Surveillance for *Ixodes scapularis* 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 geographic distributions of *Ixodes scapularis* (the blacklegged tick or deer tick) and its associated pathogens are expanding, putting an increasing number of Americans at risk for acquiring Lyme disease, anaplasmosis, babesiosis, *Borrelia miyamotoi* disease and other, less common *I. scapularis*-associated illnesses. The primary objective of this document is to provide guidance for surveillance of *I. scapularis* 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 greatest local concern.

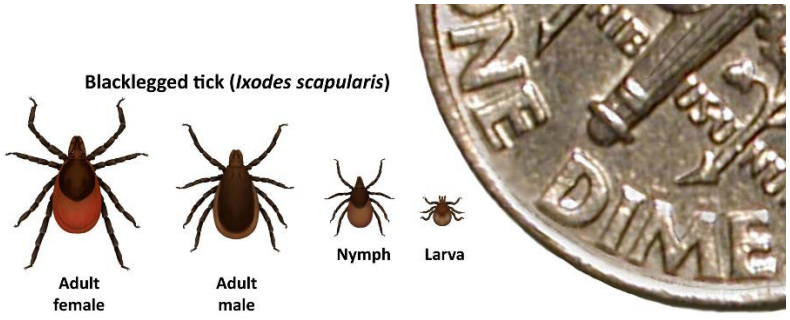

Figure 1. Active life stages of the blacklegged tick, *Ixodes scapularis*.

Public Health Importance of *Ixodes scapularis*

Of the nearly 50,000 cases of locally-acquired vector-borne disease cases reported annually from states and the District of Columbia to the Centers for Disease Control and Prevention, nearly 95% are caused by pathogens spread by ticks ((Adams et al. 2016); Figure 2). The majority are Lyme disease cases, with approximately 30,000 cases reported annually, which is an approximately 10-fold under-estimate of the nearly 300,000 Lyme disease cases diagnosed annually (Hinckley et al. 2014, Nelson et al. 2015). Since becoming a notifiable condition in 1991, the number of Lyme disease cases reported annually has roughly tripled and cases have been reported over an expanding geographical region (Kugeler et al. 2015, Mead 2015) (Figure 3).

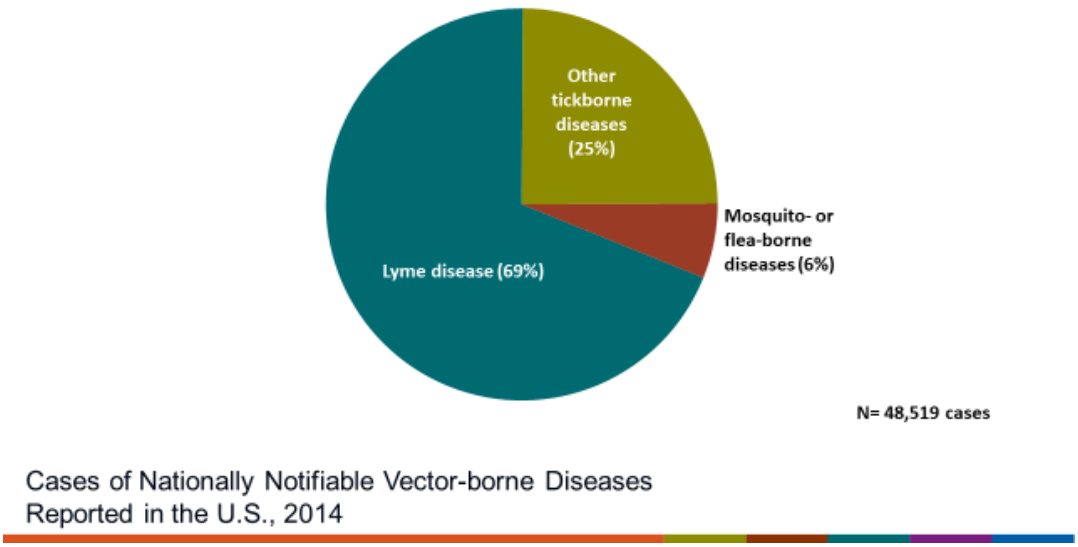

Figure 2. Reported vector-borne diseases, United States, 2014.

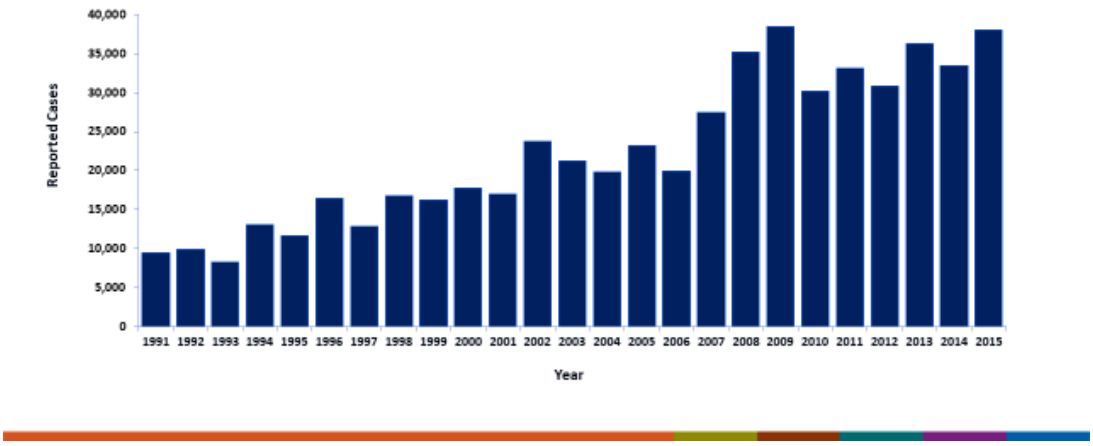

Figure 3. Number of Lyme disease cases reported per year.

Since 1970, when *Babesia microti* was first reported to be a human pathogen, six additional *I. scapularis*-borne human pathogens have been described (Eisen and Eisen 2018) (Table 1; Figure 4). Moreover, annual case counts have increased over time for notifiable *I. scapularis*-associated diseases, including Lyme disease, anaplasmosis and babesiosis (Eisen et al. 2017). In the northern parts of the tick's range, *I. scapularis* nymphs are considered the primary vectors of the agents causing Lyme disease, anaplasmosis and babesiosis.

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

Disease	Pathogen(s)	Life stages infected
Anaplasmosis	*Anaplasma phagocytophilum*	Nymphs, Adults
Babesiosis	*Babesia microti*	Nymphs, Adults
Borrelia miyamotoi disease	*Borrelia miyamotoi*	Larvae, Nymphs, Adults
Ehrlichiosis	*Ehrlichia muris eauclairensis*	Nymphs, Adults
Lyme disease	*Borrelia burgdorferi* sensu stricto, *Borrelia mayonii*	Nymphs, Adults
Powassan virus disease	Powassan virus (lineage II/deer tick lineage)	Larvae, Nymphs, Adults

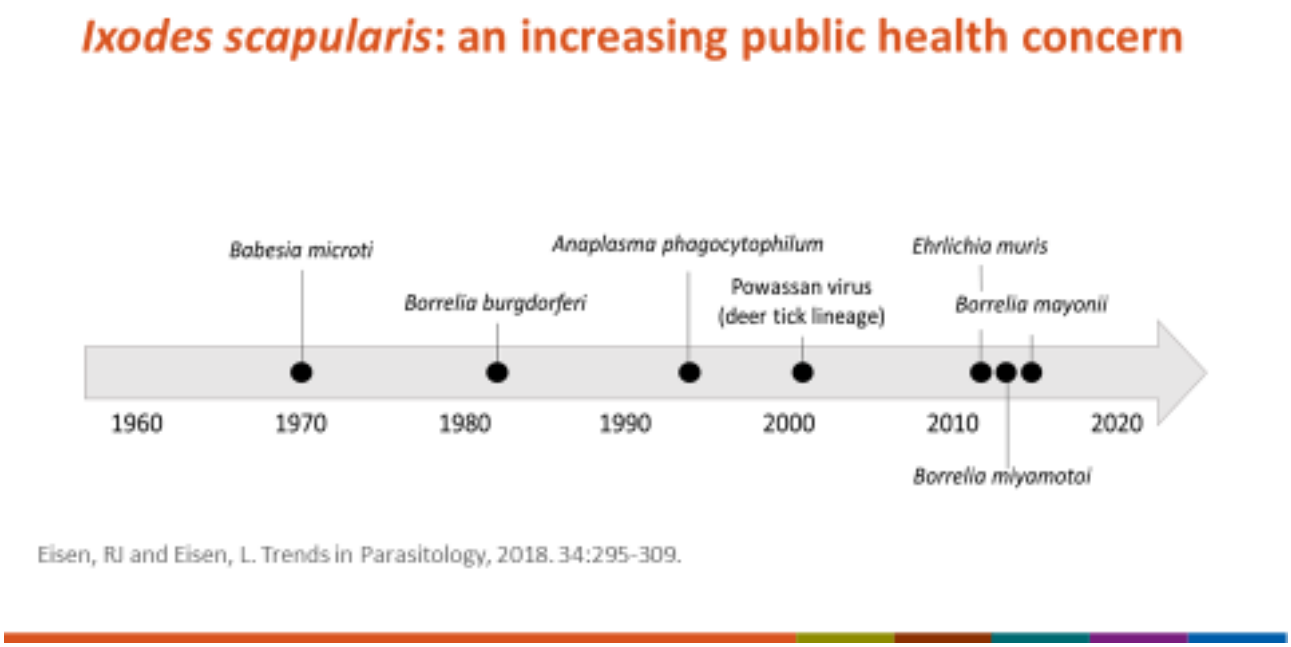

Figure 4. Timeline showing when various *I. scapularis*-borne agents were demonstrated to be human pathogens.

Tick surveillance is not standardized or routine. Nonetheless, available collection records indicate that the geographic distribution of *I. scapularis* has expanded markedly over the past two decades. Specifically, from 1996 through 2015 the number of counties in which *I. scapularis* is considered to be established has more than doubled (Eisen et al. 2016) (Figure 5). Moreover, recent models indicate that potentially suitable habitat for the blacklegged tick is wide-spread in the eastern United States, suggesting that either the distribution of the tick is currently under-reported or there is potential for additional range expansion (Hahn et al. 2016, 2017) (Figure 6).

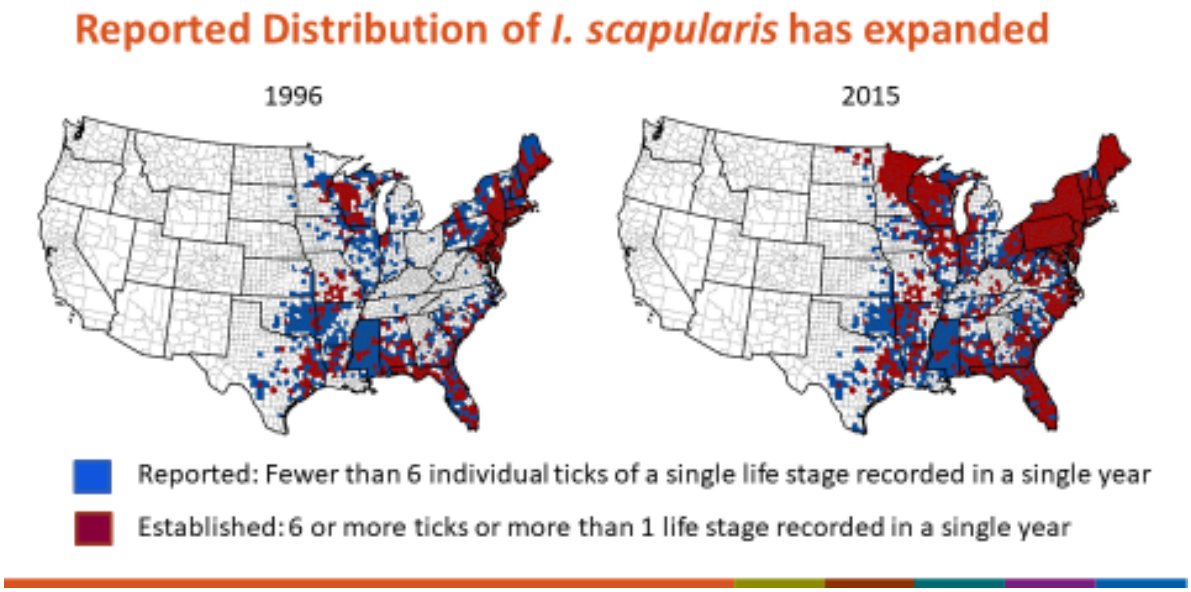

Figure 5. Distribution of counties with reported or established populations of *I. scapularis* in 1996 (Dennis et al. 1998) and 2015 (Eisen et al. 2016).

Figure 6. Distribution of potentially suitable habitat for *I. scapularis* (Hahn et al. 2016, 2017).

Because the distributions of ticks and tick-borne pathogens change over time, human risk of exposure to ticks and their associated pathogens also change. Tick surveillance is intended to monitor changes in the distribution and abundance of ticks and the presence and prevalence of tick-borne pathogens in order to provide actionable, evidence-based information to clinicians, the public and public health policy makers. Key questions address when and where humans are at risk for exposure to ticks and tick-borne pathogens.

Life Cycle of *Ixodes scapularis*

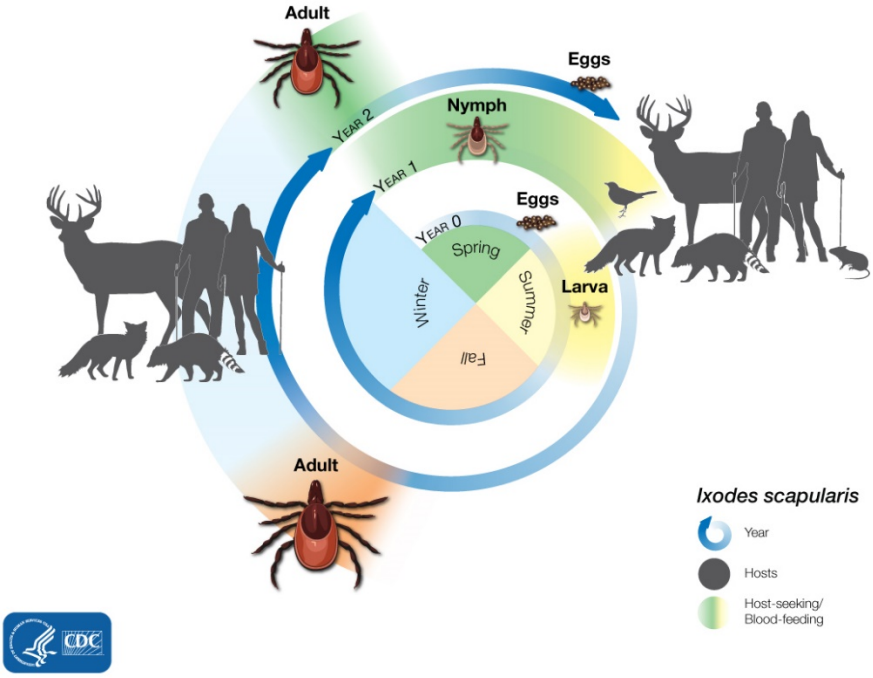

Figure 6. Generalized life cycle of *Ixodes scapularis*.

Ixodes scapularis is a primarily woodland-associated tick. It has a 2-3 year life cycle consisting of four life stages: egg, larva, nymph, and adult (Yuval and Spielman 1990). 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 small and medium-sized mammals including, but not limited to, white-footed mice, chipmunks, voles, and shrews. However, they can also infest birds, lizards and larger 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. Male ticks do not blood-feed. With the exception of Powassan virus and the relapsing fever spirochete *Borrelia miyamotoi*, *I. scapularis*-borne human pathogens have not been demonstrated to be transmitted transovarially (vertical transmission from infected females to their offspring) (Costero and Grayson 1996, Rollend et al. 2013). The other *I. scapularis*-borne pathogens 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 in the fall and spring, but can be active also in the winter months in settings where daytime temperatures are above freezing and there is little to no snow cover, allowing for tick activity. Females

typically lay eggs in the late spring but hatched larvae do not seek hosts actively until months later, in summer. After blood-feeding, larvae over-winter and molt to nymphs. Nymphs begin host-seeking in the spring with peak activity typically observed from May through July, depending on location. After blood-feeding, nymphs molt to adults and seek hosts in the fall (Figure 6). In some localities, particularly in colder-regions, the life cycle may be extended to 3-4 years (Hamer et al. 2012a).

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 public health 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. scapularis*: established, reported, or no data available
2) classify county status for presence of specific pathogens in *I. scapularis* ticks: present or no data available

Additional objectives include the following: (3) generate estimates for local prevalence of specific pathogens in relevant *I. scapularis* 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. scapularis* 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. scapularis*-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 better 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 better estimates of human encounters with infected host-seeking nymphs than simple measures of tick/pathogen presence or abundance (Mather et al. 1996, Pepin et al. 2012, Eisen and Eisen 2016). Similar arguments can be made for the relative value of estimating infection prevalence in and abundance of female ticks, particularly in areas of the eastern United States where host-seeking behavior of nymphs limits human-tick contact and where human encounters with female ticks are more common than nymphal tick encounters (Stromdahl and Hickling 2012, Arsnoe et al. 2015, Hickling et al. 2018).

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. scapularis* ticks.

Criteria for classifying county establishment status for *I. scapularis* 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. Additional information can be found in subsequent sections of this document.

Classify County Status for *Ixodes scapularis*

- **Objective: Update the *I. scapularis* 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: ≥ 6 *I. scapularis* of a single life stage or > 1 life stage collected per county within a 12-month period
    - Reported: < 6 *I. scapularis* 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, Durden and Keirans 1996)
- 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. scapularis*-borne pathogens.

Identify Presence and Prevalence of Human Pathogens in *Ixodes scapularis* Ticks

- **Objective: Map the county level distribution of human pathogens in *I. scapularis* 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. scapularis*-borne pathogen of interest has been detected in *I. scapularis* ticks or in natural hosts of *I. scapularis.* 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. scapularis* 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 decreases. Typically, testing 50 nymphal or adult ticks per site gives reasonable confidence limits for most *I. scapularis*-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. 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 scapularis* 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. scapularis* nymphs.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking nymphs collected per 100 m^2 or displayed as the inverse showing the distance covered before expected encounter with a nymph.
    - Requires at least 750 m^2 drag sampled per site for density estimate; drags should be inspected for ticks at least every 10-20 m; sampling should be timed to coincide with the peak in nymphal host-seeking activity; 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 scapularis* 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. scapularis* nymphs**.
  - Data display and minimal sampling requirements include:

        - Displayed in categories based on number of host-seeking infected nymphs collected per 100 m^2 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. scapularis* females.**
    - Data display and minimal sampling requirements include:
        - Displayed in categories based on number of host-seeking females collected per 100 m^2 (DOF) or displayed as the inverse showing the distance covered before expected encounter with a female tick.
        - Requires at least 750 m^2 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. scapularis* females**.
    - Data display and minimal sampling requirements include:
        - Displayed in categories based on number of host-seeking infected females collected per 100 m^2 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 scapularis* Ticks

- **Objective: Describe when *I. scapularis* ticks are actively host-seeking (phenology).**
- Data display and minimal sampling requirements include:
    - Displayed as state (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. For more information, see "Describing Host-Seeking Phenology of *Ixodes scapularis* Ticks."

Tick Collection Methods

Several methods can be used to collect *I. scapularis* 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. scapularis* or *I. scapularis*-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 better 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)) Drag sampling is the single most reliable method for quantifying the density of host-seeking (infected) *I. scapularis* nymphs (Falco and Fish 1992).

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

Collection Method	**Objective: Classify county status**	**Objective: Presence/Prevalence of pathogens in ticks**	**Objective: DON/DIN or DOF/DIF**	**Objective: Phenology**
Dragging/Flagging	Acceptable	Acceptable	Acceptable	Acceptable
Walking	Acceptable	Acceptable	Not Acceptable	Acceptable
CO2 traps	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable
Ticks collected from deer	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable
Ticks collected from small- or medium-sized mammals, birds, lizards	Acceptable	Acceptable for presence, but not prevalence	Not Acceptable	Acceptable
Ticks from people/pets	Acceptable, if travel history is accounted for	Acceptable for presence, but not prevalence	Not Acceptable	Not Acceptable

Drag Sampling or Flagging

Background and methods

Drag sampling and flagging are similar methods used to collect host-seeking ticks (Daniels and Fish 1990, Carroll and Schmidtmann 1992, Falco and Fish 1992). 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 scapularis*
- 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). 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 is similar in efficiency to flagging or dragging for adult ticks, but apparently yields fewer nymphs than drag sampling or flagging (Ginsberg and Ewing 1989). 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 scapularis*
- 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). Developed originally for collection of lone star ticks (*Amblyomma americanum*) which display a more aggressive and mobile host-seeking behavior compared with *I. scapularis*, 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 for this tick species. 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 scapularis*
- Identifying presence and prevalence of pathogens in ticks (all active life stages)

Tick Collection from Deer

Background and methods

White-tailed deer serve as important hosts for adult *I. scapularis* ticks. Inspection of hunter-killed deer brought into check stations is a cost-effective means of detecting changes in the distribution of *I. scapularis*, particularly in areas where the tick is emerging. However, owing to the home range of deer, it is spatially non-specific and 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 scapularis*
- 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. scapularis* 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. scapularis* immatures are seldom collected on drags, host sampling may be an effective means of demonstrating establishment of *I. scapularis* populations and documenting host-seeking phenology.

Acceptable to use to address the following key surveillance objectives:

- Classifying county status for *Ixodes scapularis*
- 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 d 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 scapularis* (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 scapularis* Ticks

Where to Sample

Ixodes scapularis is primarily a woodland-associated tick. Therefore, sampling will typically focus on forested or wooded settings, including their edges. 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. scapularis*
- counties where *I. scapularis* is newly established
- counties (or counties neighboring areas) where incidence of *I. scapularis*-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. scapularis*-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, or 3) measuring the collectors stride length and walking a fixed number of strides prior to checking the flag or drag. 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, Diuk-Wasser 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 cloth check and include any ticks recorded on their person. 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 for 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^2 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 decreases. 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. scapularis* 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. scapularis* nymphs collected per total area sampled. DON can be scaled per 100 m^2 by multiplying the total number of *I. scapularis* nymphs collected per sampling session by 100 m^2, 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. scapularis* females collected per total area sampled. DOF can be scaled per 100 m^2 by multiplying the total number of *I. scapularis* females collected per sampling session by 100 m^2, 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 scapularis* Ticks

Where to Sample

Because *I. scapularis* 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. scapularis* or pool of *I. scapularis* is positive for *Borrelia burgdorferi* s.s., *Borrelia mayonii*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum, Babesia microti, Ehrlichia muris eauclairensis*, or Powassan virus 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. scapularis* 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 (bacteria and hemoprotozoan parasites) or family (viruses) 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. scapularis* (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 (Schotthoefer and Frost 2015, Pritt et al. 2016, Margos et al. 2017a). At least 4 of those have been detected in field- collected *I. scapularis*: *B. burgdorferi* s.s., *B. mayonii*, *B. kurtenbachii*, and *B. andersonii* (Margos et al. 2010, Hamer et al. 2012b, Eisen et al. 2017). Of the *I. scapularis*-associated *B. burgdorferi* s.l. species, only *B. burgdorferi* s.s. and *B. mayonii* have been culture-confirmed as human pathogens in the United States (Stanek and Reiter 2011, Pritt et al. 2016).

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.). Ideally, 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. kurtenbachii* and *B. andersonii*. If you will be testing ticks from the upper Midwestern United States, testing should also demonstrate that the assay does not detect *B. mayonii*.

To demonstrate that an assay is ***B. mayonii* specific**, it should be tested against a panel that includes *B. burgdorferi* s.s. and other non-target *B. burgdorferi* s.l. species, ideally including *B. kurtenbachii* and *B. andersonii*.

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*, it is important to confirm that *A. phagocytophilum* primer and probe target sites are not conserved across Anaplasmataceae or Rickettsiaceae, as *I. scapularis* can harbor at least one rickettsial endosymbiont and at least one *Ehrlichia* species (Kurtti et al. 2015, Pritt et al. 2017). Assays should be tested for specificity against *Rickettsia* and *Ehrlichia* spp. as well as against other *Anaplasma* spp., ideally including *A. bovis* and *A. marginale*.

Molecular assays designed to detect *A. phagocytophilum* usually cannot differentiate the *A. phagocytophilum* human-active strain (*A. phagocytophilum*-ha), which causes disease in humans, from other variants that are not known to infect humans, including *A. phagocytophilum* variant 1 (*A. phagocytophilum*-v1) (Keesing et al. 2014). *Ixodes scapularis* may be infected with either *A. phagocytophilum*-ha, variant 1, or both, but the relative abundance of the two strains can vary dramatically between sites and years (Keesing et al. 2014). The relative abundance of *A. phagocytophilum*-v1 also tends to be higher among female ticks that have fed on deer than among males collected from deer, consistent with findings that white-tailed deer are likely a reservoir for *A. phagocytophilum*-v1 but not for *A. phagocytophilum*-ha (Courtney et al. 2003). You should interpret PCR-based *A. phagocytophilum* testing results with this in mind. It is possible to differentiate the two strains by amplifying and sequencing select targets (i.e., the *msp4* gene (de la Fuenta et al., 2005), the *ank* gene (Massung et al. 2007), or a segment of the 16S rRNA gene (Massung et al., 2003). 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. phagoctophilum* ha-positive tick.

Important considerations for *Ehrlichia muris eauclairensis* testing

To demonstrate that an assay is specific to *Ehrlichia muris eauclairensis*:

- At a minimum, BLAST analysis should be used to confirm that primer and probe target sites are not conserved across Anaplasmataceae or Rickettsiaceae, as *I. scapularis* can harbor *A. phagocytophilum* and at least one rickettsial endosymbiont (Kurtti et al. 2015).
- Ideally, the assay should be tested against a panel including other ehrlichial species as well as Rickettsiales. See, for example, (Allerdice et al. 2016).

Important considerations for *Babesia microti* testing

In the United States, *I. scapularis* is the vector of the parasite *B. microti*, which is the most common etiologic agent of human cases of babesiosis in this country. To date, *I. scapularis* has not been established to be the vector of any of the other pathogens that have caused documented U.S. zoonotic cases of babesiosis. However, the tick vectors have not been identified for all such agents, let alone for all of the many other *Babesia* species that infect non-human animals and that might be found to have zoonotic potential.

Ixodes scapularis may be infected with *B. odocoilei*, a parasite of white-tailed deer and other cervids, and *Theileria cervi*, another parasite in the same order as Babesia spp. (Prioplasmida) (Steiner et al. 2006, Fritzen et al. 2014). Neither of these has been documented to cause infection in humans.

To demonstrate that an assay is ***B. microti-specific***, it should be tested – at a minimum – against *B. odocoilei*, and ideally against a panel comprising other *Babesia* species as well as *T. cervi*.

Important considerations for Powassan virus testing

Powassan virus comprises 2 lineages: Powassan virus (POWV) lineage I, for which *Ixodes cookei* serves as the vector, and Powassan virus lineage II, or deer tick virus (DTV), for which *I. scapularis* serves as the vector (Telford et al. 1997, Kuno et al. 2001).

Powassan virus is a positive-sense RNA virus.

- Sample preservation, nucleic acid extraction, and nucleic acid storage requirements for RNA are generally more stringent than those for bacterial or protozoan DNA. If you want to include Powassan virus testing in your tick surveillance plan, you may need to collect and store one set of ticks for DNA testing and a second set for RNA testing. Alternatively, you may optimize your sample preservation, nucleic acid extraction, and nucleic acid storage protocols to allow for both DNA and RNA testing. In this case, it is important to ensure that your preservation, extraction, and storage procedures do not compromise assay sensitivity to any of your RNA or DNA pathogen targets.
- PCR-based assays designed to detect or identify this virus must incorporate a reverse transcription step.

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.

In Counties Where the Pathogen of Interest has Never Been Identified

In counties where *Borrelia burgdorferi* s.s., *Borrelia mayonii*, *Borrelia miyamotoi*, *Anaplasma phagocytophilum* or *Babesia microti* 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 ≥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 ≥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* have 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 ≥25 individuals are submitted per site per county.
- Host-seeking females (collected from vegetation, walking samples or tick traps) where ≥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. scapularis* 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:

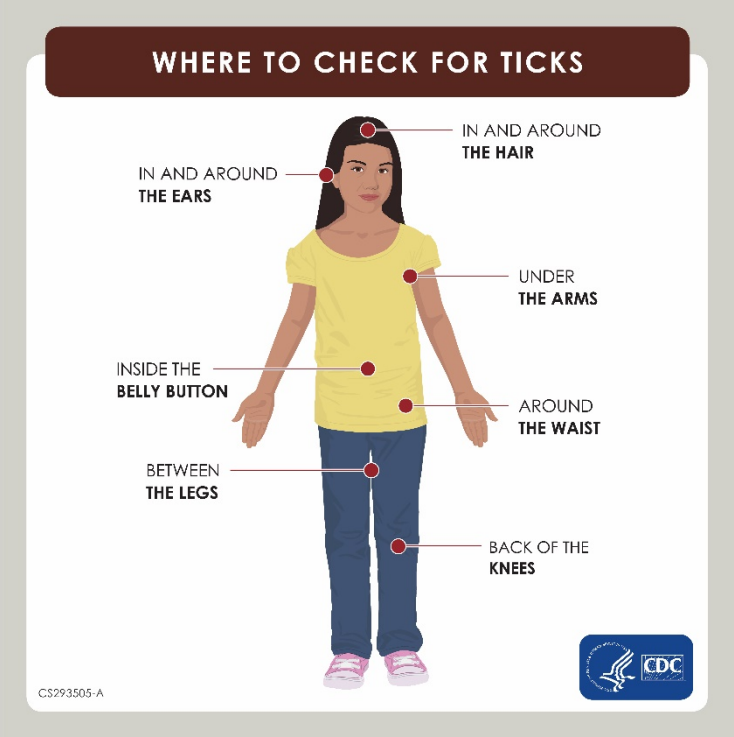
While You Are Outdoors

- **Know where to expect *I. scapularis* ticks.** Spending time outside playing in the yard, gardening or doing yard work, walking your dog in the neighborhood, camping, or hunting could bring you in contact with ticks seeking a host. Many people get bites by *I. scapularis* ticks in their own yard or neighborhood, where the ticks occur commonly in wooded portions and along wooded ecotones in yards or greenbelts (shaded, moister microhabitats), but less commonly on open, sunny and drier lawns.
- **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. scapularis* 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.

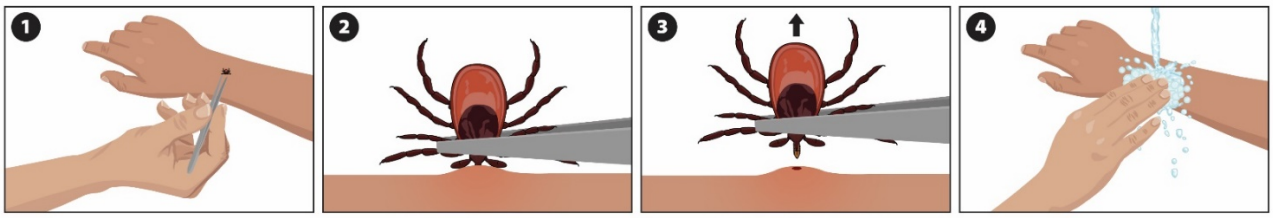
- **Even if not showering, check your body for ticks after being outdoors**. Conduct a full body check upon return from potentially tick-infested areas, including your own backyard. Use a hand-held or full-length mirror to view all parts of your body. Tick can attach anywhere on the body, but especially 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, ¼ oz. size

Sewing instructions

For each flag:

Step 1: *Preparing the materials*

From the rubberized cotton flannel material, cut:

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

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)

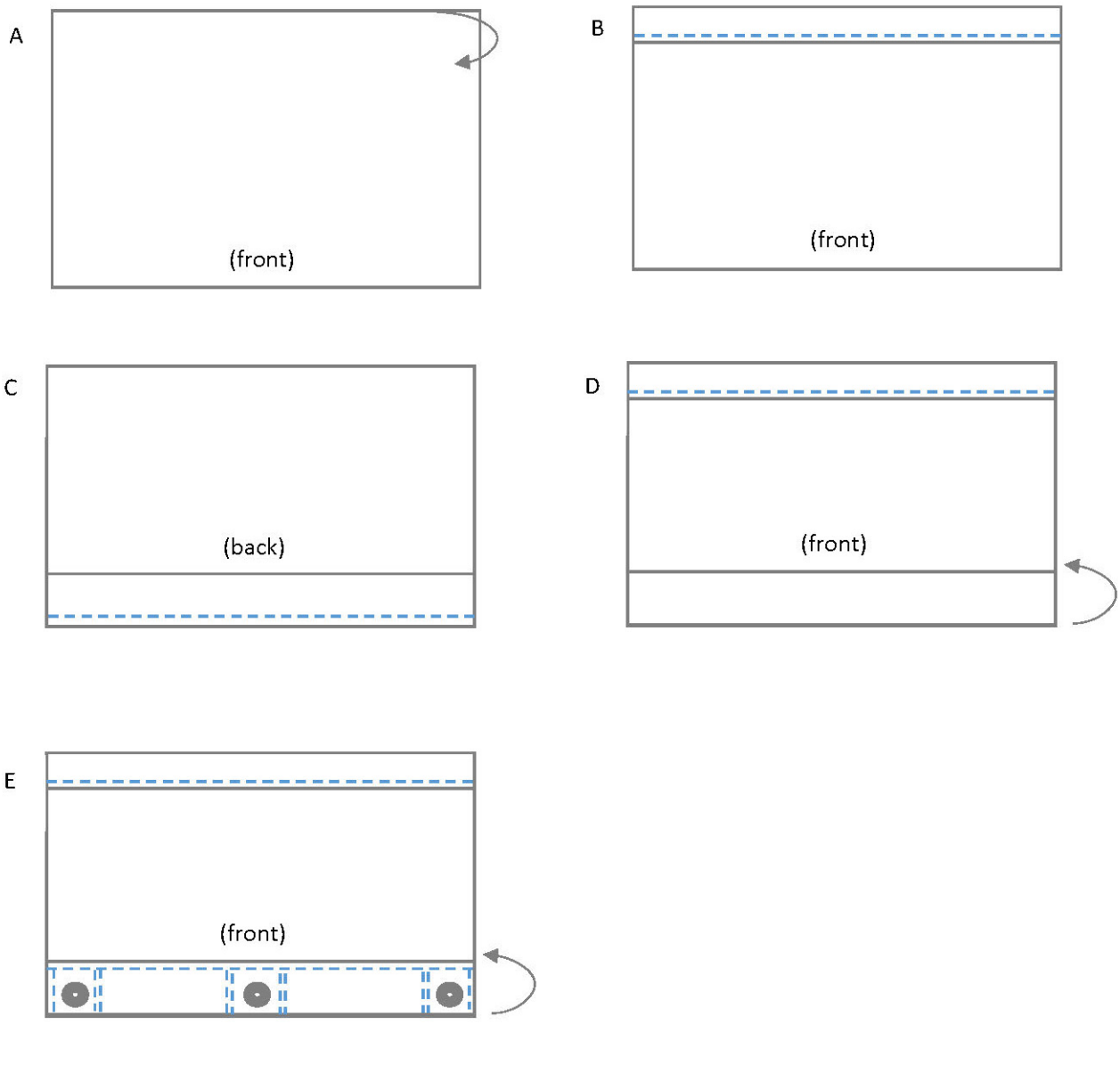
Step 3 (flat drag): *Adding the weights*

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