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BEPREP STANDARD OPERATING PROCEDURE

Short title, ID:	BEPREP_SOP-12	**Category:**	Field SOP
Title:	Tick sampling in European Case Study Areas		
Version:	1.0	**Created on:**	2023-03-10
Status:	In use	**Pages:**	6
Related SOPs:			
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1. Abbreviations

SOP	- standard operating procedure
CSA	- case study area
PPE	- Personal Protective Equipment
BACI	- Before, After, Control, Impact

2. Aim and scope

The purpose of this SOP is to collect hard tick specimens to evaluate their presence/absence, species diversity, abundance and to identify pathogens (protozoa, bacteria, viruses) of medical and veterinary importance.

This SOP is targeted for the collection of ticks in European CSAs and of ticks belonging to the *Ixodes ricinus complex*, in particular, *I.ricinus* and *I.persulcatus*. These are the most common vectors in Europe of pathogens of medical and veterinary relevance such as: *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia* spp., *Rickettsia* spp. and Tick-borne encephalitis virus. Ticks collected in the non-European Case Study Areas require a different sampling strategy and will be described in a different SOP.

3. Safety measures and potential hazards

The first and most efficacious safety measure is to carefully check (self-check or ask assistance to a colleague) body and clothes during and after the field work.

While collecting ticks, wear long trousers with the legs tucked into the socks and, if possible, a long sleeve t-shirt. Light-coloured clothes help spotting the ticks. Use of acaricides or permethrin-impregnated clothes are recommended but **self-checking for ticks after the field work is essential**.

Wearing gloves is recommended to avoid contaminating tick samples with human DNA and protect against pathogens present, however follow your local (personal protection) protocols. Use tweezers to transfer ticks into tubes, never hands.

Be aware that ticks may have attached to your clothes, so when you are back from the field change clothes and perform a thorough tick check. If possible, leave clothes and boots outside under the sun or put them in the freezer (-20 °C or -80 °C) for at least one hour.

Washing the clothes using a washing machine is recommended at high temperature (90 °C), since at lower temperatures ticks remain viable. Depending on the local situation, vaccination again TBE (FSME vaccination) is recommended.

In case of physical complaints, notify your doctor of (potential) tick bites!

4. Materials and equipment

Equipment needed: blankets, tweezers (advice: add a string to attach the tweezers to your trousers because you can easily lose them), gloves, tubes, plastic bag, marker pen, metric chord, GPS, steel tape measure, tent peg.

Item	Purpose/Note/Link
1x1 m blankets/flannel	For collecting the ticks
Tweezers	Attach a string to the tweezers to enable finding them if dropped, or attach to trousers
Disposable gloves	PPE
0.2ml PCR tubes	for storage on individual ticks
Plastic Ziplock bag	For storing all ticks from the same transect
Marker	Labelling
Metric cord	
GPS device	
Steel tape measurer, 100m	
Tent peg	to secure the tape measurer
Masking tape	to remove tick larvae

5. Field procedures

5.1. Questing tick sampling

Questing ticks, i.e., ticks from the environment with ambushing behaviour as *Ixodes ricinus*, are usually sampled by so called dragging. Dragging consists of pulling a white flannel blanket with a surface area of 1 m^2 along a predefined route (transect).

One side of the blanket ends with a 1 metre pole that helps keeping the fabric open and tight above the soil or vegetation. A piece of rope is attached to the side of the stick to allow the operator to pull the blanket behind while walking. To increase contact with the vegetation, a metal chain can be added to the other end of the cloth to add weight.

When dragging it is essential to makes sure that the pulling string is long enough so that the whole 1m^2 of the blanket touches the ground. Standard tick dragging transect line is 100 m. Ideally, transects are set out using a steel tape measure. The tape can be secured using a conventional tent peg to prevent any unintentional movement of the transect. **If possible, perform cloth-dragging on the side of the transect tape that you did not walk on when setting out the tape to minimize disturbance.**

According to the type of environment, stop and check the blanket for ticks every 5-10 m. The blanket must be checked on both sides at every stop.

Adults and nymphs are placed in individual 0.2 ml Eppendorf PCR-tubes at every check, while larvae will be removed at the end of the transect, but not collected or counted.

When tick dragging:

- favourable habitats are forests, ecotones, borders of tracks, animal paths
- avoid places where vegetation is too high and shrubby, and open meadows or exposure to direct sun
- avoid tick dragging if it's raining, if vegetation is wet or if it's too windy. If possible, use a new dragging sheet (without larvae) when starting the dragging in a new area.

Record the geographical coordinates of the centre of the transect or the starting and ending points.

According to tick phenology, the sampling by dragging should be performed during the known peak(s) for that region and repeated at least twice, ideally three times (months) every year (for example before, during and after the peak of abundance).

The minimum number of 100 m-transects should be three per CSA and in respective BACI framework. The minimum number of ticks to be analysed per CSA should **ideally** be of 500 nymphs or adults per study site and year. If not enough ticks are collected in the standard transects, collect extra ticks in nearby areas (keeping within CSA and type (BACI)) until the minimum threshold is reached.

5.2. Feeding Tick sampling

Tick samples can also be collected from hosts and can be either engorged or not. Specimens from the same animal can be stored in the same tube, either empty if cold chain is maintained or in DNA/RNA Shield.

Feeding ticks must be removed with sterilised tweezers that should be cleaned with sodium hypochlorite followed by ethanol after each animal.

6. Sample storage and sample labelling

6.1. In the field

Ticks (nymphs and adults) collected are stored individually in 0.2 ml Eppendorf PCR-tubes (e.g., Safe lock., order nr 951010022). **All strips of 1 transect go into 1 bag.**

The bag is labelled with: BP-CSA-T-running number; for example, BP-02-T-001

Additional information to be collected for each transect is:

- collection date (format: YYYY-MM-DD)
- location (see rodent SOP for reference)
- trapping site (see rodent SOP for reference)
- transect ID

- transect coordinates (see above)
- tick collector
- number of collected ticks split by life stage and for adults by sex
- if the transects were not enough to collect 500 ticks, also note down how many came from transect dragging and how many from additional dragging

During transportation from the field to the laboratory, keep the tubes at cool temperatures with ice packs or using a portable refrigerator (about 4°C).

Please see document xx for details on sample labelling and tick SOP folder for example of field data forms and long-term data collection spreadsheets.

6.2. In the laboratory

Once back from the field, the samples are stored immediately (preferably the same day, at least within 24 hours) at -80°C. **Double check the labels and add an extra label on the "inside" of every bag (to avoid labels detaching in -80° C)**.

7. Reference laboratories and analyses

RIVM will perform screening of ticks sampled in EU for *Borrelia* spp. and other (bacterial) pathogens (e.g. *Anaplasma* spp.).

FLI will perform:

- screening of ticks samples in the EU for TBEV, if this is done (to be decided after testing of rodents for TBEV)
- screening of ticks collected from non-EU partners for all pathogens deemed relevant.