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## Contents

- 1.0 Purpose/Scope
- 2.0 Responsibilities
- **3.0 Definitions**
- 4.0 Prerequisites
- 5.0 Precautions
- 6.0 Procedure
- 7.0 Implementation and Training
- 8.0 References
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- **10.0 Documentation**



#### 1.0 <u>Purpose/Scope</u>

This procedure provides a standardized method for the collecting ticks to measure the tick population density in a given area. It should be used in conjunction with the SBMS Subject Area *Natural Hazards in the Environment*.

Employee exposure to the hazards of tick borne diseases presents a risk for BNL workers assigned to tasks that occur outdoors in grassy & wooded areas and to BNL employees and visitors who access wooded areas on the BNL campus. This procedure offers a standardized sampling technique to be used to quantify the risk of tick exposure in an area. It also provides the safety measures to ensure the SHSD personnel performing the tick sampling are not exposed to undue risk of contracting a tick borne disease.

## 2.0 <u>Responsibilities</u>

- 2.1 Use of the procedure is to be limited to persons who act under the direction of a competent hazard assessment person and have demonstrated the competency to satisfactorily use the procedures and its safety measures, as evidenced by experience and training, to qualification criteria set by BNL. See Section 7.
- 2.2 Personnel that perform exposure monitoring with this procedure are responsible to follow all steps in this procedure.

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# 3.0 Definitions none

# 4.0 <u>Prerequisites</u>

- 4.1 Area Access: Use appropriate PPE for outdoor area, see Section 5.
- 4.2 Complete TQ-Lyme1.

# 5.0 Precautions

#### 5.1 Hazard Determination:

- 5.1.1 By its very nature, this procedure may be used in areas with a high tick population that pose a risk to the sampler.
- 5.1.2 The procedures do not generate Hazardous Waste. Ticks that are collected are to be preserved in household grade Isopropanol. No waste Isopropanol is generated by this sampling. All Isopropanol is consumed in preservation of the tick samples, but is recycled and not disposed of.

#### 5.2 Personal Protective Equipment:

5.2.1 See attachment 9.1 for the **<u>REQUIRED</u>** PPE to perform this field testing.

# 6.0 <u>Procedure</u>

#### 6.1 **Equipment:**

- 6.1.1 Stereo Microscope
- 6.1.2 Isopropanol (household grade is adequate. This is used as a preservative for tick specimens).
- 6.1.3 Fine tipped Tweezers
- 6.1.4 1" x 3" polyethylene zip lock storage bags or 1 dram glass vials or 3 ounce plastic jars.
- 6.1.5 100 meter tape measure.
- 6.1.6 Dry Ice





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- 6.1.7 Carbon Dioxide Bait Sampler
- 6.1.8 Sampling Flags (1 meter x 1 meter [or 36" x 36"] white flannel.



- 6.2 Done the recommended and required PPE described in Attachment 9.1 prior to entering areas with vegetation. Apply tick repellants as described in Attachment 9.1.
- 6.3 Measure out the sample plot using the fiberglass tape measure. The ideal sample plot is 1 meter wide by 30 meters (100 feet) or 1 meter wide by 50 meters (164 feet) long. When sampling is done to quantify the tick population for research or to determine the need for controls, the results need to be normalized to a specific sample area. 50m<sup>2</sup> is an ideal plot size.
  - 1 meters = 3.2808399 feet
  - 1 feet = 0.3048 meters
- 6.4 Plan sampling for maximum effectiveness in determining tick populations:
  - Observe the seasonality of ticks and know the life stage of tick present at any time of year.
  - Avoid high wind periods.
  - Avoid extreme cold periods.
  - Avoid sampling in the heat of the afternoon.
  - Do sample in the mid-morning and early evening.
  - When sampling to determine the need for controls in an area, repeat the same sample plot 3 to 4 days in a row to maximize the effectiveness of the tick population density measurement.
- 6.5 **Drag Sample:** Drag the flannel cloth with the short rod and extension string along low grass, lawns, tall grass and very low shrubs. Allow the flag to fully contact the surface. Cover the pre-measured distance of the sample



plot in a single pass 1 meter wide. [Note: When using the drag as a tick population control technique, repeat the drag several times over the same plot to capture more of the ticks that are questing. Control by this method is about 10% on only one pass.]

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- 6.6 **Flag Sample:** Using the flannel cloth flag on a pole, wave and insert the flag 1 meter deep through low braches, tall grass, shrubs, and low tree branches. Flag all levels of the low level plant canopy (up to shoulder height) one-meter deep over the length of the sample plot. Cover the pre-measured distance of the sample plot. [Note: When using the flagging as a tick population control technique, repeat the flagging of the plot several times to capture more of the ticks that are questing].
- 6.7 **Carbon Dioxide Baited Flannel Cloth Sampling:** Place a 1 square meter flannel flag on the ground in the area to be sampled. In the center of flannel cloth, place about 250 ml of dry ice pellets or a chip of 200 cc size onto the cloth. Return for the cloth in 12-24 hours and transfer ticks into plastic bags or into vials/jars containing Isopropanol.
- 6.8 **Carbon Dioxide Tape Trap Sampling:** Place clear 2-inch packing tape around the perimeter of the base of the trap, with the sticking side facing down. Place about 250 ml of dry ice pellets or a chip of 250 cc size into the plastic container in the trap. Place the trap in the desired sample area. Return for the trap in 12- 24 hours and transfer ticks trapped in the tape into plastic bags or into vials/jars containing Isopropanol.
- 6.9 **Removal of ticks from Flag/Drag Samples:** At the end of sampling using, place the flannel on a flat surface and carefully remove all ticks from both sides of the flannel. Use a hand magnifier to assist in viewing larva and nymph stages. Place the captured ticks into a plastic bag or in a jar or vial containing Isopropanol.
  - Avoid using picnic tables and other surfaces that people will occupy later as the surface for counting, unless the surface is covered with Kraft paper or plastic that is removed at the end of the counting session.

#### 6.10 **Documenting Field Sample Information**:

- 6.10.1 Record the sample number on the bag/vial/jar.
- 6.10.2 Record the locations samples, time of day, weather conditions, number of ticks collected, tick species, and the life stage of each specimen on the Field Sampling form (Attachment 9.3).

#### 6.11 **Preparing the flannel for the next sample:**

- If a large number of larvae are encountered on a sample plot, the collection of all the larvae from the flannel is not likely in the field. It is best to use a new flag for the balance of sampling.
- The exposed flag should be exposed to high heat (left on a paved surface in the

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direct sun for several hours) or cleaned with a lint roller and then stored prior to the next sampling round.

# 6.12 **Post decontamination of sampling personnel:** Within 15 minutes of the end of sampling:

- 6.12.1 Remove and closely examine all outer clothing (shoes, socks, pants, shirt, coats, and hat). Check skin & hair for the presence of ticks.
- 6.12.2 Change into non-exposed clothing and shoes.
- 6.12.3 Handle the exposed clothing by one of these techniques:
  - Place the clothing on a paved surface in the direct sun for several hours.
    - Clean the fabric with a tape lint roller.
    - Place the clothes in a laundry dryer and run for at least 30 minutes on heated cycle.
    - Spray the clothing with a Permethrin spray, allow the spray to dry, and store in a cardboard box for at least 24 hours before using.
    - If the clothing was pre-treated with a Permethrin spray within the last two weeks and the clothing has not been wetted, store the clothing in a cardboard box or plastic bag until the next use without additional spray.
    - Place the clothing into a gallon size zip lock bag or plastic bag with a tie and store for at least 10 days.
    - Place the clothing into plastic bag that has a desiccant (such as calcium sulfate or silica gel) and store for at least 5 days.
    - Place the clothing into a cardboard box and store in a location that limits the spread of ticks to occupied areas. Store for at least 20 days before the next use.
- 6.13 **Laboratory analysis of field sampled ticks:** For the nymphal and larval stages of ticks, return specimens to the laboratory for microscopic evaluation of the tick under 10 to 40 x magnification using the stereo microscope. Complete the field sampling form (Attachment 9.3) to document identification results. Use Attachment 9.2 as a reference in the identification of specimens.
- 6.14 **Lab analysis of specimens submitted for ticks attached to personnel:** For the adult, nymphal, larval stages of ticks, examine the specimens in the laboratory with microscopic evaluation under 10 to 40 x magnification using the stereo microscope. Record the analysis on the Lab Analysis form (Attachment 9.4) to document identification results. Use Attachment 9.2 as a reference in the identification of specimens.

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- 6.15 **Disposal of specimens after sampling and identifications:** Do not dispose of any live ticks, either back into the environment or into the trash stream. Keep ticks within a sealed container when disposing of them.
  - The plastic bag will kill the tick specimens within a few days. Hold these samples until the ticks are dead (usually 5 days) before disposal of the sealed bag with ticks in the trash stream.
  - The Isopropanol in jars/vials will kill the ticks within an hour of the immersion on the tick into the Isopropanol. Remove the ticks from the Isopropanol with tweezers and dispose of the dead ticks in a sealed zip lock bag in the trash stream. Recycle the used Isopropanol for the next tick specimens to avoid need to dispose of it as hazardous waste.
- 6.16 **Documenting Sampling Data and Work Conditions readings:** Use the *BNL Field Survey Form* (Attachment 9.3) to record field events and information including the locations samples, time of day, weather conditions, number, species, and life stage of the ticks.
- 6.17 **Results interpretation**: When sampling has been requested by the occupants/owner of an area, a competent person should write a hazard evaluation report that evaluates the survey data and summarizes the potential for occupational exposure to ticks. Ensure that a copy of the hazard evaluation report is sent to the IH Laboratory and is included in the ESHQ Directorate Recordkeeping system.

## 7.0 <u>Implementation and Training</u>

- 7.1 Training prior to using this meter includes a demonstration of proper operation of the instrument based on training, education, and experience. All persons must have met the qualification criteria for IH89 Tick Sampler set in *IH50300 BNL IH Program and IH Group Training & Qualification Matrix*.
- 7.2 Personnel are to document their training using Attachment 9.5, the Job Performance Measure Completion Certificate. Qualification on this JPM is required on a 3 year basis, providing the professional is monitoring noise sources frequently.
- 7.3 Complete web based class TQ-Lyme1 and review Job Risk Assessment SHSD-JRA-05.

## 8.0 <u>References</u>

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- 8.1 US Air Force Technical Information Memorandum No. 26: Lyme disease Vector Surveillance and Control, March 1990.
- 8.2 Web page: <u>www.paru.cas.cz</u>, E. Bouman, Institute of Parasitology, CV|BDV 2001 *Ixodes ricinus* breeding.
- 8.3 Falco, R.; Grounds Maintenance: Controlling deer ticks, 2/1/2002.
- 8.4 National Foundation for IPM Education, Pesticide Environmental Stewardship Program, *Risk Reduction: Demonstration of the '4-Poster' Device for Control of Ticks*, U.S. Dept. of Defense. August 22, 2002.
- 8.5 National Foundation for IPM Education, Pesticide Environmental Stewardship Program, *Deer-Target Application of Pesticides for Control of Ticks*, U.S. Dept. of Defense. August 22, 2002.
- 8.6 Hubalek, Z, et. Al: Medical and Veterinary Entomology (2003) 17, 46-51: Longitudinal surveillance of the tick Ixodes ricinus for borreliae.
- 8.7 Keirans, J.E. and T. R. Litwak, Key to Adult Ixodidae East of Mississippi River, Journal of Medical Entomology, Vol. 26, No. 5

## 9.0 <u>Attachments</u>

- 9.1 Required Personal Protective Equipment
- 9.2 Tick Identification
- 9.3 Field Sampling form
- 9.4 Lab Analysis form
- 9.5 Job Performance Measure: Qualification record

## 10.0 Documentation

Document Development and Revision Control Tracking		
PREPARED BY: (Signature and date on file)	REVIEWED BY: (Signature and date on file)	APPROVED BY: (Signature and date on file)
R. Selvey Date: 04/24/06	E. Lacina Date: 04/27/06	R. Selvey IH Manager Date: 04/28/06
ESH Coordinator/ Date:	Work Coordinator/ Date:	SHSD Manager / Date
none	none	none
QA Representative / Date:	Training Coordinator / Date:	Filing Code:
none	none	IH52

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Facility Support Rep. / Date:	Environ. Compliance Rep. / Date:	Effective Date:
none	none	04/26/06
ISM Review - Hazard Categorization ☐ High ☐ Moderate ☐ Low/Skill of the craft	Validation: Formal Walkthrough Desk Top Review SME Review Name / Date: R. Selvey 4/27/05	Implementation: Training Completed: Tracked in BTMS Procedure posted on Web: 04/28/06 Hard Copy files updated: 04/28/06

Revision Log		
Purpose: 🗌 Temporary Change 🗌 Change in Scope 🗋 Periodic review 🗋 Clarify/enhance procedural controls		
Changed resulting from: Cnvironmental impacts Federal, State and/or Local requirements Corrective/preventive actions to non-conformances on the above		
Section/page and Description of change:		
SME Reviewer/Date:	Reviewer/Date:	Reviewer/Date:

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# Attachment 9.1

# **Required & Recommended Personal Protective Equipment**

Required =	Minimum, mandatory PPE
Recommended =	Optional clothing/PPE that enhances protection
Alternative =	Optional mechanism that replaces [Required] with additional protection
Prohibited =	Clothing that is not allowed

HEAD Light colored hat (Recommended)	
UPPER BODY Light colored, short sleeve shirt (Required) Light colored, Long sleeve shirt (Recommended) Light colored, nylon or polyester wind jacket (Recommended) Tyvek or Kleenguard coverall (Alternative) PVC rain suit (Alternative) Spraying external surfaces with Permethrin Spray (Recommended)	
LOWER BODY Light colored, long pants (Required) Light colored, nylon or polyester wind pants(Recommended) Tyvek or Kleenguard coverall (Alternative) PVC rain suit (Alternative) Spraying external surfaces with Permethrin Spray (Recommended) Short pants (Prohibited)	Fund I have
FEET White socks (Required) Socks tucked into pants leg (bloused) (Required) Socks taped to pants leg (Alternative) Nylon hose bridging shoes to pants (Alternative) Rubber boots taped to pants (Alternative) Tyvek or Kleenguard suit with attached booties (Alternative) Spraying external surfaces with Permethrin Spray (Recommended) Sandals or open toed shoes (Prohibited)	X

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# Attachment 9.2 Tick Identification Principles

# **Taxonomy Key for Long Island Ticks**

Adapted fron: Keirans, J.E. and T. R. Litwak, Key to Adult Ixodidae East of Mississippi River, Journal of Medical Entomology, Vol. 26, No. 5



**Identification of Female versus Male** 

The only official copy is on-line at the SHSD IH Group website. Before using a printed copy, verify that it is current by checking the document issue date on the website.

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Phylogenetic Classification of The Deer Tick: Genus Ixodes

Phylum <u>Arthropoda</u> - Arthropods Subphylum <u>Chelicerata</u> Class <u>Arachnida</u> - Arachnids Order <u>Acari</u> - Mites Suborder <u>Ixodides</u> - Ticks Family <u>Ixodidae</u> - Hard Ticks Genus <u>Ixodes</u> Species <u>scapularis</u> - Deer Tick

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# **Taxonomic Key**

# Adapted from: US Air Force Technical Information Memorandum No. 26: Lyme disease Vector Surveillance and Control, March 1990.

#### (ADULTS)

J. E. Keirans (unpublished, 1986; revised 1987) Acarology Summer Program The Ohio State University

1. Eyes present (may be difficult to see in some male Boophilus and Anocentor nitens)
2. Festoons absent; anal groove distinct, extending anteriorly around the anus; Prostriata (cosmopolitan, common)
3. Scutum ornate or, uncommonly, inornate; palpi elongate, subcylindrical (almost always on reptiles)

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5. Spiracular plate with irregular ridges and partially ornamented with ivory coloration; festoons number 9 (Africa, rare)
<ol> <li>Palpi much longer than basis capituli, palpal segment II much longer than broad</li></ol>
7. Scutum and palpi ornamented, palpal segment III with a dorsal and ventral flange; male with paired adanal, accessory and subanal plates (India, rare
8. Scutum inornate; male with adanal and usually with subanal plates; festoons irregular, partially coalesced (India, Mideast, Africa, common)
9. Palpi extremely short, ridged dorsally and laterally; anal groove indistinct, festoons absent (cosmopolitan, cattle ticks)
10. Festoons absent; leg IV of male greatly enlarged (Africa, rare)
11. Festoons number 7; spiracular plate round with goblets few (ca. 8) and large (Neotropical, common on horses)
12. Basis capituli rectangular; usually ornate (cosmopolitan, common)
13. Scutum usually inornate (4 ornate species); adanal and accessory plates present in the male; coxa IV of male not much larger than I-III, without two long spurs, two short pointed spurs absent on female coxa IV

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# Identification of hard tick Life stages Adapted from: University of Lincoln: Frank L. Ruedisueli & Brigitte Manship



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Adult Identification Step 3: Size of palpi				
On the <u>capitulum</u> :		If so:		
The <u>palpi</u> (A & B) are longer than wide and tick ornate	Amblyomma hebraeum	The tick is of genus: <u>Amblyomma</u> male Amblyomma hebraeum dorsal view A. Article II B. Article III The tick is of genus: Article III Description: The tick is of genus: Article III The tick is of genus: Article III Article III A		
The <u>palpi</u> (A & B) are wider than long and tick has decorations on the <u>scutum</u>	Rhipicephalus evertsi Rhipicephalus evertsi A B dorsal view A. Article II B. Article III C. Basis capituli	The tick is of genus: Dermacentor male Dermacentor reticulatus		

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# TICK IDENTIFICATION GLOSSARY

Adapted from: University of Lincoln: Frank L. Ruedisueli & Brigitte Manship

Anal groove Part of the genitalia Anus Part of genitalia	A. Anal groove B. Anus
<ul><li>Basis capituli Attaches head to body can be various shapes, such as rectangular or hexagonal. Usually comprises porose areas like eyes</li><li>Capitulum Head or mouthpart of the tick made up of palpi and three segments or articles</li></ul>	A. Capitulum C. Basis capituli
Coxa Base of the legs, attachment to body	Internal   Coxal External   Spurs Genital apron Genital groove Coxa Trochanter Spiracular plate

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Eyes Are present at margin of <u>scutum</u>	A. Eyes
<b>Festoons</b> Wrinkles located at the bottom of the back	A. Festoons
<b>Genitalia</b> Reproductive organ of the tick, composed of the genital apron (A), genital groove (B), anal groove (D), postanal median groove (E) and the anus (C)	A. Genital apron B. Genital groove C. Anus D. Anal groove E. Postanal median groove
Haller's organ Sensory structure sensitive to humidity and odors situated at the tip of the first tarsus of the first walking leg	
<ul> <li>Hypostome Extension of the basis capituli, found between palpi</li> <li>Articles or segments of palpi or legs</li> <li>Palpi Part of mouthpart made up of four segments</li> </ul>	A. Article II of palpi B. Article III of palpi C. Hypostome

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<ul><li>Pulvilli Pads present at the end of the legs</li><li>Claws At the end of pads help tick to attach to host</li></ul>	A. Pulvilli B. Claws
Scutum Hard shield found on the back of the tick. Expands over the whole back in males, but only 1/3 of the back in females	A. Female scutum B. Body

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Ticks of Long Island PhotosAdapted from BNL Photos and Internet Site: Entomological Society of AmericaDeer TickDog TickLone Star Ticl

Deer Tick	Dog Tick	Lone Star Tick		
Ixodes scapularis	Dermacentor variabilis	Amblyomma americanum		
Adult Female	Adult Female	Adult Female		
Adult Male	Adult Male	Adult Male		
Nymph	Nymph	Nymph		
Larvae	Larvae	Larvae		



# BROOKHAVEN NATIONAL LABORATORY DEER TICK SAMPLING

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# IH89200 Attachment 9.3

File Code: IH62

DATE:	METHOD: White flannel (3' x 3') SAMPLE SIZE: 100 ft length by 3 ft width	SURVEYOR(S):	
Sky:SunnyPartial CloudyOvercast	Wind:fpmmph	Temp: shade°F; sun°F	RH:% DewPt:°F

Sample #	Time of Sample	Location of Sample	Field ID of Ticks Ix= Ixodes scapularis Dv= Dermacentor variabilis Aa = Amblyomma americanum				
			Total	Adult Male	Adult Female	Nymph	Larvae

Field ID of Ticks Location of Sample Sample Time of lx= lxodes scapularis Dv=Dermacentor variabilis Sample # Aa = Amblyomma americanum Total Adult Adult Nymph Larvae Male Female

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# Tick Laboratory Identification and PCR Test Record

	DATE:	ANALYSIS BY:
Presented By:	NAME:	BNL#:
Patient:	Same BNL Employee	Family Member Name:
Location:	DEPT/DIV:	BLDG:
Contact Info:	BNL phone Number	Email:Outlook Other:
Where At BNL:		Date Of Attachment:

#### ANALYSIS UNDER MAGNIFICATION

	Dermacentor	Ixodes	Amblyomma	
Genus	variabilis	scapularis	americanum	
	Dog Tick	Deer Tick	Lone Star	
Life Stage	Adult Female	Adult Female	Adult Female	
	Adult Male	Adult Male	Adult Male	
	Nymph	Nymph	Nymph	
	Larvae	Larvae	Larvae	
Signs Of	Status Of Hypostome:			
Attachment	IntactPartia	alMissing		
Signs Of	None Partia	al Full		
Engorgement	Blood in Body	Blood Streaki	ng	

### PCR TESTING

Date Sent:		Results Rec	eived:
Sample Id:	BNL MMDDYY ORG	INITIALS #	
Sent To:	Igenex	Imugen	Other:
Analysis For:	Borrelia Burgdorferi	Babesia	Ehrlichiosis
Results	Positive	Negative	Inconclusive



SHSD Industrial Hygiene Program

# IH89200 Attachment 9.5 HP-IHP-89200

# Tick Field Sampling Job Performance Measure (JPM) Completion Certificate

Candidate's Name

Life Number:

# Practical Skill Evaluation: Demonstration of Evaluation Methodology by Oral Exam & Demonstration of Methodology

Criteria C		Qualifying Performance Standard		Recov.	Satisf.
1.	Hazard Analysis	Understands the tick hazards and exposure potential to the self as sampler and workers in the area.			
2.	Personal Protective Equipment	Understands the need for PPE. Demonstrates the correct use of protective clothing and the proper treatment and storage of exposed clothing.			
3.	Sampling Equipment	Knows where equipment needed for the procedure is located and how to properly use it.			
4.	Field Sampling methods	Demonstrates correctly setting up sample plots.			
5.	Field Sampling methods	Demonstrates correctly dragging of ground vegetation & flagging vegetation in the low canopy.			
6.	Field Sampling methods	Demonstrates the correct set up of CO2 Bait traps			
7.	Field Sampling methods	Demonstrates the proper method of recovering ticks from the flannel sample cloth.			
8.	Lab Analysis	Demonstrates knowledge of the principles of identification of tick species, and life stages.			
9.	Specimen disposal	Demonstrates the proper method to dispose of tick specimens and recycle the Isopropanol used as a preservative.			
10.	Documentation	Demonstrates correctly filling out IH monitoring forms.			

# I accept the responsibility for performing this task as demonstrated within this JPM and the corresponding SOP.

Candidate Signature:

I certify the candidate has satisfactorily performed each of the above listed steps and is capable of performing the task unsupervised.

Evaluator Signature:

Date:

Date:

IH-SOP-89200 JPM Form (Preparation Date: 04/2006)