

# Guide to the Surveillance of Metastriate Ticks (Acari: Ixodidae) and their Pathogens in the United States



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## Intended Audience and Objectives

This guidance is intended to provide recommendations for the collection and processing of ticks for tick and pathogen surveillance efforts. There are several existing guidance documents for tick surveillance (listed separately in references) and these may be consulted for additional information. Herein, we provide a concise guide for those who are starting tick surveillance activities or those who wish to standardize practices and methods in support of public health.

There are three families of ticks (Acari: Ixodidae), with two families (Argasidae and Ixodidae) in the United States. General guidance on the surveillance of soft ticks (Argasidae) is available from Manzano-Roman et al. (2012). CDC tick surveillance guidance was issued for prostriate hard ticks (*Ixodes* spp.) in September 2018 (revised in April 2019; see [www.cdc.gov/ticks/surveillance/](http://www.cdc.gov/ticks/surveillance/)). The guidance herein serves to expand surveillance guidance to the metastriate or non-*Ixodes* hard tick genera (Acari: Ixodidae). This diverse group of ticks may be found in a wider variety of habitats and environmental conditions. This guidance defines the information desired from tick surveillance including where these ticks occur, when the

different stages are most active during the year, and which human pathogens are of greatest local concern.

# Public Health Importance of Metastriate Ticks

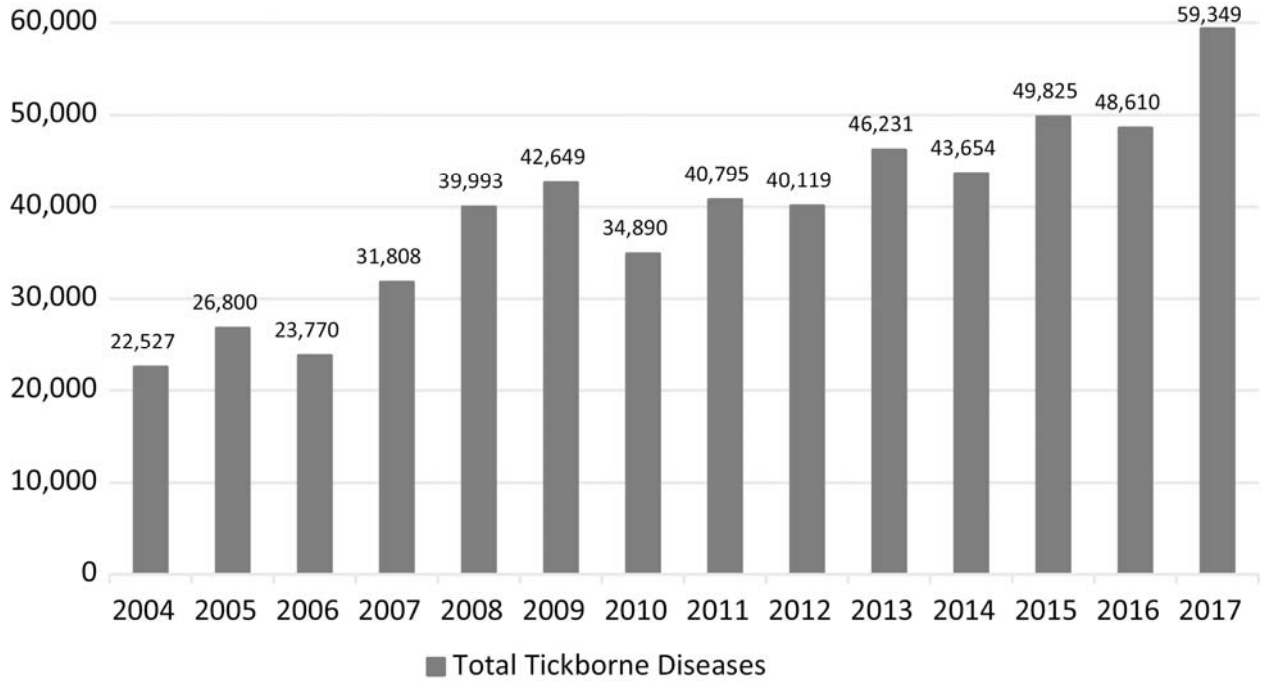
*Amblyomma* (*A. americanum* and *A. maculatum*), *Dermacentor* (*D. andersoni*, *D. occidentalis*, and *D. variabilis*), and *Rhipicephalus sanguineus* are some of the most important metastriate ticks that bite humans and transmit pathogens in the United States. Pathogens of public health concern are associated with each of the metastriate tick genera described above resulting in thousands of illnesses each year. Additional microorganisms associated with ticks are of unknown pathogenicity or have not been definitively isolated from humans. In addition to these ticks, other tick species will occasionally bite humans but will not be considered in detail for this document. The surveillance methods described herein can be applied to a wider range of metastriate ticks but may require host-targeted collection efforts to find these ticks in immature stages.

Recently, an invasive metastriate tick, *Haemaphysalis longicornis*, the Asian longhorned tick, was identified in the United States, and rapidly recognized in an expanded range. In its native habitat, as well as previously known areas of invasion, this tick is reported to harbor or transmit a number of human and animal pathogens (Heath 2016). *Haemaphysalis longicornis* has not yet been identified as carrying any human pathogens in the United States. Recent studies showed this vector to be an incompetent vector of *Borrelia burgdorferi* (Breuner et al. 2019) but appears to maintain and transmit *Rickettsia rickettsii* efficiently in experimental settings (Stanley et al. 2020. In press). Although *H. longicornis* have been found on a variety of large and medium-sized mammals, it has been found to be averse to feeding on white-footed mice, an important reservoir for tickborne pathogens in the United States (Ronai et al. 2019). Only a few human bites by *H. longicornis* have been documented in the United States to date, but the public health importance of this tick is undergoing further evaluation. Therefore, increased surveillance for this tick is needed to address medical and veterinary concerns.

## Increased reporting of tickborne diseases

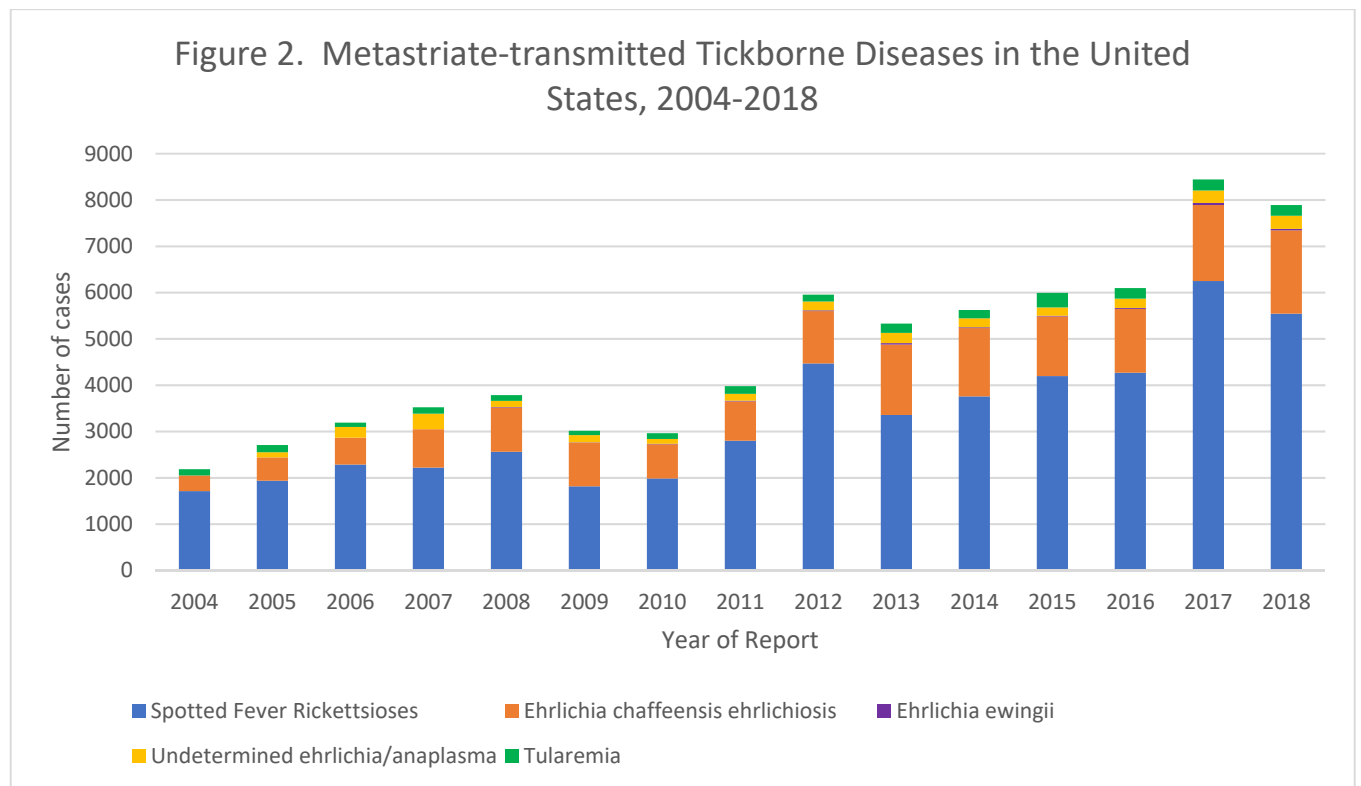
There has been a steady increase in tickborne disease reporting over the last decade (Fig. 1), with increases in all bacterial diseases and new identification of viral tickborne diseases.

**Figure 1. Total number of tickborne disease cases reported to CDC, United States, 2004-2017.**



While the numbers of Lyme disease cases make up much of this chart, other tickborne diseases have also been on the rise. Of the nearly 50,000 cases of tickborne disease reported in 2018, nearly 8,000 cases were due to pathogens associated with metastriate ticks (Fig. 2).

**Figure 2. Metastriate-transmitted Tickborne Diseases in the United States, 2004-2018**

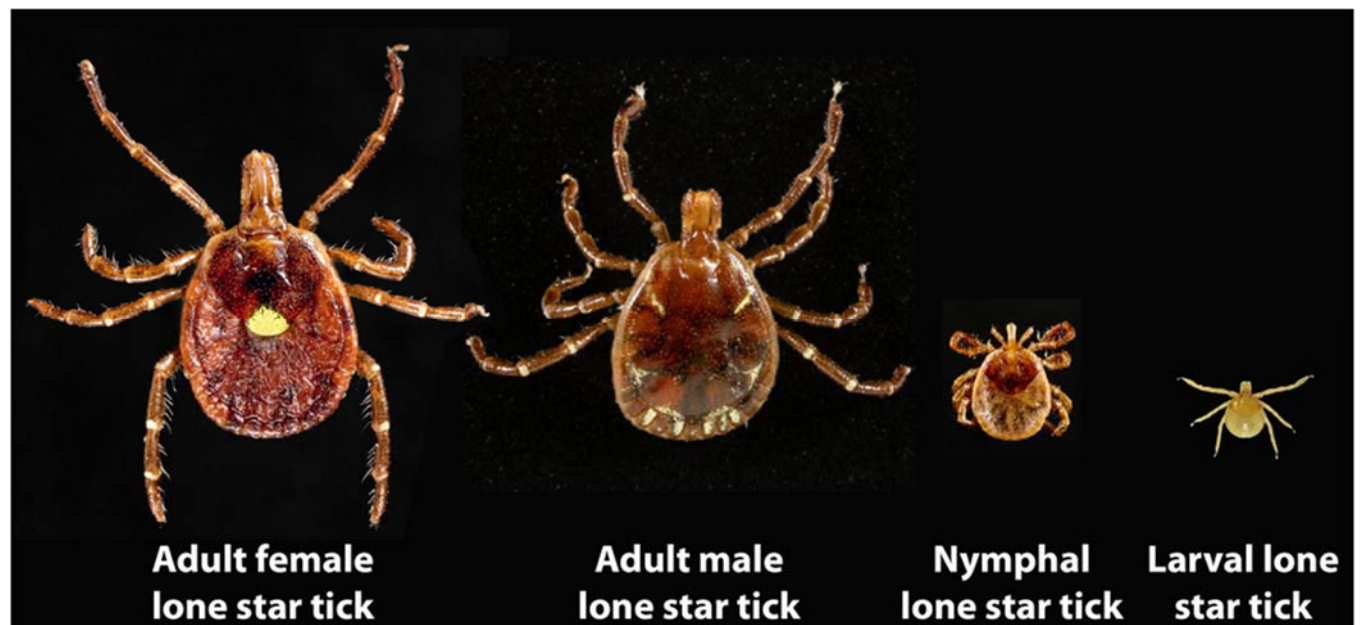


New areas have seen the expansion of certain species through both active and passive surveillance efforts. *Amblyomma americanum*, the lone star tick, has been shown to expand its range northward, with subsequent recognition of lone star tick-associated pathogens. The Gulf Coast tick, *Amblyomma maculatum*, has expanded well beyond its traditional range and is important as a vector of *Rickettsia parkeri*. Passive surveillance efforts, including citizen science efforts have provided useful information on the occurrence of various tick species and their pathogens. These approaches can provide a valuable supplement to standardized surveillance methods described here.

## Life Cycles of Metastriate Ticks

Metastriate ticks may have life cycles with feeding patterns characterized as one-, two-, or three-host strategies. All of the metastriate ticks that are of public health importance are three-host ticks, whereby three separate hosts are utilized throughout their life cycle (Fig. 3). Larvae hatching from eggs feed upon the first host, which may be large-, medium-, or small-sized mammals or birds. The engorged larvae detach and later molt into nymphs. The nymphs typically feed on medium- to large-sized mammals and detach when engorged. They molt into adults that then attach to larger mammals. Each tick has a range of animals on which they may feed; some are quite broad in their host selection, while other species feed on very specific hosts (Table 1). Examining these additional hosts may enhance your ability to detect these species or life stages, while flagging or dragging may only pick up certain stages, often adults, of several species.

Figure 3. Motile life stages of the lone star tick, *Amblyomma americanum*.



**Table 1. Typical hosts of metastriate ticks of public health importance.**

Tick species	Immature hosts	Adult hosts	Typical habitat
<i>A. americanum</i>	Medium and large mammals, birds	Medium and large mammals	Wooded ecotones, mixed conifer-deciduous forests
<i>A. maculatum</i>	Small mammals and birds	Large mammals	Grassland, drier environments (xerophilic)
<i>D. andersoni</i>	Small mammals, especially ground squirrels and chipmunks	Large mammals	Chaparral, (elevations of 4,000-10,500 ft.) open shrubby grasslands, sagebrush
<i>D. occidentalis</i>	Small mammals, especially rodents	Large mammals	Open areas, grasslands
<i>D. variabilis</i>	Small and medium-sized mammals	Medium and large mammals	Ecotones along old field meadows, mixed forests; often along trails and roadways
<i>H. longicornis</i>	Medium and large mammals; occasionally birds	Medium and large mammals	Open pastures, shrubby brush, wooded areas
<i>R. sanguineus</i>	Canine species, especially domestic dogs; occasionally additional animals such as large mammals and brown rats	Canine species, occasionally large mammals	Kennels, dog pens, peridomestic habitats in SW USA

## Tick Surveillance Objectives

Tick surveillance is intended to monitor changes in the distribution and abundance of ticks and to assess the presence and prevalence of tickborne pathogens to provide actionable, evidence-based information on infection risk to clinicians, the public, and policy makers. The following objectives will provide information to 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 as established, reported, or no data available for the following metastriate tick species:
  - a. *Amblyomma americanum* (lone star tick)
  - b. *Amblyomma maculatum* (Gulf Coast tick)
  - c. *Dermacentor andersoni* (Rocky Mountain wood tick)
  - d. *Dermacentor occidentalis* (Pacific Coast tick)
  - e. *Dermacentor variabilis* (American dog tick)
  - f. *Haemaphysalis longicornis* (Asian longhorned tick)
  - g. *Rhipicephalus sanguineus* (brown dog tick)



- 2) classify county status for presence of specific human pathogens in these ticks: present or no data available
  - a. *Ehrlichia chaffeensis*
  - b. *Ehrlichia ewingii*
  - c. *Rickettsia rickettsii*
  - d. *Rickettsia parkeri*
  - e. *Rickettsia* sp. 364D
  - f. *Francisella tularensis*
  - g. Heartland virus
  - h. Bourbon virus
  - i. Colorado tick fever virus
- 3) generate estimates for local prevalence of specific targeted pathogens in relevant life stages of the ticks listed above 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 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 tickborne 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.

Tickborne 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 density of host-seeking adults (DOA), 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 DOA. Measures of density require quantifying the sampling effort. Overall, acarological risk measures such as pursued in **Objective 3** that combine the density of host-seeking nymphs or adults and local estimates of infection prevalence provide better estimates of human encounters with infected host-seeking ticks than simple measures of tick/pathogen presence or abundance (Mather et al. 1996, Pepin et al. 2012, Eisen and Eisen 2016).

Recognizing that acarological risk measures often differ by life stage, documenting when each life stage is actively host-seeking helps identify when humans are at greatest risk for exposure to tick bites and tickborne pathogens. Therefore, **Objective 4** aims to document host-seeking phenology (seasonal variation of host-seeking activity) of larval, nymphal, and adult metastriate ticks of public health importance.

Criteria for classifying county establishment status, 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, and regularly updated. State health departments and other CDC public health partners may submit data through ArboNET ([wwwn.cdc.gov/arboNET/](http://wwwn.cdc.gov/arboNET/)). For additional information on ArboNET submissions, and to download a fillable datasheet, please see ArboNET tick module. Additional information can be found in subsequent sections of this document.

## **Obj. 1. Classify county status for metastriate tick species of public health importance**

- **Task: Update distribution maps for metastriate tick species of public health importance based on county level establishment criteria.** Data will be displayed at: [www.cdc.gov/ticks/surveillance/](http://www.cdc.gov/ticks/surveillance/)
- County status classification criteria are as follows (after Dennis et al. 1998):
  - Established:  $\geq 6$  ticks of a single life stage or  $> 1$  life stage collected per county within a 12-month period
  - Reported:  $< 6$  ticks of a single life stage collected per county within a 12-month period
  - No records (note: should not be interpreted as absence of occurrence):
- For this objective and all others, ticks should be identified to species and life stage using published taxonomic keys (see Tick Identification section for resources)
- For counties reporting new records, voucher specimens supporting the status change should be archived in curated collections (e.g., U. S. National Tick Collection; see Resources section).
- 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 on pathogen presence and prevalence and assessments of acarological risk of human exposure to tickborne pathogens.

## **How to estimate distribution of metastriate ticks**

### **Where to sample**

Metastriate ticks may be found in a variety of habitats and associated with a variety of typical vertebrate hosts (see table 1). Surveillance to extend the county-level knowledge is a goal of this effort. Documenting distribution and monitoring for changes is desirable. Generalized distribution maps have been produced by CDC, but standardized tick surveillance will generate improved maps.



*Amblyomma americanum* is generally distributed across the southeastern and south-central United States. The range of this tick is expanding and moving northward and westerly. All three motile life stages may be readily collected by drag, flag, walking surveys, carbon dioxide traps, and host examination.



*Amblyomma maculatum* is generally distributed in the southeastern United States and extends northward along the eastern states. Populations have established further north in Oklahoma. A similar species *A. triste* (which may in fact be the same species) exists in areas of Arizona, New Mexico, and Texas. Generally, only adults are collected using drags or flags. Carbon dioxide traps or host collections can detect immatures.



*Dermacentor andersoni* is found in the Rocky Mountain region of the western United States. Generally, only adults are collected using drags or flags. Carbon dioxide traps or host collections can detect immatures.



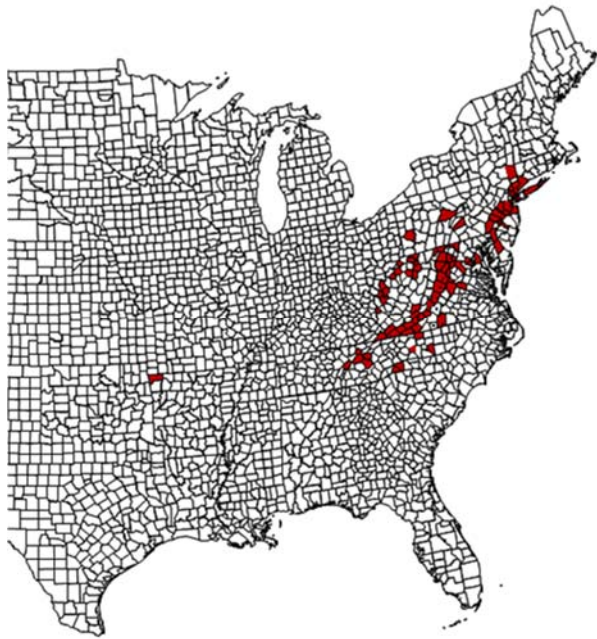
*Derma-centor occidentalis* is found in 54 of California’s 58 counties and its range extends from Oregon into Baja California in Mexico. Generally, only adults are collected using drags or flags. Carbon dioxide traps or host collections can detect immatures.



*Derma-centor variabilis* is widely distributed in the eastern United States. A separate western range includes California and other far western states. The adults may be collected by drag, flag, walking surveys, and carbon dioxide traps. Host collections can detect immatures.



*Rhipicephalus sanguineus* is thought to occur across the United States, having been distributed with its canine hosts. All motile life stages can be obtained by host examination. The adults may be collected from kennels and other dog-associated habitats. Adults may be occasionally picked up by drag or flag, but carbon dioxide traps provide a convenient method for the detection of all stages.



*Haemaphysalis longicornis* has been introduced into the United States and has been found in 97 counties in 12 states (as of March 2020). Monitoring the ongoing recognition of this invasive species in the United States is a desirable goal. As the established populations are parthenogenetic (asexual reproduction), males are not found. Adults, nymphs, and larvae can occur in high numbers and may be collected by using drags, flags, and host examination. Workers have reported that carbon dioxide traps are useful, but ticks must be picked from the traps frequently as they do not appear to stay long at the traps and are not caught using sticky tape.

### Sampling areas of interest

Specific sampling sites should focus on areas considered to be a public health concern and might include, but are not limited to, the following:

- case patient properties
- novel areas of potential human exposure to human-biting ticks
- counties where certain targeted species have become newly established
- counties (or counties neighboring areas) where incidence of tickborne 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 tickborne infections are prevalent

### Size of area to sample

The density of host-seeking nymphal (DON) or adult (DOA) ticks varies spatially and temporally. To obtain a representative sample of the density of host-seeking (infected) nymphs or adults, 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). 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). (Borgmann-Winter & Allen 2020)

## When to sample

- Sampling should be conducted during the perceived peak of nymphal or adult tick activity. This information may be available from previous phenology studies conducted in the region, timing of onset of human tickborne 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/flag, or when it is unseasonably cold or extremely windy.

## How many sites to sample

Sampling numerous sites per county improves 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 adequate sampling is conducted for only a single site per county. It may be helpful to conduct preliminary surveys at several sites before deciding upon sites for ongoing surveillance.

## Obj. 2. Identify presence and prevalence of human pathogens in metastriate tick species of public health importance

- **Task: Map the county level distribution of human pathogens in metastriate tick species of public health importance.** Data will be displayed at: [www.cdc.gov/ticks/surveillance/](http://www.cdc.gov/ticks/surveillance/)
- Data to be mapped include:
  - Shading counties where pathogens of interest have been detected in ticks collected from the environment or from their natural vertebrate hosts. 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 (for example: 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). Infection in ticks collected from the environment (by dragging,

flagging, walking sampling, or trapping) or infection in ticks collected from trapped mammals (provided their home ranges, as known, 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 a tick species collected from the environment or from a natural vertebrate host (this information will be updated annually on [www.cdc.gov/ticks/surveillance/](http://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](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html) ([www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html)). 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. Some tick species may have very low prevalences of a particular pathogen (e.g., *Rickettsia rickettsii* in *Dermacentor variabilis*), and may require testing of hundreds of ticks to get reasonable confidence limits. More common pathogens can be assessed with smaller sample sizes. 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. This gives 80% power to detect a prevalence of 6.2% or higher. Infection prevalence and associated 95% confidence intervals should be calculated for data submitted to ArboNET.
- Target sample sizes should be determined prior to surveillance for a specific pathogen.

## How to estimate infection prevalence in host-seeking ticks

In planning surveillance of pathogens in host-seeking ticks there typically is some expected level of confidence in declaring that a pathogen is absent from a site or a county. The primary challenge is that, by chance alone, we may collect a sample of ticks that are not infected, even though the pathogen does occur in the population. The less common a pathogen, such as *Rickettsia rickettsii*, the more likely this is can occur. As prevalence in ticks increases, we need to sample relatively fewer ticks to rule out this possibility. Statisticians refer to this issue when they discuss power calculations or minimum effective sampling. The needed, minimum sampling size therefore relies on the number of ticks collected (and tested), the prevalence of infection and the desired certainty of detecting the pathogen. Traditionally, this certainty is set at 80% (4 of 5 times we want to detect the pathogen if it is present).

The required sample size is valuable for planning resource allocations and sampling efforts. During case investigation this is somewhat less critical because we have presumptive knowledge of the location(s) of exposure that we want to target. However, in prospective surveys for pathogens (Objective 2) the question is more open-ended. Here is a thought example to illustrate the issues. Consider a pathogen such as

*Rickettsia parkeri* and in the population the infection prevalence is 0.35 (35%). This implies that the probability that any one tick will not be infected is 0.65 (65%). If we collected eight ticks and tested them, basic statistical rules tell us (making some assumptions about statistical independence) that the chance we would find no infected ticks is  $0.65^8 = 0.032$ . So, the chance that none of the ticks were infected if the prevalence was really 0.35 is only about 3/100 times. Now, consider what happens when the infection prevalence is 0.002 (0.2%). In this case the probability a tick is not infected is 0.998 and using the same rules, the chance that we could find no infected ticks in a sample of eight, even if prevalence was 0.002 is  $0.998^8 = 0.984$ . Thus, it is nearly certain that we would not detect the pathogen in a sample of eight ticks when the pathogen prevalence is low.

This pattern leads to the use of power calculations and various software packages, such as EpiInfo ([www.cdc.gov/epiinfo](http://www.cdc.gov/epiinfo)), to provide straightforward calculations to determine how large a sample needs to be collected to have 'enough' power. The issues of study design and power represent entire topics of research interest but an electronic calculator, and the example outlined in the above paragraph also can be used for quick estimates. In these two examples, the probability of finding no infected ticks even though the true prevalences were 0.35 and 0.002 represent  $1.0 - (\text{power})$ . Thus, the power of testing 8 ticks when the prevalence is 0.35 is  $1.0 - 0.032 = 0.968$  (nearly 97%) while the power of testing 8 ticks when the true prevalence is 0.002 is  $1.0 - 0.984 = 0.016$  (1.6%). Traditionally, many groups target a power of 80% in their studies. In the first case, when prevalence is 35% we have too high a power (we don't need to test eight ticks to detect the pathogen if the prevalence is really 0.35), while in the second example the power is too low. The goal is not to have exactly a power of 80% because the vagaries of sampling field specimens (and other causes of negative results in diagnostics) may be substantial. Rather, the intent is to establish an estimate of how many samples are needed to reach a satisfactory level of certainty in our efforts and whether we need to sample fewer locations more intensively or spend less effort at individual sites and sample more locations.

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<sup>2</sup> 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 adult densities but can be included in assessing site-specific estimates of pathogen prevalence.

Pathogen detection assays should meet the minimal requirements described above (See "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](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html) ([www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html)). 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 sufficient numbers of ticks 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.



### **Obj. 3. Estimate the density of host-seeking (infected) metastriate tick species of public health importance**

For each of the objectives listed below, when sufficient data have been submitted to ArboNET, CDC will post annual surveillance reports at [www.cdc.gov/ticks/surveillance/](http://www.cdc.gov/ticks/surveillance/).

- **Task: Map the county level density of host-seeking nymphs or adults.**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking nymphs or adults of individual species collected per 100 m<sup>2</sup> or displayed as the inverse showing the distance covered before expected encounter with a nymph or adult of that species.
    - Requires at least 750 m<sup>2</sup> drag sampled per site for density estimate; drags should be inspected for ticks at least every 10m to avoid loss due to drop-off of ticks; sampling should be timed to coincide with the peak in nymphal or adult host-seeking activity; ideally, estimates of nymphal and adult 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 Ticks.”
    - Requires at least 1 site sampled per county, otherwise county will be displayed as “no records”. Document when sampling was attempted, even if unsuccessful.
    - In ecologically diverse counties, sampling at multiple sites representing the range in suitable habitat for the tick is recommended; different tick species may be found at different altitudes as well. When multiple sites are sampled per county, average and range will be calculated.
    - Although timed sampling (e.g., dragging for fixed amounts of time, rather than fixed distances) is a valid sampling approach and used by many public health jurisdictions, in the interest of comparability among localities, we will only accept distance-based assessments of DON/DIN or DOA/DIA for ArboNET submission.
  
- **Task: Map the county level density of host-seeking infected nymphs or adults**
  - Data display and minimal sampling requirements include:
    - Displayed in categories based on number of host-seeking infected nymphs or adults collected per 100 m<sup>2</sup> (with a 1m<sup>2</sup> drag) or displayed as the ticks per distance flagged for infected nymphs or adults.
    - Calculated by multiplying the estimated density of nymphs or adults by infection prevalence (both described above).
    - When multiple sites are sampled per county, average and range will be calculated.
    - 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 DOA and DIA for ArboNET.

## 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 nymphal ticks collected per total area sampled. DON can be scaled per 100 m<sup>2</sup> by multiplying the total number of 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 infection prevalence (% of ticks infected or the point estimate derived using the Pooled Infection Rate Add-In [[www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html)]). Because infected ticks are not spatially distributed in a homogenous manner, confidence intervals can be large. 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 adults (DOA) is estimated as the total number of adult ticks collected per total area sampled. DOA can be scaled per 100 m<sup>2</sup> by multiplying the total number of adult ticks 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 (DIA) is estimated by multiplying DOA by infection prevalence (% of ticks infected or the point estimate derived using the Pooled Infection Rate Add-In [[www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html](http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html)]). Like nymphs, adult densities can have large confidence intervals. To include a confidence interval, DOA should be multiplied by the lower infection prevalence confidence limit and then by the upper infection prevalence confidence limit.

## Obj. 4. Document host-seeking phenology of metastriate tick species of public health importance

- **Task: Describe when 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 specific months of the year or not, or no records if phenology studies were not reported from a particular state or its neighboring states).
  - Based on weekly, every two weeks, 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 Metastriate Ticks."

## Describing host-seeking phenology of metastriate ticks

### Where to Sample

Because tick species are found in various habitats, phenology study sites should be situated in the appropriate habitats for the targeted species, ideally in an area where the tick is abundant in order to accurately assess temporal changes in density. Sites with low density are susceptible to stochastic (random) variation. 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 estimating 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, every two weeks, or monthly 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.

## Limitations to Tick Surveillance

- Merely documenting the presence of certain tick species within a county may be a poor indicator of human disease risk. For example, *D. variabilis* has been reported in many counties in the southeastern United States, but *Rickettsia rickettsii* infection rates are typically low. The limited contact between people and infected *D. variabilis* substantially reduces risk.
- Although county estimates of the density of host-seeking infected nymphs or adults may be a better predictor of human disease occurrence compared with simple measures of tick presence or density of host-seeking ticks, DIN/DIA and DON/DOA do not always accurately estimate risk of tickborne diseases in humans. This may relate to spatial heterogeneity (differences by region) 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 unknown factors.



# Preparation for Tick Surveillance

## Permission and permits

Tick surveillance is often conducted at sites that can readily be re-visited several times within the season and over multiple years. Specific areas may be chosen based on likelihood of human-tick contact, such as previous case patient properties or publicly utilized areas. Sites should contain suitable tick habitat (mowed areas are not always productive areas to search). Pilot sampling might be done to identify suitable areas for repeated collections. If these sites are private properties, it may be prudent to employ standardized permission forms to document permission to collect on owned land. It would also be prudent to develop standardized permission forms for collection of specimens from owned animals (pets or livestock). Working with local authorities can facilitate identification of appropriate sites and owner contact information. State parks and state-owned land may require permission from the local park supervisor or a permit from the appropriate state authority (Department of Conservation or Department of Natural Resources). Collection of any wildlife, including invertebrates in some states, will require a scientific collection permit. Depending on the state, you may be required to obtain one permit for the entire collecting team, or individual permits for each collector. Please contact the appropriate authorities to determine the requirements for each state and submit applications early as it can take multiple weeks for approvals.

## Personal protection for persons conducting tick surveillance

Use personal protective procedures when conducting tick surveillance:

- **Use Environmental Protection Agency (EPA)-registered tick repellents** containing DEET, picaridin, IR3535, Oil of Lemon Eucalyptus (OLE), para-menthane-diol (PMD), or 2-undecanone when skin application is desired. EPA's helpful search tool ([www.epa.gov/insect-repellents/find-repellent-right-you](http://www.epa.gov/insect-repellents/find-repellent-right-you)) can help you find the product that best suits your needs. Always follow product instructions.

- **Treat clothing and gear** with products containing 0.5% permethrin. Permethrin can be used to treat boots, clothing and gear and remain protective through several washings. Application prior to a collection trip is conducted and the clothing allowed to dry. Upon drying, the clothing is safe to handle and the treatment will last multiple launderings. This is the method used to treat military uniforms and it has been shown to be effective in reducing tick bites and reducing exposure to pathogens (Vaughn et al. 2014). Permethrin should not be applied to skin.
- **Wear long-sleeved shirts tucked into pants and long pants tucked into the socks** to provide a barrier to tick access to skin. Tick and chigger gaiters (e.g., Forestry Suppliers), especially when treated with permethrin, provide a very effective additional barrier when placed over the pant-boot junction. Light colored clothing facilitates detection of crawling ticks, but one study showed enhanced numbers of *Ixodes ricinus* on lighter clothing (Stjernberg & Berglund 2005). Tyvek coveralls have been used by some collectors but are not advised in hot weather due to chances of overheating and dehydration.
- **Inspect for ticks on your clothes and skin routinely** after tick collection attempts. If an attached tick is discovered remove by grasping tick with forceps close to the skin at the skin-tick interface and pull tick steadily backward until removed.



Because various species of game animals may be seasonally harvested throughout the year, it is recommended that surveillance workers wear safety vests and caps in blaze orange or other bright color, particularly in areas where public hunting is permitted. The bright clothing has an additional benefit in making it easier to locate other workers in heavily wooded areas.

## Biosecurity

It is important to consider biosecurity when conducting tick surveillance so that inadvertent transport of ticks from one site to another is avoided. This can spread infestations of tick species, especially of exotic ticks, and can compound your assessments of sites in both species diversity and numbers. When site collection is complete, all workers should closely examine clothing and gear to remove any motile ticks. The cloth may be laid across a vehicle hood and a masking tape lint roller or duct tape used to remove any ticks from flags or drags before moving to other sites. The roller is used repeatedly until no ticks are seen (this works best with larvae). Some workers carry additional flags/drags for use at each site. If the flag or drag is made to be removable (using clips or Velcro), the cloth can be removed and placed into a 1-gallon sealable bag for later examination and a new cloth attached for the next collection site.

# Collection and Transport of Ticks

Regardless of the method of collection, workers need some basic equipment. Fine-tipped forceps are suitable for collection from flags/drags, carbon dioxide traps, and hosts. It is helpful to attach a brightly colored lanyard or plastic survey flagging to the forceps so that they can be located if dropped. A short

piece of plastic tubing can be used to protect the sharp tips from becoming damaged, while retracting reels can be added to make the forceps readily accessible.

Often, it may be easier to maintain live ticks for an extended field trip and then preserve them (ethanol or frozen) when they are transported back to the laboratory. Vials containing a base of plaster of Paris and capped by a latex cover work well for initial collections. Fine-tipped forceps are closed and push through the latex to make a small slit into which the collected individual ticks can be pushed. The plaster is wetted with a few drops of water to maintain humidity within the vial. Activated charcoal can be added to the plaster of Paris when preparing vials to provide anti-fungal activity and serve as a color indicator of water having been added. Vials may be placed into Whirl-Pak bags containing a slightly wetted cotton ball, labeled by sample site, and sealed. The vials can be stored in a cooler for the duration of a field survey. Be sure to avoid placing ticks directly in contact with cold packs or ice (separate them physically by a piece of Styrofoam).

If ticks are to be frozen, they can be placed into dry ice bucket, but this requires maintenance of the cold chain through the field survey to the laboratory. Dropping ticks directly into ethanol after collection often causes the ticks to draw their legs inward and this can make morphologic features less visible for identification. Dropping the ticks first into hot water or Boardman's solution makes the ticks extend their legs and then the ticks can be transferred to ethanol for preservation (Cooley 1938; Boardman 1944).

## Methods of Tick Collection

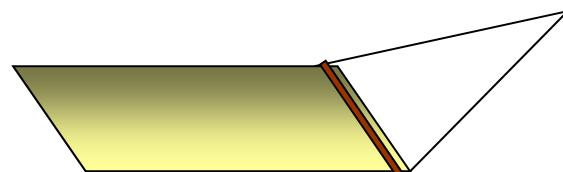
Numerous methods can be used to collect ticks; however, some are better suited than others for addressing specific surveillance objectives (Table 2). For example, all of the methods described below can be used to demonstrate the presence of tickborne pathogens in a county of interest. Demonstrating that both the vector and pathogen are present within a county provides fundamental data for assessing the risk of human encounters with infected ticks. However, unlike Lyme disease, which is most commonly acquired through the bite of infected nymphs (Eisen & Eisen 2016), entomologic metrics have not been determined for many diseases transmitted by metastriate ticks, and this would be a worthy goal of research for other tick species. Estimates of the density of pathogen-infected host-seeking nymphs or adults may be a predictor of the risk of human disease associated with metastriate ticks. Assessing the presence of the tick or pathogen, developing quantitative measures of the density of host-seeking nymphs or adults, and determining the infection prevalence in the nymphs or adults are excellent first steps toward making this association.

**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 DOA/DIA	Objective: Phenology
Dragging/Flagging	Acceptable	Acceptable	Acceptable	Acceptable
Walking	Acceptable	Acceptable	Not Acceptable	Acceptable
CO <sub>2</sub> 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

### Sampling by dragging or flagging

Dragging and flagging are similar methods used to collect host-seeking ticks whereby a cloth is waved across and through vegetation. This method has been in use since at least 1911 and has proven to be a very effective method (Smith 1990, Sonenshine 1993). It has been used for a variety of ixodid species (Carroll and Schmidtman 1992, Falco and Fish 1992; Gladney 1978). The terms dragging and flagging are used somewhat interchangeably in the scientific literature which can cause confusion. For the purposes of this guidance document, dragging will refer to cloth being attached to a dowel and pulled along behind the operator by a cord attached at each end of the dowel. A flag is attached to the end of a longer pole in the manner of a banner and swept in front or to the side of the operator. A combination drag-flag providing function of each has been described by Newman et al. (2019).



Both formats typically use a wool, flannel, denim, muslin, corduroy, or other sturdy white fabric with sufficient texture for ticks to grip. Texture and weight of the cloth affect the ability to collect ticks (De la Mendonca 2018, Newman et al. 2019, Vassallo et al. 2000). The cloth is usually light in color to readily detect ticks. The size of the cloth has varied among workers yet is often 1m wide x 1m-1.5m long in size. A particularly good cloth is that described by Carroll & Schmidtman (1992) which is a waterproof baby crib cloth with two layers of flannel cloth laminated to a vinyl rubber center sheet. It can be pulled through briars and heavy vegetation with no tearing. Flags and drags made from this material last multiple years of use.



When using drags, contact between the fabric and vegetation can be increased by sewing weights (e.g., metal washers or chains) into the trailing edge and/or the trailing edge may be cut into “fingers” or “strips” rather than using a solid cloth. There does not appear to be any significant differences between drags utilizing strips versus the whole cloth (Siegel et al. 1991; Petney et al. 2018). Modified handles or extensions may be used to increase maneuverability (e.g., the tick sweep, Carroll & Schmidtman 1992, Newman et al. 2019).

Flagging is most effective when the flag is waved or moved in a figure eight pattern just ahead of the operator. The area covered by each pass can be estimated for transect purposes, or a pattern or sampling quadrants can be established at each site. Flags can help protect users from tick bite or snakes as the flag precedes the user. Flags have been supplemented with carbon dioxide to increase yields, but this method may be more complicated (Gherman et al. 2012).

For additional details on how to make tick drags and flags, see the “How to Make Tick Drags” and “How to Make Tick Flags” supplemental information. The tick drag is moved horizontally across vegetation (drag) or wiped across vegetation in more vegetated areas. Flags allow collection at various heights of vegetation and may be found to be more maneuverable than drags around high-density scrubs and other vegetation. These methods of sampling provide good spatial precision for documenting the occurrence or abundance of ticks in a county.

Flagging and dragging can be used to estimate abundance by either moving the cloth across a measured transect distance or for a measured period of time (Sonenshine 1993, Wilson 1994). The distance method is usually 100 meters per transect, checking the drag by flipping it over every 10 m. A handheld counter may be used to keep up with the number of 10m segments while dragging/flagging. It has been estimated that some ticks may be wiped off the drag after being collected if frequent checks are not conducted (Borgmann-Winter & Allen 2020). Flags may be swept alongside the operator or waved in a figure-eight pattern ahead of the worker, so it is being flipped at each wave and examined. A search image will develop over time so that additional time is not needed for examination. A defined area may be demarcated and sampled at each timepoint. Some workers use ticks per person per unit time as an estimate of tick abundance as it serves as a surrogate for tick encounters (e.g., Gleim et al. 2014). For both drags and flags, there is individual variation in walking speed, choice of path, and time spent picking ticks from the cloth over directional movement in the habitat.

For the purposes of ArboNET submissions, ticks per distance unit will be used.



Flags and drags are acceptable to use to address the following key surveillance objectives:

- Classifying county status for each tick species
- Identifying presence and prevalence of pathogens in ticks (all active life stages)
- Estimating the density of host-seeking (infected) nymphs or adults
- Documenting host-seeking phenology

## Walking sampling

Walking sampling entails an investigator walking at a leisurely pace (estimated to be one pace per second) through tick habitat and checking his/her clothing and body for crawling ticks (Carey et al. 1980, Schulze et al. 1986). A flannel suit has been described for this work or white cotton coveralls may be worn to readily locate any walking ticks that climb onto the worker (Chapman et al. 2000). 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 suggested 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. Walking sampling may be more relevant 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 adults but may result in lower yield than drag sampling or flagging for nymphs (Ginsberg and Ewing 1989). This method of sampling provides good spatial precision for documenting the occurrence and/or abundance of ticks in a county.

Walking sampling is acceptable to use to address the following key surveillance objectives:

- Classifying county status for each tick species
- Identifying presence and prevalence of pathogens in ticks (all active life stages)
- Documenting host-seeking phenology

## Carbon dioxide–baited tick traps



Carbon dioxide or dry ice traps work on the premise that ticks have well-developed chemo-receptors and are attracted to carbon dioxide to find a host (Garcia 1962). As carbon dioxide is 2.9 times as dense as air, it dissipates and disperses away from the source typically along the ground. One format of the traps consists of a solid container to hold dry ice (a solid form of carbon dioxide) within an insulating material that is surrounded by a board with a border of sticky tape to capture ticks attracted to the carbon dioxide released as the dry ice sublimates (Wilson et al. 1972). A simple version can be constructed with a disposable plastic container sitting on a 1 m<sup>2</sup> piece of flannel (see photo). The flannel is checked regularly to gather ticks or it is allowed to operate for a certain

time period and the flannel collected into a sealable bag for transport to the laboratory. Another version of the trap utilizes lactic acid and calcium carbonate to generate carbon dioxide (Guedes et al. 2012); the catches for *Amblyomma* spp. were comparable to those obtained by dry ice traps. Different tick species and stages display variable responses to CO<sub>2</sub> traps and travel at different speeds to reach the traps. These traps work particularly well for lone star ticks (*Amblyomma americanum*) (Wilson et al. 1972). *Ixodes scapularis* are attracted to CO<sub>2</sub> traps, but these traps appear to be less effective than drag sampling or flagging (Ginsberg and Ewing 1989, Falco and Fish 1992), possibly due to the speed of movement. The CO<sub>2</sub> traps work best on windless days and early in the morning or later in the day when there is less wind. Dry ice traps more effectively captured adult *A. americanum* than nymphs, but efficacy did not vary by habitat. It has been suggested that a standardized period of 24 hours be employed (Kensinger & Allan 2011), but shorter time periods (2-3 hours) can be employed for targeted assessments (Nicholson et al. 2006). Dry ice traps set for *Haemaphysalis longicornis* should be monitored frequently as the ticks that are initially attracted may soon move away after finding no source of a bloodmeal. Carbon dioxide trapping is generally less labor-intensive and may be used to supplement dragging or flagging collections for assessments of host-seeking densities of metastriate tick species. This method of sampling provides good spatial precision for documenting the occurrence or presence or prevalence of pathogens in a county.

Carbon dioxide traps are acceptable to use to address the following key surveillance objectives:

- Classifying county status for each tick species
- Identifying presence and prevalence of pathogens in ticks (all active life stages)
- Documenting host-seeking phenology

### **Tick collection from deer**

White-tailed deer serve as important hosts for adult *Amblyomma americanum*, *A. maculatum*, and *Haemaphysalis longicornis* ticks. Inspection of hunter-killed deer brought into check stations is a cost-effective means of detecting changes in the distribution of these tick species, particularly in areas where the tick is emerging. This may have to be specially arranged in states that have moved to telephone or digital reporting. 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. Additionally, *A. americanum* nymphs and adults are not active during the fall and early winter deer hunting season (adults are usually collected from deer from February to late winter). Based on known *H. longicornis* phenology, this species might also be dormant during the fall/winter hunting season.

Tick collection from deer is acceptable to use to address the following key surveillance objectives:

- Classifying county status for each tick species
- Identifying presence but not prevalence of pathogens in ticks (all active life stages) as blood from the host is likely present

## Tick collection from small- or medium-sized mammals, and birds



Small- and medium-sized mammals, and birds often serve as hosts of larval, nymphal, and adult metastriate ticks. Trapping and inspecting these animals for ticks can provide useful information on the presence and infestation levels of ticks and presence of associated pathogens, 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 other sampling, however, in areas where immatures are seldom collected on drags/flags, host sampling may be a very effective means of demonstrating establishment of certain tick populations and

documenting host-seeking phenology. Trapping of animals for tick examination may require special collection permits from state wildlife officials.

NOTE: Prevalence of hantaviruses in rodents has been documented in many areas across the United States indicating a need for personal protective equipment to prevent investigator exposure in these regions (Mills et al. 1995). Personal protective equipment should consist of a back-closure gown, latex or nitrile gloves, and an N95 or HEPA-filtered respirator when handling these animals.

Tick collection from animals is acceptable to use to address the following key surveillance objectives:

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

## Ticks found on people and their pets

Pets, especially dogs, can be important sentinels for the detection of pathogen activity in the vicinity of humans. Individual pets or pets coming to veterinary clinics or shelters provide convenient sites for tick surveillance. Many public health entities have established citizen science programs whereby ticks are submitted for identification or testing. When accompanied by appropriate training in specimen requirements and accurate data collection, these programs can be very informative.

Identification of ticks collected from people or companion animals can be a useful means of assessing human- or pet-tick encounters. However, because people and their pets often travel varying distances, ticks collected from these hosts should only be included in assessments of county status when travel history is available and 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. Free-roaming or stray animals may provide some information on host-tick interaction, but their unknown travel history render them uninformative for mapping specific occurrence.

Testing of ticks from people is sometimes requested. Because it provides little actionable information, *CDC does not recommend testing ticks from people for human clinical diagnostic purposes or for making a*

*treatment decision.* Positive results from a tick do not necessarily mean that a person has been infected with that same pathogen and negative results can cause false assurance.

Acceptable to use to address the following key surveillance objectives:

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

## Tick Identification

A vital component of any tick surveillance is the accurate identification of ticks to stage and species. This expertise is not always readily available at the local level, especially when first starting tick surveillance. Assistance may be provided by state university personnel or through workshops organized for states or regions.

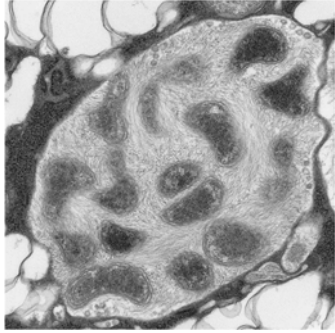
It is important to retain a portion of the collections from a site, season, and morphological type of ticks as voucher specimens. These can be deposited into curated collections so that researchers can verify identifications, or corrections may be made if taxonomic classification changes in the future. See Peterson (2010) for a discussion of vouchers.

The importance of dichotomous keys to tick surveillance is paramount. State and regional keys should be chosen based on the tick species found in the area. There are many good tick identification keys for regional tick species, but these may not be readily available. Consult with local public health entomological personnel, cooperative extension service entomologists, or entomology faculty at local universities for assistance in tick identification or for the availability of locally relevant tick identification keys. CDC may be able to help determine appropriate keys.

Molecular identification of ticks via genes for 16S rDNA, ITS, etc. may be useful, particularly on engorged or damaged specimens where morphologic identification is not possible. Often a leg may be pulled from an unidentifiable tick and the rest of the body retained for reference using morphological features. The primers 16S+1 and 16S-1 have been used routinely for molecular tick identification in the Rickettsial Zoonoses Branch (Black and Piesman 1994).

Taxonomic assistance and resources may be available from local health departments or entomology departments in state universities. The CDC Rickettsial Zoonoses Branch is able to assist in identifications by contacting:

Ecology and Entomology Team  
Rickettsial Zoonoses Branch  
Centers for Disease Control and Prevention  
Email: [ticksurveillance@cdc.gov](mailto:ticksurveillance@cdc.gov)



# Detection of Pathogens in Metastriate Ticks

## **Recommended tick samples and preservation for pathogen testing**

Pathogen testing in unfed (flat) ticks is recommended for the following surveillance objectives:

- Identifying presence and prevalence of pathogens
- Calculating density of infected nymphs (DIN) and density of infected adults (DIA)

Results from pathogen testing in fed ticks or from small mammal host tissue should generally not be used for surveillance data because: 1) ticks can acquire pathogens from host blood and become infected, but may not be able to maintain infection through the molt, 2) although infected, ticks may not be a competent vector of the pathogen, and 3) 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 small mammal host 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 (higher concentrations are generally better)
- RNALater™ (Invitrogen) or other RNA stabilization buffer
- Frozen at -80°C without preservatives

## **Recommended laboratory practices**

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

## Minimum criteria for acceptability of pathogen detection assay

In order to report that an individual or pool of targeted tick species is positive for *Ehrlichia chaffeensis*, *E. ewingii*, spotted fever group rickettsiae, tularemia bacteria, or a pathogenic tickborne 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 species. To demonstrate that an assay is 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 that particular tick species.

OR

- An assay or assays that detect a genus or family gene target followed by sequencing to identify the pathogen to species-level or to at least to confirm or rule out a target species. If a 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 target. Those amplicons that cannot be determined to species can be included as “<genus> spp.” in a report.

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, conventional or nested PCR target in the presence of tick nucleic acid (DNA or RNA as needed for that agent). 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 nucleic acid from other pathogens targeted by the sample assay.
- An internal control or tick-nucleic acid reactive assay that can be used to confirm the presence of amplifiable DNA or RNA in each specimen. A specimen that does not contain amplifiable nucleic acid should not be included in infection prevalence calculations.

For examples and considerations of testing strategies, see Graham et al. (2018) and Fenollar & Raoult (2004).

## Important considerations for *Rickettsia* testing

The *Rickettsia* genus is comprised of three major clades: the spotted fever group *Rickettsiae* (SFGR), the typhus group *Rickettsiae*, and the ancestral group; some authors divide these further. All three groups have been reported from metastriate ticks, but only the SFGR will be considered in these guidelines. There are at least 28 recognized named species within the traditional SFGR in the United States, with a number of candidate species identified, but not formally named. Many of these taxa are non-pathogenic to humans and exist as endosymbionts in ticks. At least three of those associated with ticks have been confirmed as human pathogens in the United States; *Rickettsia rickettsii*, *R. parkeri*, and *Rickettsia* 364D

are the primary species to be considered. A number of PCR assays have been published in the literature, and these assays vary in their sensitivity and specificity. Targeting the specific tick species and their pathogens will reduce overall costs of surveillance in areas where non-pathogens are abundant.

Detection strategies include the use of broad coverage amplification targets followed by nucleotide sequencing (Portillo et al. 2017). The 16S rRNA and 17-kDa gene sequences lack enough variation to discriminate species. Identification has more often used the *gltA* (citrate synthase), *ompA* (rickettsial surface protein A), and *ompB* (rickettsial surface protein B) genes. The gene “D” (*sca4*) has also been very useful for identification. Recognition of a new species may be possible but would require sequences from at least five genes (*rrs*, *gltA*, *ompA*, *ompB*, and *sca4*). Both nested and real-time PCR assays have been described for various targets.

An adjunct or alternative approach is to utilize species-specific PCR assays. Both nested and real-time assays have been described and used for tick testing. The user must be cautious in their use as some assays have been described in the literature as species-specific and are found in practice to amplify other species. Testing against a panel of species found in an area would be prudent to verify specificity in your laboratory.

- To demonstrate that an assay is specific for SFGR, it should be tested against the targeted SFGR species, and multiple strains of those species. Two assays have been described in the literature to be broadly reactive with *Rickettsia* species and sensitive enough for routine use (Stenos et al. 2005; Kato et al. 2013).
- To demonstrate that an assay is *R. rickettsii* specific, it should be tested against *R. rickettsii* (multiple strains if possible) and a panel including non-target SFGR species. One example of a species-specific assay for *R. rickettsii* is that described by Kato and associates (2013) or Kakumanu et al. (2018).
- Prevalence of pathogenic *Rickettsiae* can vary greatly in the primary tick vector species. *Rickettsia rickettsii* is found in about 1% of *Dermacentor variabilis* or *D. andersoni* but can be found in as many as 4-10% of *Rhipicephalus sanguineus* in affected areas. *Rickettsia* 364D is found in approximately 1% of *D. occidentalis* ticks in northern California, but 6-7.7% in southern California. *Rickettsia parkeri* is found in up to 45% of *A. maculatum* ticks in parts of the eastern United States.

## Important considerations for testing for Family Anaplasmataceae

There are three species of the genus *Ehrlichia* found to infect humans in the United States: *Ehrlichia chaffeensis*, *E. ewingii* and *E. muris eauclairensis*. These species, along with *Anaplasma phagocytophilum* in *Ixodes* spp., may be targeted in tick surveillance of the Family Anaplasmataceae. One strategy is to amplify DNA from the family and sequence the product to determine species. Assays targeting the 16S rRNA (Li et al. 2002), citrate synthase (*gltA*), and heat shock genes have been useful for family-level testing. Species-specific PCR assays have been described in both nested and real-time formats and provide another avenue for testing.

- To demonstrate that an assay is broadly reactive with the family Anaplasmataceae, it should be tested with a panel of species and strains for both genera targeted in surveillance. Some assays will also react with other genera and the candidate genus “*Neoehrlichia*”, which may be encountered during tick surveillance.

- Sequence analysis of broadly reactive gene targets may provide the power for identification.

Species-specific assays have been described in the literature and have been used for tick testing. These assays should be verified in your laboratory as specific for the targeted species by testing against that species (ideally multiple strains) and a panel of related family members.

- *Ehrlichia chaffeensis* is detectable by using general Anaplasmataceae PCR assays and may be distinguished either by sequencing or use of a specific assay. Ideally, any species-specific assay should be tested against a panel including other ehrlichial species found in the United States to verify performance.
- *Ehrlichia ewingii* is detectable by using general Anaplasmataceae PCR assays and may be distinguished either by sequencing or use of a specific assay. Ideally, any species-specific assay should be tested against a panel including other ehrlichial species found in the United States to verify performance.

### **Important considerations for *Francisella tularensis* testing**

*Francisella tularensis* exists in two subspecies, *F. t. tularensis* (type A) and *F. t. holarctica* (type B). The organism may be transmitted to humans through contact with infected animal fluids, ingestion of contaminated food or water, inhalation of aerosols, or through the bite of an ixodid tick (Petersen et al. 2009). In the United States, most tick-transmitted cases occur in the Missouri-Arkansas region and involve *Amblyomma americanum* and *Dermacentor variabilis* ticks (Eisen 2007). A focus of local transmission was identified on Martha's Vineyard, Massachusetts (Goethert et al. 2004). Ticks and animal tissues may be tested by using PCR assays. Many ticks, particularly *Dermacentor* species have been found to harbor endosymbionts that can amplify using certain PCR assays (Kugeler et al. 2005). Assay performance regarding specificity should be carefully considered to avoid misinterpretation.

- *Francisella tularensis* is listed as a tier 1 select agent and all live organism work must be conducted under the rules and regulations of select agents.
- To demonstrate that an assay is *F. tularensis*-specific, it should be tested using specific *F. tularensis* DNA and *Francisella*-like endosymbiont DNA controls.

### **Important considerations for tickborne virus testing**

**Heartland virus** (HRTV) is a single-stranded RNA virus in the genus *Phlebovirus*, Family Phenuiviridae, for which *A. americanum* serves as the vector (Savage et al. 2013; Savage et al. 2016). The average infection rate of pooled nymphs has been estimated at 1 per 559 ticks, while the estimated infection rate of adults is estimated at 1/885. Raccoons and white-tailed deer may play important roles in the maintenance of this virus in nature (Riemersma & Komar 2015). Ticks and animals may be tested using RT-PCR assays described by McMullan et al. (2012) or Savage et al. (2013).

**Bourbon virus** (BRBV) is a single-stranded RNA virus in the genus *Thogotovirus*, Family Orthomyxoviridae, for which *A. americanum* may serve as the vector (Savage et al. 2017). The estimated infection rates of nymphs and adults are 1/3125 and as high as 1/3226, respectively. Raccoons and white-tailed deer may



serve as amplifier hosts in nature (Jackson et al. 2019). Ticks and animals may be tested using a RT-PCR assay described by Savage et al. (2017).

**Colorado tick fever virus (CTFV)** is a double-stranded RNA virus in the genus *Coltivirus*, Family Reoviridae. The tick vector in the United States is *D. andersoni*. The virus persists in nature through a tick-small mammal transmission cycle and humans serve as a dead-end host. CTFV variants found in black-tailed jackrabbits have been found to infect humans. Detection of the virus may be accomplished by using RT-PCR assays, such as that described by Lambert et al. (2007).

Metastriate tickborne virus detection considerations include:

- 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 Heartland or Bourbon 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 bisect the tick for separate extractions. It is also possible to 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 these viruses must incorporate a reverse transcription step.
- Because of the low infection rates demonstrated thus far in nature, ticks may be pooled for detection and cost-efficiency of testing.

## CDC Assistance in Testing for Pathogens

In support of metastriate tick surveillance efforts, CDC has limited resources available to support pathogen detection in ticks submitted from public health partners. Recommended assay protocols, positive DNA controls, and technical consultation are available. Training for laboratorians in tick testing will be planned on an ad hoc basis. Limited testing may be available in the CDC laboratories, but may be limited to sites associated with human cases, or in areas of vector or pathogen emergence. Contact the Tick Surveillance Email ([ticksurveillance@cdc.gov](mailto:ticksurveillance@cdc.gov)) to discuss assistance in pathogen testing. Samples will not be accepted for testing from the general public.

### **In counties where the pathogen of interest has never been identified**

In counties where *E. chaffeensis*, *E. ewingii*, *R. rickettsii*, *R. parkeri*, or *Rickettsia* 364D have not been previously identified in ticks or their hosts (consult ArboNET database for current status), CDC may be able to assist in the testing of samples submitted by collaborating public health partners for presence of pathogens. *Francisella tularensis* and tickborne virus testing may not be available but interested agencies may contact the Tick Surveillance Centralized Email ([ticksurveillance@cdc.gov](mailto:ticksurveillance@cdc.gov)). The samples of interest for rickettsial testing include:

- 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 adults (collected from vegetation, walking samples or tick traps); pathogen prevalence will be estimated if sample size is  $\geq 25$  individuals 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 assay not being optimized for that purpose.

### **In counties where the pathogen of interest has been identified**

In counties where *E. chaffeensis*, *E. ewingii*, *R. rickettsii*, *R. parkeri*, or *Rickettsia* 364D have been identified previously in ticks or their hosts (please consult ArboNET database for current status), CDC may be able to assist in testing 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 sufficient individuals are submitted per site per county.
- Host-seeking adults (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 DIA, we may 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.

# Further Reading

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# Additional Resources

## **Tick Surveillance and ArboNET Websites:**

[www.cdc.gov/ticks/surveillance/](http://www.cdc.gov/ticks/surveillance/)

[wwwn.cdc.gov/arboNET/](http://wwwn.cdc.gov/arboNET/)

## **United States National Tick Collection (USNTC):**

### **General website**

<https://cosm.georgiasouthern.edu/usntc/>

### **Center of Excellence Tick Workshop 2020**

<https://cosm.georgiasouthern.edu/usntc/center-of-excellence-tick-workshop-2020/>

# Supplemental Material

## How to make a tick drag

### 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:

- 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*

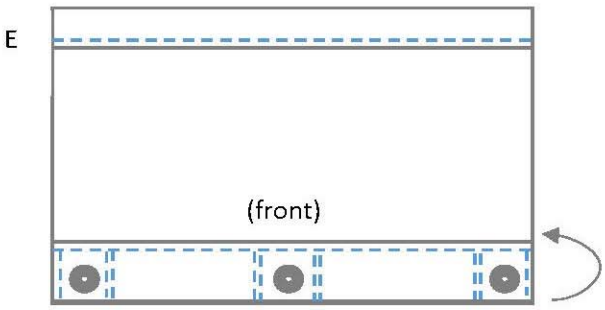
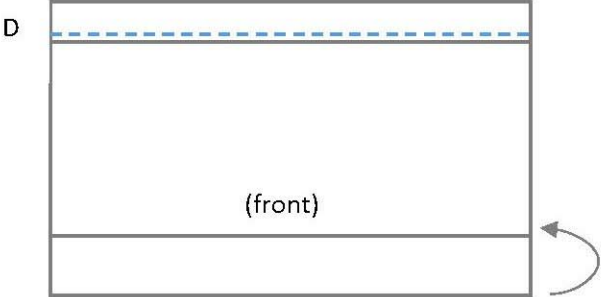
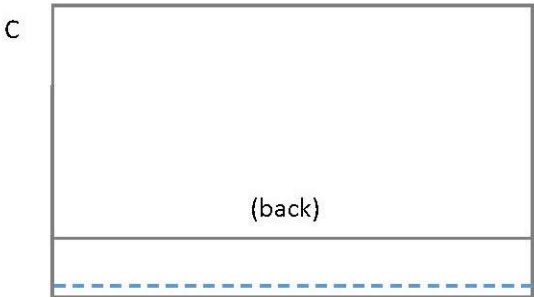
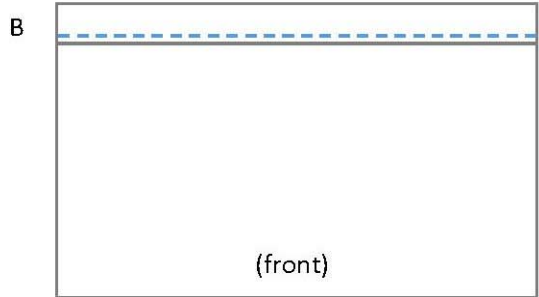
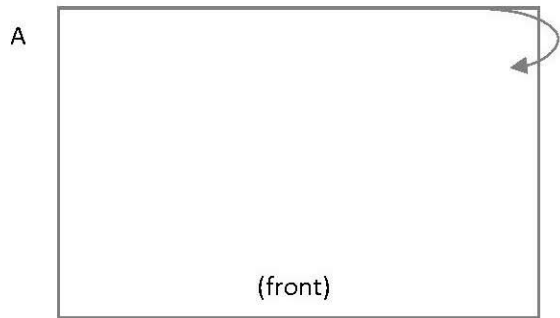
- a. Flip the panel over so that the seam from Step 2 is facing down. The panel should still be situated so that the loop is across the top of the panel.

- 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)

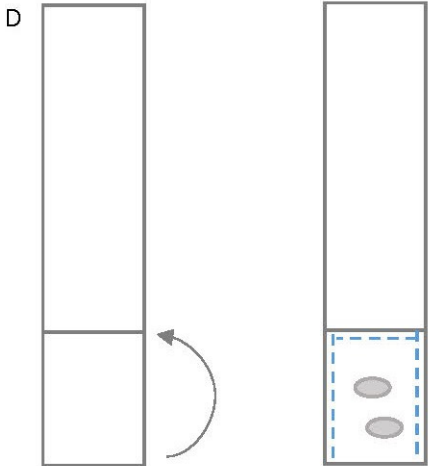
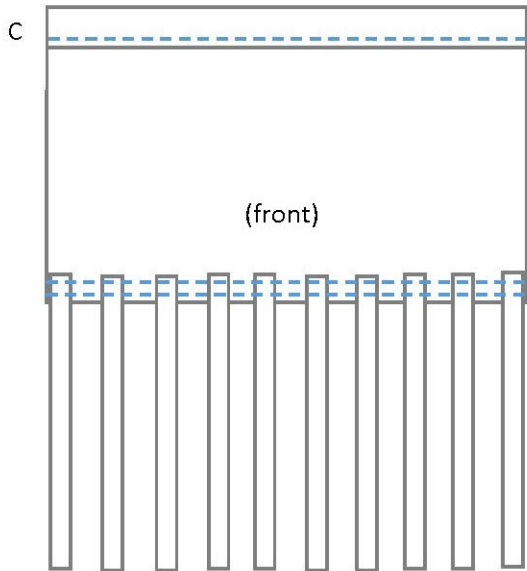
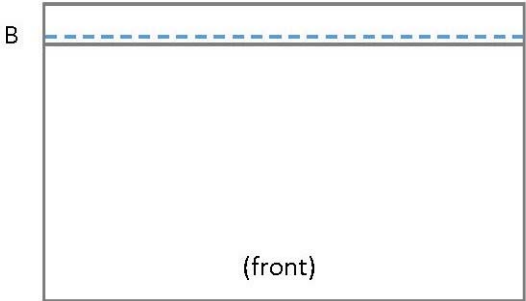
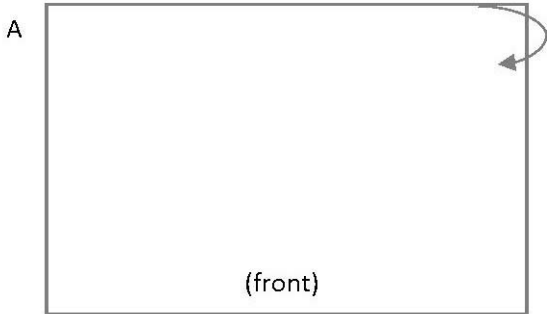
#### Step 4: *Completing the drag*

- 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



# How to make a tick flag

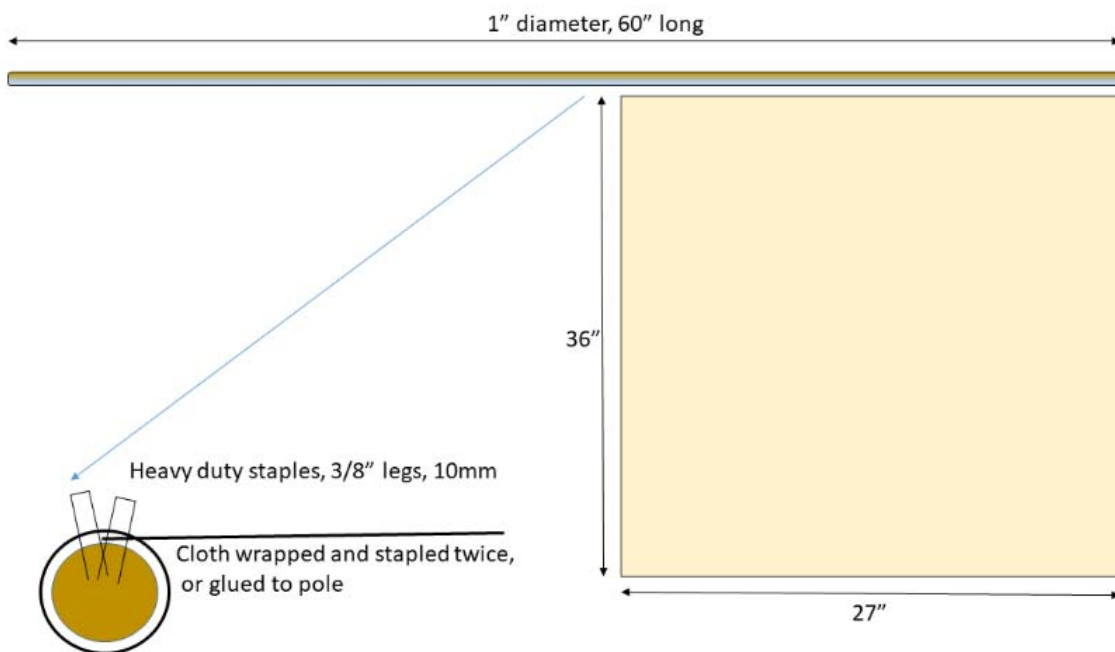
## Materials:

- 1" diameter by 60" long wooden broomstick
- Heavy duty stapler
- Heavy duty 3/8" staples
- Crib Cloth or flannel (approx. 27in x 36in)
- Hammer

## Instructions:

1. Cut your cloth material to the appropriate size (sewing or hemming will not be required if the laminated fabric is used)
2. Staple the shorter edge of the cloth to broom stick at one end along the whole edge of the cloth
3. Hammer staples down if needed so they are flat on the broomstick
4. Wrap cloth around broomstick until the cloth overlaps, staple again beside the previous layer's staples
5. Hammer staples down if needed so they are flat on the broomstick

## Assembly diagram:



# How to construct a tick drag-flag

Reference: Newman, B. C., W. B. Sutton, Y. Wang, C. J. Schweitzer, A. C. Moncayo, and B. T. Miller. 2019. A standardized method for the construction of a tick drag/flag sampling approach and evaluation of sampling efficacy. *Exp. Appl. Acarol.* 79 (3-4): 433-446.

Supplies (estimated prices as of 2019):

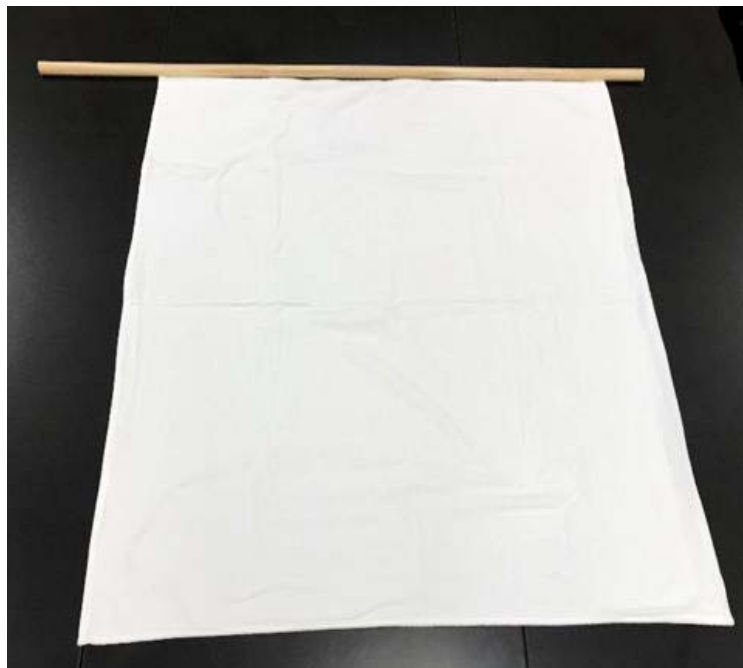
1. Cotton white flannel fabric (2 in quantity, \$3.59/ yard), or cotton bleached white muslin fabric (2 in quantity)
2. diamond braided rope bright color (4.7 mm x 22.8 m)
3. wood round dowel (pine, 2.2225 cm x 1.2192 m)
4. white industrial strength Velcro (4.57 m x 5.08 cm)
5. all-purpose stainless-steel blade scissors (20.3 cm)

\* Prior to construction of the tick drag-flag, we cut sampling fabric into four 1 x 1 m square panels (two cotton flannel and two cotton muslin).

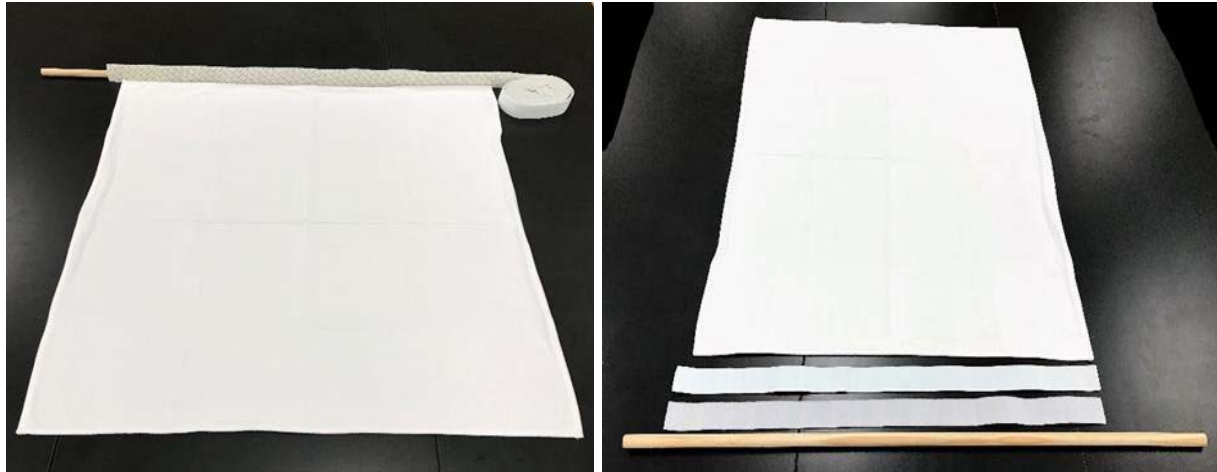
\* Of note, sewing was completed by a professional seamstress (\$9.00 per panel) as it was more cost-effective than purchasing necessary equipment (e.g., sewing machine) to ensure fabric panels were sewn in such a manner as to remain durable throughout the project. A double fold hem technique was used to sew fabric along all four edges.

Instructions for assembly:

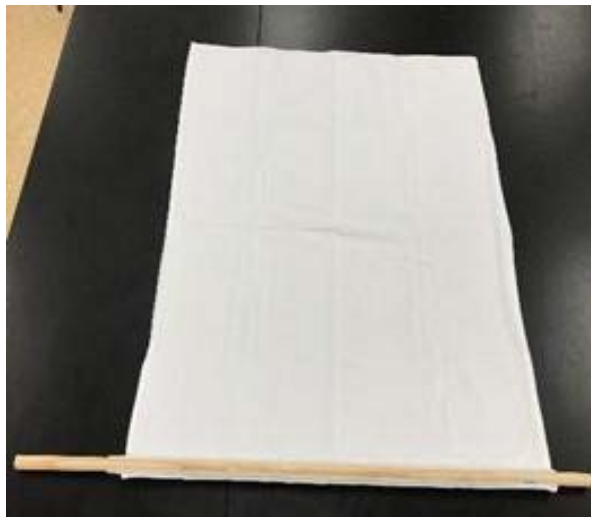
1. Gather all supplies.
2. Align fabric panel with a wood dowel such that 4 cm and 18 cm of space are left between ends of dowel and fabric. To ensure precision, we encourage marking the wood dowel at 4 cm and 18 cm from each end.



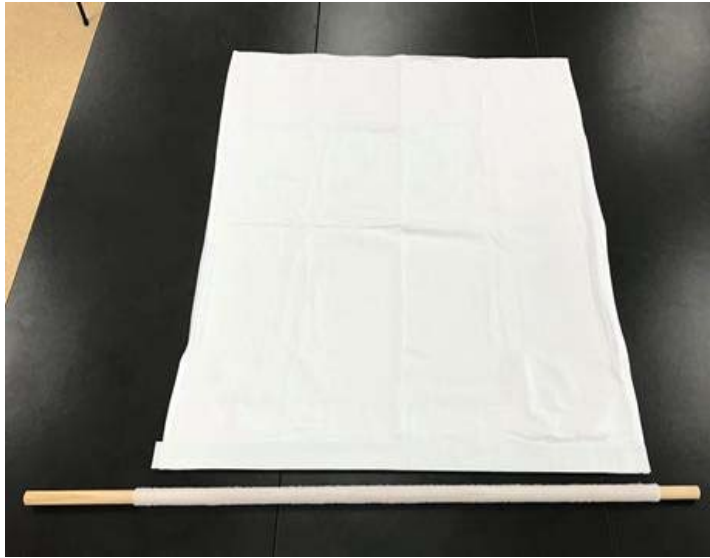
3. Align Velcro with fabric and markings on dowel ~2cm past 18 cm marking to ensure enough space is left for the adhesive side of Velcro to make complete contact with the entire edge of fabric and cut Velcro to length.



4. Remove plastic film lining the loop side of the Velcro and carefully attach to the dowel making sure no wrinkles or pockets form. As stated in the previous step, an extra 2 cm is necessary because fabric must be taut when attaching to the adhesive of the hook side of the Velcro.
5. Combine dowel and fabric panel ensuring that both hook and loop sides of Velcro adhere.



This completes the necessary steps to assemble a 1 x 1 m **tick flag**. The larger space left on the dowel (~18 cm) will serve as the handle in which to perform tick sampling via flagging technique.



6. Cut diamond braided rope bright color to ~2 m and tie each loose end of rope to wood dowel.

This completes the necessary steps to assemble a 1 x 1 m **tick drag**.

