will be provided to staff involved in tick-borne disease sampling:

1. Classroom training on field safety: to be provided by NEON EHS. Date(s), location(s), and format(s) of training are TBD.

2. Classroom training on biological and methodological background of tick-borne disease sampling: to be provided by the disease ecologist. The training will take place in June, 2012 at the D3 domain lab. General information about motivation(s) for sampling, methodologies involved in collecting and processing samples, and associated safety risks will be covered during a presentation lasting ~45 minutes.

3. Field training on implementation of sampling methods: to be provided by the disease ecologist. The training will take place in June, 2012 at the Ordway Swisher Biological Station in D3.

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling Frequency and Timing

Initial plans are for sampling using the drag method to occur once in each plot at each of the three sites. Plot locations will be provided by FSU. Sampling should commence as soon as possible following training and be completed in as short a period of time as possible given other scheduling constraints and obligations. Scheduling is flexible but should aim to minimize the amount of time that elapses between sampling at different sites. For example, it is not desirable to perform all sampling at one site in June and all sampling at another site in August as seasonal patterns in tick phenology could impact estimates of tick abundance. Sampling with a given intensity at one site (e.g., dragging in nine plots) should be followed by comparable sampling effort (nine plots) at the other two sites within two weeks. Additinoally, endeavor not to introduce bias into sampling results through daily scheduling of sampling. As an example, do not sample all plots at site one between 7:00 and 10:00 and all plots at site two between 14:00 and 17:00. Avoid similar issues with regard to the timing of sampling in particular vegetation types (e.g., always sampling one type in the early morning versus another just before dusk).

At each site, FSU will provide a list of multiple sampling plots in each of the major vegetation types. Sampling should be done in "sets" such that one haphazardly selected plot in each vegetation type is sampled in sequence (a set). For example, if three major vegetation types exist at a site, sample one haphazardly selected plot in vegetation type one, one plot in type two, and then one plot in type three (a set) before sampling a second plot in type one. Continue sampling in sets until all plots at a site have been sampled. If time permits some plots may be resampled.

Table 2

Domain	Date	Frequency

10.2 Contingent decisions

10.3 Field Procedure

10.3.1 Equipment and Materials

Appropriate clothing: to maintain comfort while facilitating the identification of ticks crawling on the body. At a minimum, light colored long pants and hiking boots are required. Under extreme conditions (exposure to large numbers of ticks) Tyvek pants and rubber wading/rain boots may be worn. A light colored, long-sleeved shirt with tight/snug fitting cuffs is also recommended.

Duct tape: for sealing the seam between pants and footwear/socks as needed and for removing ticks from clothing.

Lint rollers: an optional method for removing ticks from drag cloths (esp. larval stages) and from clothing of field technicians.

Masking tape: for covering vent holes of CO_2 trap insulated coolers prior to deployment and for capturing ticks on the base of the CO_2 trap during sampling.

Stopwatch: to time drag sampling.

Drag cloth: for collecting ticks using the dragging method. A drag cloth is a $1m^2$ piece of white flannel cotton with a circular dowel (wood or PVC) slightly longer than 1m-long attached to one end. A rope or cord is attached to the dowel, allowing the cloth to be pulled along the ground. Drag cloths are not commercially available and so need to be custom fabricated. At least one template cloth will be provided to each domain manager and can be used as a model to fabricate additional cloths. White flannel cotton can be purchased at a fabric store and should be washed and dried prior to construction to prevent subsequent shrinkage (the dimensions of the cloth must be $1m^2$). Once washed and dried, take the white flannel cotton to a tailor and request that a) three edges of the cloth be hemmed, and b) the fourth have a sleeve sewn into it large enough to accommodate the circular dowel (generally not more than 3cm in diameter). Remember to specify that the final dimension of the cloth must be $1m^2$. Insert the circular dowel through the sleeve of the finished cloth and attach one end of a 2-4m long rope/cord to either end.

Weights: for keeping drag cloths in contact with the ground. Under windy conditions drag cloths can be blown upwards and loose contact with the ground. To prevent this, weights can be temporarily attached to the cloth. Fishing weights can be purchased from a sporting goods or tackle shop, and these can be attached to black metal office stationary clips using epoxy. The weighted clips can then be attached to or removed from drag cloths as needed.

CO_2 traps: for collecting ticks using the CO_2 trap method. In the current prototype design a trap will dispense CO_2 from dry ice stored in a 0.5 gallon cylindrical Igloo cooler (same model and type as used in the NEON CO_2 mosquito traps). These coolers will either be purchased from the same vendor from which the mosquito traps are procured or from any hardware or houseware store. Coolers purchased from the latter sources will need to be modified in-house in the same way as the coolers sold for use in mosquito trapping (e.g., by drilling a hole in the bottom and mounting a plastic mason jar lid with holes drilled in it on the bottom of the inside of the cooler using stainless steel screws). The coolers will be deployed on top of a square plastic platform with beveled edges. Thick food service trays flipped upside down serve the purpose well. A 5-inch plastic pot (from a plant nursery) is attached to the center of the platform with Velcro, and the Igloo cooler is inserted into this pot, allowing for easy removal of the cooler and disassembly of the trap. The plastic pot should have a number of drain holes around the base.

Dry ice: for baiting CO_2 traps.

Transect tape: for measuring out drag transects. Tape should be at least 100m in length.

Pin flags: for marking the path walked during drag sampling.

Forceps: for removing ticks from drag cloths.

Hand lens: for locating/identifying ticks on drag cloths.

Sample storage vials: for storing collected adult and nymphal ticks. These vials should have a screw top lid. Volumes will vary depending on the number of ticks typically collected at a site or at the time of sampling. 10ml or 50mL Falcon tubes seem like likely storage vial choices.

Ziplock bags: for holding/transporting masking tape with attached ticks from CO_2 traps.

Fragrance-free laundry detergent: for washing drag cloth and other sampling equipment as necessary.

Datasheets: to record data

Pencils/pens: to write on datasheets

Insecticide: for protection from biting insects including fleas, ticks, and mosquitoes. A product containing DEET is recommended. Note: apply insecticide, especially around wrists, ankles, and neck, at least 30 minutes prior to arriving in the field.

10.3.2 Preparation

At least one week prior to a sampling bout:

1. Make sure that all supplies are available and functionally ready for use. Procure replacement supplies as necessary.

Prior (1-2 days) to the start of a sampling bout:

2. Map sampling locations: the location of sampling plots will be determined by FSU. Prior to going into the field, use a map to identify the locations of these points at the site and determine how to access them.

Just prior to heading to the field:

3. Bait CO_2 traps: fill the insulated coolers of the CO_2 traps with dry ice and place masking tape over their vent holes to minimize sublimation prior to deployment.

4. Apply insecticide, especially around wrists, ankles, and neck, at least 30 minutes prior to arriving in the field. Clean the palms of hands (e.g., with soap/water) before handling any sampling equipment.

10.3.3 Sample Collection in the Field

Two sampling methods will be used as part of site characterization activities in Domain 3 during spring and summer of 2012. Sampling will be done in teams of two. Before heading out to begin sampling put on appropriate clothing and apply insecticide as previously specified.

Drag sampling

Drag sampling will be performed at least once in every plot at each of the three sites to estimate the presence/absence and abundance of adult and nymphal ticks.

1. Travel to the first sampling plot and make a mental note of the path by which you arrive at the plot.

2. Once at the sampling plot haphazardly determine the direction of the path you will walk for drag sampling. You 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. Additionally, the path you walk during sampling should not overlap with the path by which you arrived at the plot.

3. Before you being 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 that may have become attached prior to the start of sampling (grab ticks by a leg). Place these ticks into an empty sample vial.

4. Place the drag cloth on the ground, start the stopwatch, and commence dragging. 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). Counting "1 Mississippi" for each step forward is a good approximation of appropriate cadence. 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). The other team member 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 snagged on plants or rocks.

5. Every 20-40 seconds, stop the stopwatch and inspect the drag cloth for ticks. This is made easier by holding the cloth vertically. Carefully check every inch of the cloth, scanning in a systematic manner and examining the lower surface of the cloth before the upper surface. Remove any nymphal and adult ticks with forceps and place them into the sample vial, recording the number of each on the datasheet. Be mindful of the fact that if you touch or hold the drag cloth during this process ticks may crawl onto your hands or arms. Additionally, both team members should inspect their bodies from the waist down for ticks. Any attached nymphal or adult ticks should also be removed, placed into the sample vial, and scored as data.

6. Re-start the stopwatch and resume dragging as before, stopping at 20-40 second intervals to check the cloth and remove/count ticks. Continue this for 20 minutes of stopwatch time (20 minutes of actual dragging of the cloth). You can walk a straight line path if possible or follow a haphazard path that meanders through the plot so long as you a) do not cross your own path (resample an area) and b) remain in the vegetation type associated with the patch.

7. At the end of the 20 minutes of sampling perform a final tick count of ticks on the cloth and your clothing and then release all nymphal and adult ticks in the sample vial.

8. On the field datasheet be sure that you have recorded all relevant sampling information.

9. A few notes:

   A. Although the drag lengths are based on time it would be great if you could measure their total distance in meters. An approximate measurement is fine (e.g., 52m). In the most simple case (the drag is linear) placing a pin flag at the start and end points of the drag will allow you to easily measure the distance with a transect tape. If you drag along a meandering path, place a pin flag at each point where you considerably change your heading and then use the transect tape to measure the distance between each pair of flags along the sampling path, summing the interval distances to estimate the total. It would be good to know how far you are travelling, and how much this varies among individuals, vegetation types, etc.

   B. You will note that the plan is to record only the number of nymphal and adult ticks. Given that larval ticks are frequently present in very high numbers, counting and collecting them seems too difficult and time consuming. There are at least two changes to the initial training

plan that result from this. First, please record exact numbers of nymphs and adults rather than using the categorical scoring. Second, consider it part of your prototyping charge to devise one or more ways to quantify and/or collect the larval ticks. As a first past, would be great if we could just count them, even if approximately. Taking photos of the drag cloth has been suggested…my primary concern is how to remove the larvae from the cloth after the photo so that there is no carry over to the next drag cloth inspection (don't want to double count). Removing larvae from the cloth with tape sort of defeats the purpose since that will probably kill them and prevent their preservation in a manner suitable for long-term archiving and future examination (hard to remove the larvae from the tape without crushing them). So…see if you can come up with any solutions.

CO_2 sampling

CO_2 sampling will be performed opportunistically as part of prototyping of equipment and sampling strategies. Many aspects of this sampling protocol are TBD and there is room for prototyping on multiple fronts (see below). I imagine that most or all of this work will be conduct at the D3 core site at Ordway Swisher since preliminary sampling suggests that ticks are very uncommon at both of the relocatable sites. CO_2 sampling does not have to be conducted in every plot and should be performed opportunistically. Traps can be deployed overnight in areas to get a sense for presence/absence of ticks, their fine scale distribution within plots (e.g., close to vs. far from ecotones) and potentially their diurnal patterns of activity. Additionally, traps should be deployed in areas where ticks are known to occur in order to prototype trap design and deployment options. I might check with Ordway Swisher staff to inquire about the possibility of conducting this work at the location near the Butler building where training was done. At present this location is not a formal plot but there are clearly lots of ticks there.

1. Prior to departing for the field to deploy traps fill the igloo cooler of each trap full of pellet dry ice. Screw the lid of the cooler down tightly and cover the vent hole on the bottom with a piece of masking tape.

2. At the site of deployment place the plastic base platform down on the ground and adjust its position so that all or as much of the bottom edge of the platform is in contact with the ground as possible.

3. Attach a strip of masking tape to the upper portion of the bevel on each side of the plastic base platform. Between ½ and ¾ of the width of the tape should extend away from upper surface of the platform, overhanging the beveled edge of the platform, with sticky side down. Be careful not to move the platform or kick dirt/leaves onto the tape during this process.

4. Attached the plastic flowerpot to the base platform using the Velcro tape.

5. Remove the masking tape covering the vent hold of the trap's igloo cool and place the cooler in the flowerpot. The trap is now set.

6. When retrieving the trap, first check the igloo cooler and note a) whether an ice plug has formed in the vent hole and b) if there is much dry ice left in the cooler (a small quantity of 50-100g is to be expected). Remove the tape strips from the trap's base platform and count the number of

attached larval and nymphal ticks. Record these numbers as being caught by the trap. Place the tape in a ziplock bag or other storage implement for transport back to the lab (where you can prototype methods of removing ticks from the tape).

7. Pick up and move the trap out of the way and place a drag cloth directly over the deployment site. Gently press the cloth down so that it makes contact with the ground. Peel the cloth up and count the number of attached nymphal and adult ticks. Record these numbers as being caught by pressing. Remove these ticks from the cloth with forceps and place in an empty sample vial.

8. Use the drag cloth to drag the area within a 2m radius of the CO_2 trap deployment site. Check the cloth a few times, counting the number of nymphal and adult ticks and recording these numbers as being caught by dragging. Remove these ticks from the cloth with forceps and place into the sample vial.

9. Release all nymphal and adult ticks in the sample vial.

10. On the field datasheet be sure that you have recorded all relevant sampling information. You can distinguish between the number of ticks captured by trapping, pressing, and dragging in the notes section of the datasheet….or come up with a datasheet design that works better for you.

10. A few notes…..quite a number of opportunities for prototyping here, some of which are detailed below:

A. Trap deployment: based on initial results it seems that the volume and sublimation rate of dry ice in the coolers will allow for 24-hour deployments. I would suggest setting traps out in the AM and collecting in the AM, but you can experiment with other options (e.g., noon to noon, evening to evening). You could also set traps and periodically count ticks captured at intervals during the deployment to quantify diurnal variation in tick activity.

B. Trap design: a few things here. First, we need to come up with a rain cover of some sort. When the tape gets wet it tends to loose stickiness and collapse down onto the trap platform where it does not catch ticks. Second, does the flowerpot promote condensation and formation of an ice plug in the igloo cooler vent hole? Third, what sort of masking tape is best for collecting ticks? We want tape sticky enough to catch the ticks but not so sticky that it is hard to remove adult and nymphal ticks from the tape. Fourth, is there a way to remove the ticks (nymphs, adults, and larvae?) from the tape without forceps? For example, a quick soak of the tape in alcohol and then gentle brushing of the tape with a toothbrush? This could be a good solution for collection of larvae. Fifth, any other options for an object to use as the base platform? It needs to have a beveled edge, be sufficiently tall to keep the tape away from leaves and dirt and shouldn't be made of metal, which could heat up and repel ticks

C. Sample collection: in addition to collecting ticks on the masking tape of the trap you should also use the pressing and dragging methods as there will likely be ticks in the vicinity of the trap which have not made contact with it. I have somewhat arbitrarily set the dragging distance as a 2m radius around the trap but you should feel free to explore catch rates at further distances. It

would be interesting to quantify how rapidly tick numbers drop off as you move away from the traps.

10.3.4 Sample Preservation

No ticks will be collected for archiving or analysis as part of sampling activities in D3 during spring and summer of 2012. Incidental collection as part of methods development, however, will occur. As an example, ticks will become attached to the masking tape of the CO_2 traps, and this tape will be brought back to the lab to investigate methods for removing ticks from the tape. These ticks will be sacrificed but not archived.

10.3.5 Sample Shipping (may not be applicable for all Field SOPs)

No samples will be shipped as part of sampling activities conducted in D3 during spring and summer of 2012.

10.3.6 Data Handling (may not applicable for all Field SOPs)

Enter information from field data sheets into a NEON database (likely a Microsoft Excel file) as soon as possible after collection (ideally at the end of each day of sampling) or as directed by the domain manager.

10.3.7 Refreshing the Field Sampling Kit

10.3.8 Equipment Maintenance, Cleaning, and Storage

Larval ticks can be most easily removed from the drag cloth if they have been killed. This can be done by putting the drag cloth in a clothes dryer (set to medium) for ~30 minutes or in a -80C freezer for ~2 hours. If using a dryer it is best for the cloth to actually be dry first to avoid shrinking during drying. Remember that drag cloth dimensions must remain at $1m^2$ to maintain standardized sampling effort.

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 low heat setting to prevent the drag cloth from shrinking. Always make sure the drag cloth is completely dry before placing in storage.

Clean any other equipment as necessary using dilute laundry detergent and a dish sponge and once dry, store in a cool, dry container.

11 LAB STANDARD OPERATING PROCEDURE

Aside from prototyping activities to investigate methods of removing ticks stuck to masking tape on CO_2 traps there are no laboratory activities associated with this exercise.

11.1 Timing

The 2012 D3 sampling activities should commence as soon after the June 2012 training as possible and continue as time will allow until all plots in the sample set have been sampled and prototyping is

satisfactorily complete. If extra time remains additional drag sampling can be conducted. Contact the disease ecologist for details and to make plans.

11.2 Lab Procedure

Aside from the aforementioned prototyping activities, specific details of which are TBD, there are no formal laboratory procedures associated with this sampling.

11.2.1 Equipment and Materials

11.2.2 Preparation

11.2.3 Sample Processing in the Lab

11.2.4 Sample Preservation

11.2.5 Sample Shipping

11.2.6 Data Handling

11.2.7 Refreshing the Laboratory Supplies

11.2.8 Laboratory Maintenance, Cleaning, Storage

12 DEFINITIONS

13 REFERENCES

APPENDIX A Field Data Sheets

Site	Plot	Date	Staff initials	Method	Sampling effort*	# nymphs	# adults	total	Notes
				drag/CO2					
				drag/CO2					
				drag/CO2					
				drag/CO2					
				drag/CO2					
				drag/CO2					
				drag/CO2					
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				drag/CO2					
				drag/CO2					
				drag/CO2					
				drag/CO2					

* for drags, approximate distance covered - for CO_2 traps, start time and duration of deployment

APPENDIX B Lab Data Sheets

APPENDIX C Considerations for implementation

Tick sampling by dragging cannot be conducted during rain and should not be conducted after rain when the ground is still wet. Additionally, if the wind is sufficiently strong to regularly blow a drag cloth (with clip-on weights attached) off the ground it is probably advisable to postpone sampling. CO_2 traps can be deployed during rain but a rain cover should be developed.

APPENDIX D Procedure Checklist

APPENDIX E Contingent Decisions