

# Surveillance for *Ixodes pacificus* and pathogens found in this tick species in the United States

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## Intended Audience and Objectives

Public health entomologists/biologists are the intended audience for this document.

The primary objective of this document is to provide guidance for surveillance of the western blacklegged tick, *Ixodes pacificus*, and pathogens found in this tick species in order to provide health care providers and the public with current and accurate information on where this tick occurs, when the different life stages are most active during the year, and which human pathogens are of the greatest local concern.

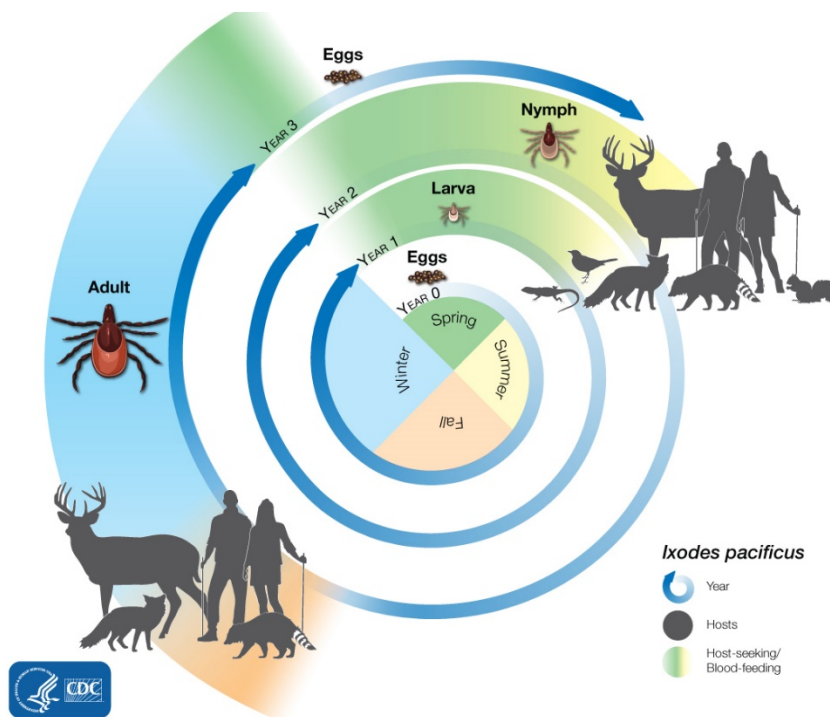
## Public Health Importance of *Ixodes pacificus*

The western blacklegged tick, *Ixodes pacificus*, is the primary vector to humans in the far western United States of *Borrelia burgdorferi* sensu stricto (Lyme disease), *Anaplasma phagocytophilum* (anaplasmosis) and most likely *Borrelia miyamotoi* (*B. miyamotoi* disease) (Table 1).

**Table 1.** Pathogens transmitted by *Ixodes pacificus*, life stages that can be infected, and the human diseases caused by infection with these pathogens.

| Disease                           | Pathogen(s)   | Life stages infected   |
|-----------------------------------|---|------------------------|
| Lyme disease                      | <i>Borrelia burgdorferi</i> sensu stricto,<br><i>Borrelia mayonii</i> | Nymphs, Adults         |
| Anaplasmosis                      | <i>Anaplasma phagocytophilum</i>                                      | Nymphs, Adults         |
| <i>Borrelia miyamotoi</i> disease | <i>Borrelia miyamotoi</i>   | Larvae, Nymphs, Adults |

## Life Cycle of *Ixodes pacificus*



### Generalized life cycle of *Ixodes pacificus*.

*Ixodes pacificus* is primarily a woodland-associated tick. In north-coastal California where the tick has been studied most extensively, it has, minimally, a 3-year life cycle consisting of four life stages: egg, larva, nymph, and adult (Padgett and Lane 2001). Larval and nymphal ticks each take a single bloodmeal before molting to the next life stage and may acquire human pathogens through blood-feeding on infectious hosts or by co-feeding transmission (infected and uninfected ticks feeding in close proximity; pathogen transmission can occur in the absence of a systemic host infection). Larvae and nymphs feed primarily on lizards, squirrels, and ground-dwelling birds. However, they can also infest other mammals including deer. Female ticks take a single bloodmeal (most commonly from deer but also from other medium-sized and large mammals), lay a large batch of eggs and then die. Based on evidence from closely related *Ixodes* species and in contrast to other human pathogens transmitted by this tick, *Borrelia miyamotoi*, is likely transmitted transovarially by *I. pacificus* (vertical transmission from infected females to their offspring). *Borrelia*

*burgdorferi* sensu stricto, and *Anaplasma phagocytophilum* (and likely *Borrelia miyamotoi*) are maintained via horizontal transmission, where infected nymphal or female ticks transmit the agents to vertebrate hosts, and naïve larval or nymphal ticks then acquire pathogens while feeding on the infectious hosts.

Adults are active mainly during the cool months of the year from late fall to early spring. Females typically lay eggs in spring but hatched larvae do not seek hosts actively until the following spring. After blood-feeding, larvae molt to nymphs, but do not begin host-seeking until the following spring. Peak nymphal host seeking is typically observed in May and June, depending on location. After blood-feeding, nymphs molt to adults and seek hosts in the fall, winter or early spring, resulting in a minimum of three years to complete a life cycle (Figure 2; Padgett and Lane 2001). Notably, host-seeking phenology may differ across the geographic range of this tick species. For example, in central and southern California, the host-seeking period, particularly of juvenile ticks is truncated, with activity occurring in March and April (MacDonald and Briggs 2016).

## Tick Surveillance Objectives

Tick surveillance is intended to monitor changes in the distribution and abundance of ticks and the presence and prevalence of tickborne pathogens in order to provide actionable, evidence-based information to clinicians, the public and policy makers. Key questions address when and where humans are at risk for exposure to ticks and tickborne pathogens.

Specifically, at the spatial scale of U.S. counties, CDC aims to:

- 1) classify county status for *I. pacificus*: established, reported, or no data available, and
- 2) classify county status for presence of specific pathogens in *I. pacificus* ticks: present or no data available.

Additional objectives include the following: (3) generate estimates for local prevalence of specific pathogens in relevant *I. pacificus* life stages and local density of host-seeking (infected) nymphs or adults, which then can be aggregated and displayed at county scale; and (4) document host-seeking phenology of all *I. pacificus* life stages in strategic locations across the tick's range and display this information at state or regional spatial scales. For more details on tick sampling methods, please see the "Tick Collection Methods" section of this document.

Objective 1 provides the most basic information for risk assessment (i.e., is the tick known to be reported or established in the county of interest?). Presence of a vector tick species does not necessarily indicate presence of human pathogens, and therefore, Objective 2 provides additional information about potential exposure to *I. pacificus*-borne human pathogens. While documenting the presence of a human pathogen in a county is useful, estimates of infection prevalence in host-seeking ticks (the percentage of ticks tested that are infected) provides a better indication of the likelihood that ticks encountered by humans may be infected with the pathogen of interest.

Tick-borne infections in humans arise following the bite of infected ticks. Therefore, a measure that captures the abundance of host-seeking ticks, often referred to as density of host-seeking nymphs (DON) or females (DOF), provides more accurate information on the likelihood of human encounters than simple measures of tick presence or establishment. That is, although human behavior affects the likelihood of human-tick encounters, assuming similar human behavior across tick habitats, human-tick encounters are likely to increase with increasing DON or DOF. Overall, acarological risk measures such as pursued in Objective 3 that combine the density of host-seeking nymphs

and local estimates of infection prevalence (often referred to as the density of host-seeking infected nymphs or DIN) provide more accurate estimates of human encounters with infected host-seeking nymphs than simple measures of tick/pathogen presence or abundance (Mather et al. 1996, Eisen et al. 2006, Pepin et al. 2012, Eisen and Eisen 2016).

Finally, recognizing that acarological risk measures often differ by life stage, documenting when each life stage is actively host-seeking aids in identifying when humans are at greatest risk for exposure to tick bites and tick-borne pathogens. Therefore, Objective 4 aims to document host-seeking phenology of larval, nymphal and adult *I. pacificus* ticks.

Criteria for classifying county establishment status for *I. pacificus* and estimating infection prevalence, densities of host-seeking (infected) ticks and documenting host-seeking phenology are summarized below. CDC aims to collate tick surveillance data to make county-level data available to the public on national-scale maps that will be displayed on the CDC website. State health departments and other CDC public health partners may submit data through ArboNET. For additional information on ArboNET submissions, please see <https://wwwn.cdc.gov/arboNET> or contact us at [ticksurveillance@cdc.gov](mailto:ticksurveillance@cdc.gov). Additional information can be found in subsequent sections of this document.

## Classify County Status for *Ixodes pacificus*

- **Objective: Update the *I. pacificus* distribution map based on county level establishment criteria** (Dennis et al. 1998). Data will be displayed at: <https://www.cdc.gov/ticks/surveillance/>
- County status classification criteria are as follows:
  - Established:  $\geq 6$  *I. pacificus* of a single life stage or  $> 1$  life stage collected per county within a 12-month period
  - Reported:  $< 6$  *I. pacificus* of a single life stage collected per county within a 12-month period
  - No records
- For this objective and all others, ticks should be identified to species and life stage using published taxonomic keys (e.g., Keirans and Clifford 1978, Furman and Loomis 1984).
- For counties reporting new records, voucher specimens supporting the status change should be archived.
- Because we have greater confidence in presence than absence data, after a county is classified as “established,” it will remain so and will not regress to “reported” or “no records” status. Counties classified as “reported” may progress to “established” and counties classified as “no records” may progress to “reported” or “established” when criteria for those classifications have been met. After a county is classified as “established” surveillance efforts should focus instead on pathogen presence and prevalence and assessments of acarological risk of human exposure to *I. pacificus*-borne pathogens.

## Identify Presence and Prevalence of Human Pathogens in *Ixodes pacificus* Ticks

- **Objective: Map the county level distribution of human pathogens in *I. pacificus* ticks or in natural hosts for this tick.** Data will be displayed at: <https://www.cdc.gov/ticks/surveillance/>
- Data to be mapped include:
  - Shading counties where the *I. pacificus*-borne pathogen of interest has been detected in *I. pacificus* ticks or in natural hosts of *I. pacificus*. This is a simple binary response (pathogen detected or not). Pathogen detection assays must meet minimal assay requirements described in “Minimum Criteria for Acceptability of Pathogen Detection Assay.” Samples from which potential exposure could have occurred in other counties will not be included (ticks from people or pets are not acceptable unless travel outside of the county within 10 days prior to detection of the tick can be ruled out) but infection in ticks collected from the environment (by dragging, flagging, walking, or trapping) or infection in ticks collected from trapped mammals (provided their home ranges are limited enough

- to infer exposure occurred in the county of interest) are acceptable for documenting presence of pathogens in a county.
- For counties where the pathogen of interest already has been detected in *I. pacificus* ticks (this information will be updated annually on <https://www.cdc.gov/ticks/surveillance/>), pathogen prevalence and 95% confidence intervals can be estimated per relevant tick life stage and per collection site in Excel using the [Pooled Infection Rate Add-In](#). Inclusion of confidence intervals is recommended in addition to point estimates in order to convey the level of uncertainty in point estimates. Confidence intervals can be interpreted as “there is a 95% probability that the true infection prevalence is between [insert lower confidence limit] and [insert upper confidence limit].” As sample sizes increase, the width of the confidence intervals decrease. Typically, testing 50 nymphal or adult ticks per site gives reasonable confidence limits for most *I. pacificus*-borne pathogens. For example, when 10 of 50 tested ticks are positive, infection prevalence is estimated as 20% (95%CI: 11-33%). Likewise, if no ticks are infected in a sample of 25 or 50 ticks, infection prevalence could be as high as 13% or 7%, respectively. Although infection prevalence can be calculated for smaller sample sizes, uncertainty in estimates is high; pathogen prevalence will not be displayed unless a minimum of 25 ticks have been tested within a given county for a given life stage. Overall, estimates of infection prevalence improve with increasing sample sizes. Infection prevalence and associated 95% confidence intervals will be calculated by CDC for data submitted to ArboNET.

## Estimate the Density of Host-Seeking (Infected) *Ixodes pacificus* Ticks

For each of the objectives listed below, when sufficient data have been submitted to ArboNET, CDC will post annual surveillance reports at <https://www.cdc.gov/ticks/surveillance/>.

- **Objective: Map the county level density of host-seeking *I. pacificus* nymphs.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking nymphs collected per 100 m<sup>2</sup> or displayed as the inverse showing the distance covered before expected encounter with a nymph.
    - Requires at least 750 m<sup>2</sup> drag sampled per site for density estimate; drags should be inspected for ticks at consistent intervals and at least every 10-20 m; sampling should be timed to coincide with the peak in nymphal host-seeking activity (Eisen et al. 2010, Diuk-Wasser et al. 2012); ideally, estimates of nymphal density should be based on at least 2-3 visits to the site within the perceived seasonal peak in host-seeking (Dobson 2013). For more information on sampling, please see: “Estimating the Density of Host-seeking (Infected) *Ixodes pacificus* ticks.”
    - Requires at least 1 site sampled per county, otherwise county will be displayed as “no records.”
    - In ecologically diverse counties, sampling at multiple sites representing the range in suitable habitat for the tick is recommended; when multiple sites are sampled per county, average and range will be accessible.
    - Although timed sampling (e.g., dragging for fixed amounts of time, rather than fixed distances) is a valid sampling approach, in the interest of comparability among localities, we will only accept distance-based assessments of DON and DIN for ArboNET.
- **Objective: Map the county level density of host-seeking infected *I. pacificus* nymphs.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking infected nymphs collected per 100 m<sup>2</sup> or displayed as the inverse showing the distance covered before expected encounter with an infected nymph.

- Calculated by multiplying the estimated density of nymphs by infection prevalence (both described above).
    - When multiple sites are sampled per county, average and range will be accessible.
- **Objective: Map the county level density of host-seeking *I. pacificus* females.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking females collected per 100 m<sup>2</sup> (DOF) or displayed as the inverse showing the distance covered before expected encounter with a female tick.
    - Requires at least 750 m<sup>2</sup> drag sampled or flagged per site for density estimate; because adults drop off more readily than nymphs, drags or flags should be inspected for ticks every 10 m; sampling should be timed to coincide with the peak in adult host-seeking activity; ideally, estimates of female density should be based on at least 2-3 visits to the site within the perceived seasonal peak in host-seeking.
    - Requires at least 1 site sampled per county, otherwise county will be displayed as “no records.”
    - Sampling at three or more sites per county is recommended; when multiple sites are sampled per county, average and range will be accessible.
    - In ecologically diverse counties, sampling at multiple sites representing the range in suitable habitat for the tick is recommended; when multiple sites are sampled per county, average and range will be accessible.
    - Although timed sampling (e.g., dragging for fixed amounts of time, rather than fixed distances) is a valid sampling approach, in the interest of comparability among localities, we will only accept distance-based assessments of DOF and DIF for ArboNET.
- **Objective: Map the county level density of infected host-seeking *I. pacificus* females.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking infected females collected per 100 m<sup>2</sup> or displayed as the inverse showing the distance covered before expected encounter with an infected female tick.
    - Calculated by multiplying the estimated density of females by infection prevalence in tested adult ticks (both described above).
    - When multiple sites are sampled per county, average and range will be accessible.

## Document Host-Seeking Phenology of *Ixodes pacificus* Ticks

- **Objective: Describe when *I. pacificus* ticks are actively host-seeking (phenology).**
- Data display and minimal sampling requirements include:
  - Displayed as region of the state, or state-wide (or neighboring state) records of tick activity by life stage. This will be a categorical response (records of the tick being active for a particular month of the year or not, or no records if phenology studies were not reported from a particular state or its neighbor).
  - Based on weekly, bi-weekly, or monthly non-removal sampling over a 12-month period, excluding winter months too cold for tick activity in colder parts of the tick’s range and excluding months that are too hot for tick activity in the warmer parts of the tick’s range. For more information, see “Describing Host-Seeking Phenology of *Ixodes pacificus* Ticks.”



# Tick Collection Methods

Several methods can be used to collect *I. pacificus* ticks, however, some are better suited than others for addressing specific surveillance objectives (Table 2). For example, all of the methods described below can be used to demonstrate the presence of *I. pacificus* or *I. pacificus*-borne pathogens in a county of interest. Demonstrating that both the vector and pathogen are present within a county provides fundamental data for assessing the risk of human encounters with infected ticks. However, for Lyme disease, which is most commonly acquired through the bite of infected nymphs, estimates of the density of *Borrelia burgdorferi sensu stricto* (s.s.)-infected host-seeking nymphs are a more accurate predictor of human Lyme disease occurrence than simple measures of the presence of the tick or pathogen, or quantitative measures of the density of host-seeking nymphs or the infection prevalence in the nymphs alone (Mather et al. 1996, Stafford et al. 1998, Pepin et al. 2012, Eisen and Eisen 2016))

**Table 2.** Summary of tick collection methods that are acceptable or unacceptable for each surveillance objective.

| Collection Method   | Objective:<br>Classify county<br>status              | Objective:<br>Presence/Prevalence of<br>pathogens in ticks | Objective: DON/DIN<br>or DOF/DIF | Objective:<br>Phenology |
|---|--|--|----------------------------------|-------------------------|
| Dragging/Flagging   | Acceptable   | Acceptable   | Acceptable                       | Acceptable              |
| Walking   | Acceptable   | Acceptable   | Not Acceptable                   | Acceptable              |
| CO <sub>2</sub> traps   | Acceptable   | Acceptable for presence,<br>but not prevalence             | Not Acceptable                   | Not<br>Acceptable       |
| Ticks collected from deer   | Acceptable   | Acceptable for presence,<br>but not prevalence             | Not Acceptable                   | Not<br>Acceptable       |
| Ticks collected from small-<br>or medium-sized mammals,<br>birds, lizards | Acceptable   | Acceptable for presence,<br>but not prevalence             | Not Acceptable                   | Acceptable              |
| Ticks from people/pets  | Acceptable, if<br>travel history is<br>accounted for | Acceptable for presence,<br>but not prevalence             | Not Acceptable                   | Not<br>Acceptable       |

## Drag Sampling or Flagging

### Background and methods

Drag sampling and flagging are similar methods used to collect host-seeking *Ixodes spp.* ticks (Daniels and Fish 1990, Carroll and Schmidtman 1992, Falco and Fish 1992; Tälleklint-Eisen and Lane 2000). Both typically use a 1 m wide by 1 m long flannel, denim or other sturdy white fabric with sufficient texture for ticks to grip. To increase contact between the fabric and vegetation, weights (e.g., metal washers or chains) may be sewn into the trailing edge and/or the trailing edge may be cut into “fingers” or “strips” rather than using a solid cloth. Modified handles (e.g. wooden dowel or rope) may be used to increase maneuverability. For additional details on how to make tick drags, please see the “How to Make Tick Drags” supplemental information. The tick drag or flag is moved horizontally across

vegetation or leaf litter (drag) or more vertically (flag). This method of sampling provides good spatial precision for documenting the occurrence and/or abundance of ticks in a county.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus*
- Identifying presence and prevalence of pathogens in ticks (all active life stages)
- Estimating the density of host-seeking (infected) nymphs or females; although either dragging or flagging can be used, horizontal distance sampled should be reported to ArboNET
- Documenting host-seeking phenology

## Walking Sampling

### Background and methods

Walking sampling entails an investigator walking through tick habitat and checking his/her clothing and body for crawling ticks (Carey et al. 1980, Schulze et al. 1986, Lane 1996). The distance walked and the number of ticks encountered per distance should be recorded. Investigators typically wear light-colored clothing to more easily detect ticks on clothing. Long sleeves and long pants, tucked into socks, are required to reduce the risk for tick bites. This method of collection may be more accurate for assessing human-tick encounters than drag sampling, flagging or collection from hosts or carbon dioxide baited traps, but more so in areas with emergent vegetation for ticks to ascend than in leaf litter where tick exposures more commonly may be related to human behaviors exposing legs or hands/arms directly to the substrate (e.g., when playing or doing yardwork). Walking sampling generally yield fewer adult *I. pacificus* compared with dragging (Lane 1996). This method of sampling provides good spatial precision for documenting the occurrence and/or abundance of ticks in a county.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus*
- Identifying presence and prevalence of pathogens in ticks (all active life stages)
- Documenting host-seeking phenology

## Carbon Dioxide–Baited Tick Traps

### Background and methods

Carbon dioxide traps work on the premise that ticks have well-developed chemo-receptors and are attracted to carbon dioxide to find a host. Traps consist of a solid base to hold dry ice (a solid form of carbon dioxide) within an insulating material that is surrounded by a sticky tape to capture ticks attracted to the carbon dioxide released as the dry ice sublimates (Wilson et al. 1972). These traps were developed originally for collection of lone star ticks (*Amblyomma americanum*) which display a more aggressive and mobile host-seeking behavior compared with *I. scapularis* or *I. pacificus*. Carbon dioxide traps capture *I. scapularis*, but appear to be less effective than drag sampling or flagging (Ginsberg and Ewing 1989, Falco and Fish 1992). Carbon dioxide trapping is generally less labor-intensive than several other tick collection methods, but because of its inefficiency at collecting *I. scapularis*, it is not recommended for assessments of host-seeking densities *I. scapularis* or *I. pacificus*. However, this method of sampling provides good spatial precision for documenting the occurrence and/or presence or prevalence of pathogens in a county.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus*
- Identifying presence and prevalence of pathogens in ticks (all active life stages)

## Tick Collection from Deer

### Background and methods

Columbian black-tailed deer serve as important hosts for adult *I. pacificus* ticks. Inspection of hunter-killed deer brought into check stations is a cost-effective means of detecting changes in the distribution of *I. pacificus*, particularly in areas where the tick is emerging. However, owing to the home range of deer, it is spatially non-specific. Based on studies of closely-related *I. scapularis*, abundance of ticks on deer may not correlate well with estimates of host-seeking tick densities obtained from drag sampling (French et al. 1992, Bouchard et al. 2013, Lee et al. 2013, Raizman et al. 2013). Because infection rates derived from blood-fed ticks is not representative of infection rates in host-seeking ticks, we do not recommend assessing infection prevalence in ticks collected from deer to infer infection prevalence in host-seeking ticks.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus*
- Identifying presence but not prevalence of pathogens in ticks (all active life stages)

## Tick Collection from Small- or Medium-Sized Mammals, Birds and Lizards

### Background and methods

Small- and medium-sized mammals, birds and lizards often serve as hosts of larval and nymphal *I. pacificus* ticks. Trapping and inspecting these animals for ticks can provide useful information on the presence and abundance of ticks and presence of associated pathogens in the collected ticks, as well as data on host-seeking phenology of immature life stages, in a county of interest. Spatial precision of estimates is associated with the home-range of the target animals, with migratory birds having the greatest home-range and providing low spatial precision in estimating exposure sites to ticks. Host trapping is generally more labor-intensive than drag sampling, however, in areas where *I. pacificus* immatures are seldom collected on drags, host sampling may be an effective means of demonstrating establishment of *I. pacificus* populations and documenting host-seeking phenology.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus*
- Identifying presence but not prevalence of pathogens in ticks (all active life stages)
- Documenting host-seeking phenology

## Ticks Found on People and Pets

### Background and methods

Identification of ticks collected from people or pets can be a useful means of assessing human- or pet-tick encounters. However, because people and their pets often travel long distances, ticks collected from these hosts should only be included in assessments of county status when travel history is considered. Specifically, because ticks can remain

attached to a host for 7-10 days, samples obtained from persons or pets who traveled outside the county of residence within 10 days of tick encounter should be excluded. Likewise, records with more than one possible exposure site should not be reported. [CDC does not recommend testing ticks from people for human diagnostic purposes.](#)

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes pacificus* (if travel history is considered)
- Identifying presence but not prevalence of pathogens in ticks (all active life stages; if travel history is considered)

## Estimating the Density of Host-seeking (Infected) *Ixodes pacificus* Ticks

### Where to Sample

*Ixodes pacificus* is primarily a woodland-associated tick. Therefore, sampling will typically focus on forested or wooded settings, including their edges, and especially along trails. Nymphal ticks are more likely to be found in leaf litter and on substrates such as logs, tree trunks and rocks. Specific sampling sites should focus on areas considered to be a public health concern and might include, but not limited to, the following:

- novel areas of potential human exposure to *I. pacificus*
- counties where *I. pacificus* is newly established
- counties (or counties neighboring areas) where incidence of *I. pacificus*-borne illnesses have changed over time
- heavily used recreational areas, including those bordering on neighborhoods
- areas where novel pathogens are suspected to be circulating
- representative habitat types within counties where *I. pacificus*-borne infections are prevalent

### Size of Area to Sample

The density of host-seeking nymphal or female ticks varies spatially and temporally. To get a representative sample of the density of host-seeking (infected) nymphs or females, the sampling area should be expansive (spanning at least 750 m of linear transects, or 50 transects of 15 m dragged with a cloth measuring 1 m wide). Distance sampled can be assessed using several methods including: 1) setting fixed sampling grids where flags, stakes or other objects are used to mark the start and end points of each measured length of the transect, 2) using a measured rope or cable and dragging or flagging its full length, 3) measuring the collectors stride length and walking a fixed number of strides prior to checking the flag or drag, or 4) measuring distance sampled with a global positioning system. Because ticks can drop off from the drag or flag easily, inspecting the cloth at regular intervals is important (typically between 10-20 m; adults detach more readily than nymphs and therefore the drag or flag should be checked minimally every 10-15 m) (Diuk-Wasser et al. 2006, Eisen, Eisen and Lane 2006, Diuk-Wasser et al. 2010, Eisen et al. 2010, Diuk-Wasser et al. 2012, Johnson et al. 2017, Johnson et al. 2018a, Johnson et al. 2018b). Drags/Flags should be checked systematically and all parts of the cloth should be examined, including the leading edge, ropes, and seams. Samplers should also inspect their hands at each check and include any ticks recorded on their person while in the field. Wearing white or other light-colored clothing is recommended to more easily detect ticks on the tick collector.

## When to Sample

- Sampling should be conducted during the perceived peak of nymphal or adult tick activity. This information could be gleaned from previous phenology studies conducted in the region, timing of onset of human Lyme disease cases or data obtained from passive surveillance (submission of ticks from people or pets, etc.).
- Sampling each site 3 or more times within the perceived peak of host-seeking activity provides the most accurate density estimates, but this may not always be feasible; sampling twice improves precision over a single sample (Dobson 2013).
- Sampling should NOT be conducted when it is raining, when the vegetation is wet enough to saturate the tick drag or when it is unseasonably cold or extremely windy.

## How Many Sites to Sample

Sampling numerous sites per county provides better estimates of spatial variation in the density of host-seeking (infected) ticks within a county. Sampling multiple sites is strongly encouraged, particularly within ecologically-diverse counties. However, data will be displayed if minimum sampling requirements are met of only a single site per county.

## How to Estimate Infection Prevalence in Host-Seeking Ticks

In some situations, particularly where the densities of host-seeking ticks are low, it will not be possible to collect a reasonable sample size for pathogen testing within the defined 750 m sampling area even when combining ticks collected over multiple sampling sessions. In this case, it is recommended to collect additional ticks through drag sampling or flagging in the area surrounding the sampling plot. These ticks should not be included in estimates of nymphal or females densities, but can be included in assessing site-specific estimates of pathogen prevalence.

Pathogen detection assays should meet the minimal requirements described above (“Minimum criteria for acceptability of pathogen detection assay”). Pathogen prevalence and 95% confidence intervals can be estimated per tick life stage and per site in Excel using the [Pooled Infection Rate Add-In](#). Inclusion of confidence intervals is recommended in addition to point estimates in order to convey the level of uncertainty in point estimates. Confidence intervals can be interpreted as “there is a 95% probability that the true infection prevalence is between [insert lower confidence limit] and [insert upper confidence limit].” As sample sizes increase, the width of the confidence intervals decrease. Typically testing 50 ticks per site gives reasonable confidence limits. However, the number of ticks that need to be tested is dependent on how infection prevalence estimates will translate to public health action. Pathogen prevalence will not be displayed unless a minimum of 25 *I. pacificus* ticks of a given life stage have been tested within a given county. NOTE: infection prevalence and confidence intervals will be calculated per site upon submission of data to the ArboNET Tick Module (described below: ArboNET Tick Module).

## How to Calculate the Density of Host-Seeking (Infected) Ticks with Confidence Intervals

- Density of host-seeking nymphs (DON) is estimated as the total number of *I. pacificus* nymphs collected per total area sampled. DON can be scaled per 100 m<sup>2</sup> by multiplying the total number of *I. pacificus* nymphs collected per sampling session by 100 m<sup>2</sup>, then dividing the product by the total area sampled.
- Density of host-seeking infected nymphs (DIN) is estimated by multiplying DON by the local infection prevalence (% of ticks infected or the point estimate derived using the [Pooled Infection Rate Add-In](#)). To

include a confidence interval, DON should be multiplied by the lower infection prevalence confidence limit and then by the upper infection prevalence confidence limit.

- Density of host-seeking females (DOF) is estimated as the total number of *I. pacificus* females collected per total area sampled. DOF can be scaled per 100 m<sup>2</sup> by multiplying the total number of *I. pacificus* females collected per sampling session by 100 m<sup>2</sup>, then dividing the product by the total area sampled.
- Density of host-seeking infected adults (DIF) is estimated by multiplying DOF by the local infection prevalence (% of ticks infected or the point estimate derived using the [Pooled Infection Rate Add-In](#)). To include a confidence interval, DOF should be multiplied by the lower infection prevalence confidence limit and then by the upper infection prevalence confidence limit.

# Describing Host-Seeking Phenology of *Ixodes pacificus* Ticks

## Where to Sample

Because *I. pacificus* is a primarily woodland-associated tick, phenology study sites should be situated in woodlands, ideally in an area where the tick is abundant in order to accurately assess temporal changes in density. Sites with low tick density are susceptible to stochastic variation. Typically, significant differences in host-seeking phenology are not expected over short-distances. Therefore, this labor-intensive sampling should be conducted in strategic locations to identify regional differences in host-seeking phenology, such as in 1-2 sites per State.

## How to Sample

Drag sampling, flagging or collection of ticks from hosts trapped within a fixed area provide suitable samples for documenting when ticks are actively host-seeking.

## When to Sample

Sampling should be conducted at the same site, using the same standardized methods across sampling session. Sites should be sampled weekly or every two weeks to assess either the presence or abundance of ticks collected by life stage per visit. For drag sampling or flagging, ticks should be returned to the transect from which they were collected (non-removal sampling) to avoid artificial depletion of ticks over time in the study area due to intensive sampling.

# Pathogen Detection

## Recommended Tick Samples and Preservation for Pathogen Testing

Pathogen testing in host-seeking, unfed ticks is recommended for the following surveillance objectives:

- Identifying presence and prevalence of pathogens
- Calculating DIN and DIF

Results from pathogen testing in fed ticks, or from vertebrate host blood or tissue, should be considered with caution because: 1) in some cases ticks can acquire pathogens from hosts while feeding and become infected, but not be able to maintain infection through the molt to the next life stage, and 2) infection rates derived from blood-fed ticks or from hosts is not representative of infection rates in host-seeking ticks. Pathogen testing in fed ticks, or from vertebrate host blood or tissue, is acceptable for the following surveillance objectives:

- Documenting presence of pathogens in a county

Prior to testing, ticks or tissue samples should be preserved in one of the following:

- 70-95% ethanol (denatured ethanol should be avoided as it contains additives that may inhibit PCR)
- RNALater
- Frozen at -80°C without preservatives

## Minimum Criteria for Acceptability of Pathogen Detection Assay

To improve accuracy in estimates of infection prevalence and to enable detection of co-infections, ticks should be tested individually, rather than in pools. However, testing pools of ticks can be useful in some situations, including 1) when prevalence of infection is expected to be very low and testing resources are limited, or 2) when simply noting the presence, rather than prevalence of pathogens is the goal.

In order to report that an *I. pacificus* or pool of *I. pacificus* is positive for *Borrelia burgdorferi* s.s., *Borrelia miyamotoi*, or *Anaplasma phagocytophilum*, based on the results of molecular testing of a nucleic acid extract, that testing must include:

- A detection assay or assays (e.g., real-time PCR or standard PCR) specific to the target pathogen. To demonstrate that an assay is pathogen species-specific, it should be tested against a panel comprising genetically-similar species, ideally including any genetically-similar species that might also be found in *I. pacificus* ticks (see the specific considerations for each pathogen below). A published assay that has previously been shown not to detect genetically-similar species meets this requirement.

OR

- An assay or assays that detect a genus followed by sequencing to identify the pathogen to species or to at least confirm or rule out the target species. If a molecular target sequence is similar to homologous sequences from multiple species such that it is impossible to confirm or rule out the presence of the target species, testing must incorporate sequencing of at least one additional molecular target.

In addition to the minimum requirements listed above, we highly recommend using a molecular testing scheme that has been published in a peer-reviewed journal and includes:

- Multiple targets for each pathogen.
- Established limits of detection for each real-time and/or standard PCR target in the presence of tick DNA. If the testing scheme includes a multiplex assay designed to detect multiple pathogens, the limit of detection for each pathogen target should also be confirmed in the presence of more abundant DNA from other pathogens targeted by the same assay.
- An internal control (e.g., a segment of the tick actin gene) that can be used to confirm the presence of amplifiable DNA in each specimen. A specimen that does not contain amplifiable DNA should not be included in infection prevalence calculations.

See, for example, Graham et al. (2018).

All real-time or standard PCR testing should include no-template controls and, if possible, negative extraction controls (extracts from DNA-free water or buffer taken through the entire DNA extraction process alongside tick specimens). To limit the risk of contaminating field-collected samples with amplicons from previously processed samples, nucleic acid extraction, PCR reaction set-up, and any work with amplicons (e.g., setting up sequencing reactions) should be conducted in separate work areas, ideally with dedicated pipets.

## Important considerations for *Borrelia* testing

The *Borrelia* genus comprises two major clades: a relapsing fever (RF) group and a distinct *Borrelia burgdorferi* sensu lato (s.l.) complex. Phylogenetic analyses place *B. miyamotoi* within the RF group. To date, *B. miyamotoi* is the only RF group *Borrelia* associated with *I. pacificus* (Barbour 2014). *Borrelia miyamotoi* is known to cause human disease in the United States (Krause et al. 2015).

There are at least 9 recognized (named) species within the *B. burgdorferi* s.l. complex occurring in the United States (Schotthoefler and Frost 2015, Pritt et al. 2016, Margos et al. 2017a). At least 5 of those have been detected in field-collected *I. pacificus*: *B. burgdorferi* s.s., *B. americana*, *B. bissettiae*, *B. californiensis*, and *B. lanei* (Margos et al. 2017a, Margos et al. 2016, Fedorova et al. 2014, Rudenko et al. 2009).

## Notes on nomenclature:

- Publications may use “*Borrelia burgdorferi*” to refer to *B. burgdorferi* s.s. and/or *B. burgdorferi* s.l. If you are using a published assay that is reported to be *B. burgdorferi*-specific, it is important to determine whether it is truly specific to *B. burgdorferi* s.s., which causes human disease, or to *B. burgdorferi* s.l., which includes a number of species that are not known to cause human disease.
- Some have proposed dividing the genus *Borrelia* into two genera, with *Borrelia* continuing to encompass species in the RF group, and a new genus, *Borreliella*, to encompass species previously included in the *Borrelia burgdorferi* s.l. group (Adeolu and Gupta 2014). Investigators continue to debate this proposal (Barbour et al. 2017, Margos et al. 2017b). Those querying databases to identify specimens to species should be aware, however, that *Borreliella* was included in a validation list (no. 163: list of new names and new combinations previously effectively, but not validly, published (Oren and Garrity 2015)), and that *B. burgdorferi* s.l. species in the National Center for Biotechnology (NCBI) nucleotide databases may be identified as *Borrelia* species or *Borreliella* species.

To demonstrate that an assay is ***B. miyamotoi* specific**, it should be tested against at least one *B. burgdorferi* s.l. species (e.g., *B. burgdorferi* s.s.). It should also be shown not to detect other RF *Borrelia* species.

To demonstrate that an assay is ***B. burgdorferi* s.s. specific**, it should be tested against a panel including non-target *B. burgdorferi* s.l. species, ideally including *B. americana*, *B. bissettiae*, *B. californiensis*, and *B. lanei*.

There are a number of published assays for amplifying and sequencing *Borrelia* targets to identify *Borrelia* to species. Assays including nested PCR protocols are useful for amplifying the often scarce pathogen DNA in ticks. See Wang et al. (2014) for descriptions of and references to several approaches for molecular typing of *B. burgdorferi* s.l. Note that protocols for PCR-based RFLP can also be used to generate amplicons for sequencing.

## Important considerations for *Anaplasma phagocytophilum* testing

To demonstrate that an assay is specific to *A. phagocytophilum*, at a minimum, sequence analysis using BLAST or a similar database search tool should be used to confirm that primer and probe target sites are not conserved across Anaplasmataceae or Rickettsiaceae, as *I. pacificus* can harbor at least one rickettsial endosymbiont, *Candidatus Cryptoplasma californiense* (a novel Anaplasmataceae species) (Eshoo et al. 2015, Rounds et al. 2012), and *Ehrlichia*



*chaffeensis*, though detection of *E. chaffeensis* in *I. pacificus* has been rare and may be highly focal (Lane et al. 2004). Assays should be tested for specificity against *Rickettsia* and *Ehrlichia* spp. as well as against other *Anaplasma* spp., ideally including *A. ovis*, *A. bovis* and *A. marginale*.

Molecular assays designed to detect *A. phagocytophilum* in *I. pacificus* may not differentiate *A. phagocytophilum* strains known to cause disease in humans from strains not known to infect humans (Rejmanek et al. 2012). You should interpret PCR-based *A. phagocytophilum* testing results with this in mind. It is possible to differentiate strains by amplifying and sequencing select targets including the *ank* or *groESL* genes (Rejmanek et al. 2012). This is advisable when reporting an *A. phagocytophilum*-positive tick from a county that has never reported a human anaplasmosis case and/or has never reported an *A. phagocytophilum*-positive tick.

## Samples CDC will Test for Pathogens

In support of tick surveillance efforts, CDC has limited resources available to support pathogen detection in ticks submitted by public health partners. Samples will not be accepted for testing from the general public. We offer tick testing for the following pathogens: *Borrelia burgdorferi* s.s., *Borrelia mayonii*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum* and *Babesia microti*. By submitting ticks to CDC for testing, submitters agree to allow CDC to retain the DNA extract for our reference collection. Limited resources typically preclude us from returning aliquots from ticks for which we perform DNA extractions. For submitters wishing to retain DNA from their ticks, we ask that you extract the DNA and submit an aliquot to CDC for pathogen testing. Prior to submitting ticks or DNA for testing, public health entities should contact CDC at: [ticksurveillance@cdc.gov](mailto:ticksurveillance@cdc.gov).

### In Counties Where the Pathogen of Interest has Never Been Identified

In counties where *Borrelia burgdorferi* s.s., *Borrelia miyamotoi*, or *Anaplasma phagocytophilum* have not been identified previously in ticks or hosts, CDC will test the following samples submitted by collaborating public health partners for **presence of pathogens**:

- Host-seeking nymphs (collected from vegetation, walking samples or tick traps); pathogen prevalence will be estimated if sample size is  $\geq 25$  individuals per site per county.
- Host-seeking females (collected from vegetation, walking samples or tick traps); pathogen prevalence will be estimated if sample size is  $\geq 25$  individuals per site per county.
- Ticks collected from hosts; ticks will be tested for pathogen presence only, but prevalence will not be estimated. Blood-fed adults will not be tested due to assays not being optimized for that purpose.

### In Counties Where the Pathogen of Interest has Been Identified

In counties where *Borrelia burgdorferi* s.s., *Borrelia mayonii*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum* or *Babesia microti* has been identified previously in ticks or hosts, CDC will test the following samples submitted by collaborating public health partners for **prevalence of pathogens**:

- Host-seeking nymphs (collected from vegetation, walking samples or tick traps) where  $\geq 25$  individuals are submitted per site per county.
- Host-seeking females (collected from vegetation, walking samples or tick traps) where  $\geq 25$  individuals are submitted per site per county.

- In areas where drag sampling/flagging was conducted to assess DIN or DIF, we will test ticks from low density sites, even if the total sample size is less than 25 individuals. Collection of additional ticks from area surrounding the density sampling site should be attempted, but in some cases, collection of 25 individuals will not be feasible.

## Limitations to Tick Surveillance

- Presence of *I. pacificus* within a county may be a poor indicator of human disease risk. For example, *I. scapularis* has been reported in many counties in the southeastern United States, but *Borrelia burgdorferi* s.s. infection rates are typically low and nymphs do not commonly ascend vegetation when host-seeking, thus limiting contact between people and nymphs.
- Although county estimates of the density of host-seeking infected nymphs is a better predictor of human disease occurrence compared with simple measures of tick presence or density of host-seeking nymphs, DIN and DON do not always accurately estimate risk of tick-borne diseases in humans. This may relate to spatial heterogeneity in where ticks are found and where people spend time outdoors, human behaviors that may increase or decrease risk of exposure to infected ticks, or other factors.

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# Supplemental Material

## Avoiding Tick Bites

The best way to prevent tick-borne diseases is to prevent tick bites. To do so, [CDC recommends](#):

### While You Are Outdoors

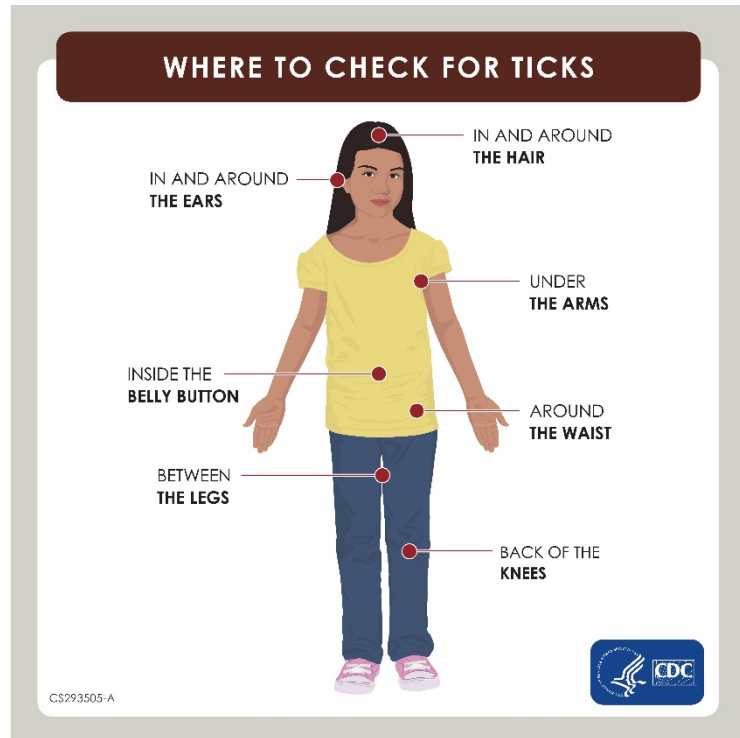
- **Know where to expect *I. pacificus* ticks.** Adult ticks are found on the tips of grasses and shrubs, often along trails. Nymphs are found in leaf litter and on rocks, logs, tree trunks or fallen branches under trees in oak woodlands or other shaded natural areas. Thus, spending time, hiking, camping, or hunting could bring you in contact with ticks seeking a host. Some people may find ticks in their own yard or neighborhood, and get bites while gardening or doing yard work.
- **Use [Environmental Protection Agency \(EPA\)-registered tick repellents](#)** containing DEET, picaridin, IR3535, Oil of Lemon Eucalyptus (OLE), para-menthane-diol (PMD), or 2-undecanone. EPA's helpful [search tool](#) can help you find the product that best suits your needs. Always follow product instructions.
  - Do not use repellent on babies younger than 2 months old.
  - Do not use products containing OLE or PMD on children under 3 years old.
- **Treat clothing and gear** with products containing 0.5% permethrin. Permethrin can be used to treat boots, clothing and camping gear and remain protective through several washings.
- **Minimize the risk of contact with *I. pacificus* ticks**
  - Avoid wooded and brushy areas with high grass and leaf litter when possible.
  - Walk in the center of trails.
- **Check your clothing for crawling ticks** frequently and remove them before they can attach and blood-feed.

### After You Come Indoors

- **Check your clothing for ticks.** Ticks may be carried into the house on your clothing. Any ticks that are found should be removed. Tumble dry clothes in a dryer on high heat for 10 minutes to kill ticks on dry clothing after you come indoors. If the clothes are damp, additional time may be needed. If the clothes require washing first, hot water is recommended. Cold and medium temperature water will not kill ticks.
- **Shower soon after being outdoors.** Showering within two hours of coming indoors has been shown to reduce your risk of getting Lyme disease and may be effective in reducing the risk of other tick-borne diseases. Showering ensures that you remove (and then presumably change into clean) clothing and also provides an opportunity to spot ticks that were crawling or attached under the clothing. Showering may help wash off unattached ticks and it is a good opportunity to do your daily tick check.

- **Even if not showering, check your body for ticks after being outdoors.** Conduct a full body check upon return from potentially tick-infested areas, including your own backyard. Use a hand-held or full-length mirror to view all parts of your body. Tick can attach anywhere on the body, but especially check these parts of your body and your child's body for ticks:

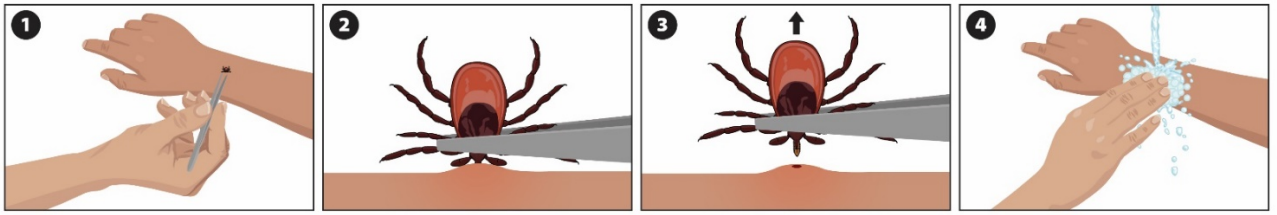
- Under the arms
- In and around the ears
- Inside belly button
- Back of the knees
- In and around the hair
- Between the legs
- Around the waist



- **Examine gear and pets.** Ticks can be transported into the home on clothing and pets, then attach to a person later, so carefully examine pets, coats, and daypacks.

## How to Remove a Tick

- Use fine-tipped tweezers to grasp the tick as close to the skin's surface as possible.
- Pull upward with steady, even pressure. Don't twist or jerk the tick; this can cause the mouth-parts to break off and remain in the skin. If this happens, remove the mouth-parts with tweezers. If you are unable to remove the mouth-parts easily with clean tweezers, leave it alone and let the skin heal.
- After removing the tick, thoroughly clean the bite area and your hands with rubbing alcohol or soap and water.
- Never crush a tick with your fingers. Dispose of a live tick by putting it in alcohol, placing it in a sealed bag/container, wrapping it tightly in tape, or flushing it down the sink or toilet.



- If you develop a rash or fever within several weeks of removing a tick, see your doctor. Be sure to tell the doctor about your recent tick bite, when the bite occurred, and where you most likely acquired the tick.

# How to Make Tick Drags

## Blanket-Style Drag

### Supplies

- 1-1/2 yd. rubberized cotton flannel sheeting, 45" wide
- 2 - zinc-plated screw eyes, size #12
- 3 - zinc-plated cut washers, 2" outer diameter, 3/4" inner diameter
- 1 - length of braided polyester clothesline, 3/16" thick
- 1 - dowel, 3/4" in diameter, 48" long
- Heavy-duty thread
- Heavy-duty sewing machine
- 20 small lead sinkers, used for weighting fishing lines, 1/4 oz. size

### Sewing instructions

For each flag:

#### Step 1: *Preparing the materials*

From the rubberized cotton flannel material, cut:

- One (1) – 39.5" x 36" rectangle for the main panel of the tick drag.
- One (1) – 39.5" x 4" strip for the pocket that will hold the washers.

#### Step 2: *Sewing the loop for the dowel*

- Laying the main panel flat so that it measures 39.5" from left to right, fold the top of the panel down approximately 3" toward the front of the panel and pin or clip in place. (Diagram A)
- Sew along the bottom edge of the fabric, leaving the two sides open to form a "loop" for the dowel. (Diagram B)

Step 3 (flat drag): *Adding the weights*

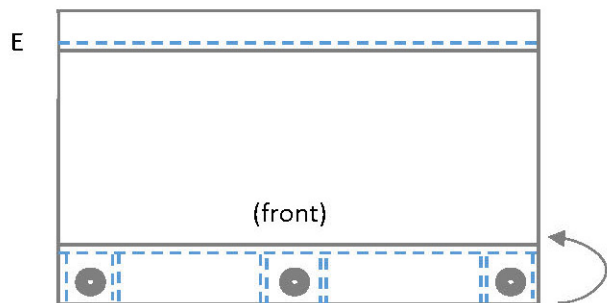
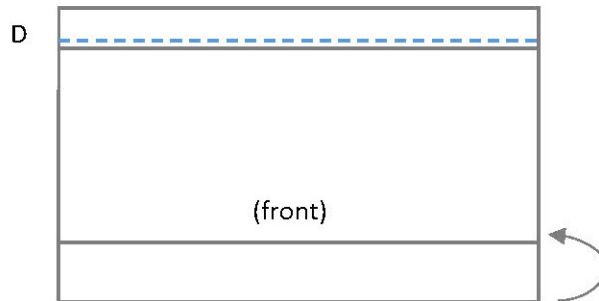
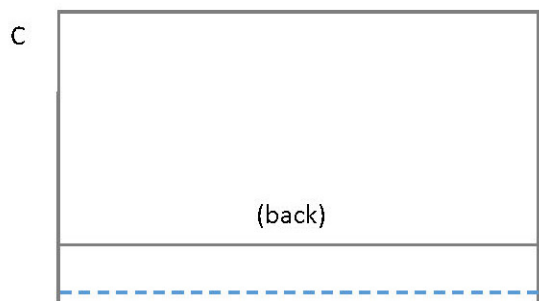
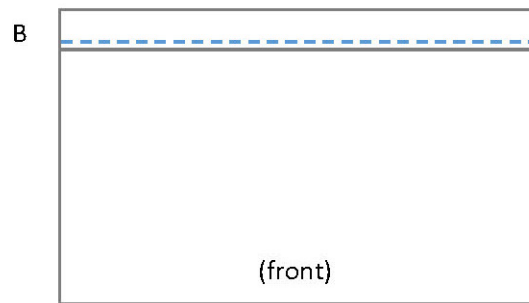
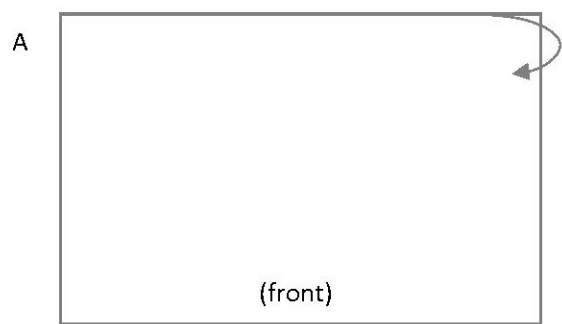
- a. Flip the panel over so that the seam from Step 2 is facing down. The panel should still be situated so that the loop is across the top of the panel.
- b. Next, pin or clip the 39.5" x 4" rectangle onto the bottom of the panel so that the long edges align. Sew the two pieces together along the bottom edge, using a generous seam allowance. (Diagram C)
- c. Flip the panel again so that the seam from Step 2 is again facing up. Turn the 39.5" x 4" strip from Step 3b to the front of the panel and pin or clip in place. (Diagram D)
- d. Following the diagram, sew the strip in place, adding the three washers as you work. (Diagram E)

Step 4: *Completing the drag*

- a. Affix one screw eye to each end of the dowel, and thread the dowel through the dowel loop from Step 2.
- b. Measure and cut a length of braided cord, and knot each end through the screw eyes to make the drag handle. The length of cord should be long enough for the front of the drag to reach the ground as the collector pulls it along the vegetation.



# Sewing diagrams



# Modified Drag with “Fingers”

## Supplies

- 1-1/2 yd. rubberized cotton flannel sheeting, 36” wide
- 2 - zinc-plated screw eyes, size #12
- 3 - zinc-plated cut washers, 2” outer diameter, 3/4” inner diameter
- 1 - length of braided polyester clothesline, 3/16” thick
- 1 - dowel, 3/4” in diameter, 48” long
- 20 - small lead sinkers, ¼ oz. weight
- Heavy-duty thread
- Heavy-duty sewing machine

## Sewing instructions

From the rubberized cotton flannel material, cut:

- a. One (1) – 39.5” x 23” rectangle for the main panel of the tick drag.
- b. Ten (10) – 23” x 2” strips for the fingers that will hold the lead weights.

### Step 2: *Sewing the loop for the dowel*

- a. Laying the main panel flat so that it measures 39.5” from left to right, fold the top of the panel down approximately 3” toward the front of the panel and pin or clip in place. (Diagram A)
- b. Sew along the bottom edge of the fabric, leaving the two sides open to form a “loop” for the dowel. (Diagram B)

### Step 3 (finger drag): *Adding the weights*

- a. Pin or clip the ten 23” x 2” fabric strips at even distances across the bottom of the rectangular piece so that each one overlaps the larger piece by approximately 1”.
- b. Sew a double line of stitches across all ten fingers, securing them to the back of the drag. (Diagram C)
- c. Fold approximately 2” of the bottom of each strip over and sew along two edges to form a pocket with an open side. (Diagram D)
- d. Insert two of the lead sinkers into this pocket and continue sewing the third side of the pocket to close. Repeat for all ten fingers. (Diagram D)

### Step 4: *Completing the drag*

- a. Affix one screw eye to each end of the dowel, and thread the dowel through the dowel loop from Step 2.
- b. Measure and cut a length of braided cord, and knot each end through the screw eyes to make the drag handle. The length of cord should be long enough for the front of the drag to reach the ground as the collector pulls it along the vegetation.

# Sewing diagrams

