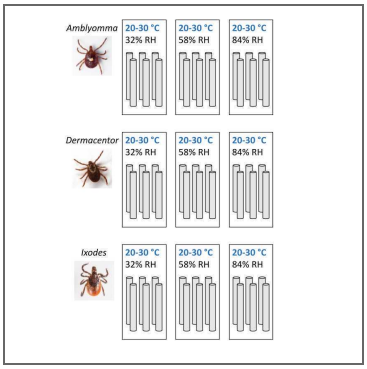

VERSION 5

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A novel laboratory method to simulate climatic stress with successful application to experiments with medically relevant ticks V.5

PLOS One ✓ Peer-reviewed method

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PLOS ONE Lab Protocols | Project Parasite 1.0

Caleb Nielebeck

ABSTRACT

This protocol details a novel method to isolate individual ticks and manipulate their environment. We successfully used this method to investigate how humidity affects survival and host-seeking (questing) behavior of three species of ticks: the lone star tick (Amblyomma americanum), American dog tick (Dermacentor variabilis), and black-legged tick (Ixodes scapularis). We placed 72 adult females of each species into individual plastic tubes and separated them into three experimental relative humidity (RH) treatments representing distinct climates: 32% RH, 58% RH, and 84% RH. For 30 days we assessed the survival and questing behavior of each tick.

The last step in this version contains a supplemental video with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

MANUSCRIPT CITATION: Nielebeck C, Kim SH, Dedmon L, Pangilinan M, Quan J, et al. (2022)A novel laboratory method to simulate climatic stress with successful application to experiments with medically relevant ticks. PLOS ONE 17(9): e0275314. https://doi.org/10.1371/journal.pone.0275314

Protocol status: Working
We use this protocol and it's working

Created: Oct 13, 2023

Last Modified: Oct 16, 2023

PROTOCOL integer ID: 89273

Keywords: Tick questing, tick behavior experiment, Amblyomma, Dermacentor, Ixodes

GUIDELINES

Questing qualifications:

- The individual is not walking but it is still with its front legs extended

Example of an *Amblyomma* tick questing

Example of a *Dermacentor* tick questing

Death qualifications:

- If any tick appears dead, lightly blow on it since ticks respond to carbon dioxide exhaled by potential hosts
- If the tick does not move at all in 2 minutes, it should be counted as dead and placed in 70% ethanol

MATERIALS

Ticks:

- 72 adult female *Amblyomma americanum*

- 72 adult female *Dermacentor variabilis*
- 72 adult female *Ixodes scapularis*

Experimental set up:

- 1 - Climate chamber (e.g. Percival I-41VL)

Equipment	
Incubator	NAME
Climate Chamber	TYPE
Percival	BRAND
I-41VL	SKU
https://www.percival-scientific.com/product/i-41vl/	LINK

- 216 - 20 cm x 2.5 cm Clear PETG plastic tubes
- 216 - 20 cm Wooden skewers
- 36 - 2 L Airtight containers
- 12 - 32 % Boveda Two-Way Humidity Control Packs
- 12 - 58 % Boveda Two-Way Humidity Control Packs
- 12 - 84 % Boveda Two-Way Humidity Control Packs
- 1 - Temperature/relative humidity data logger (e.g. ONSET UX100-003)

Equipment	
HOBO Temperature/Relative Humidity 3.5% Data Logger	NAME
Data logger	TYPE
ONSET	BRAND
UX100-003	SKU
https://www.onsetcomp.com/products/data-loggers/ux100-003/	LINK

- 70% Ethanol
- Colored dot stickers

- Sharpie

Other tools:
- Entomology forceps
- 30 cm ruler
- White surface (e.g. lab bench diaper)

SAFETY WARNINGS

Always handle ticks with blunt entomology forceps, as regular forceps can injure them.
Always handle ticks over a white surface so that they can easily be spotted in case they are dropped.

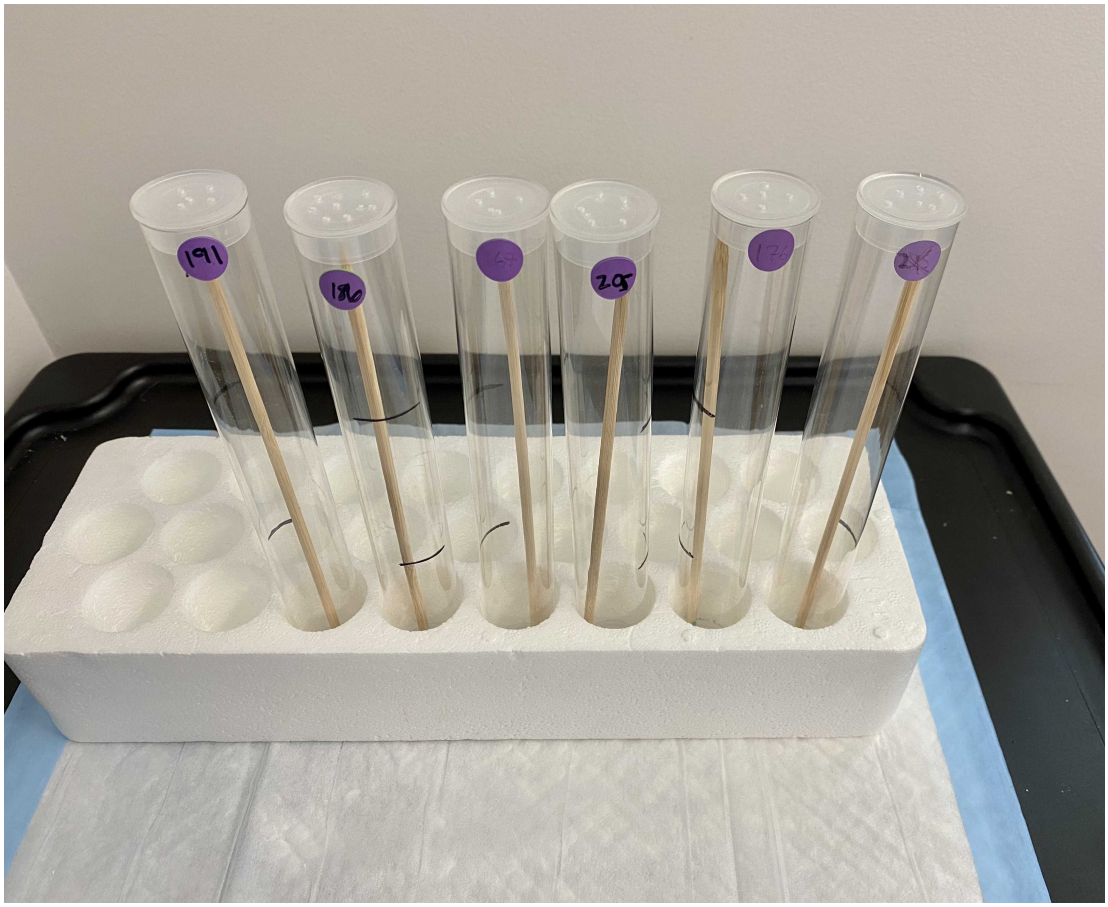
BEFORE START INSTRUCTIONS

We acquired adult female ticks from the Oklahoma State University Tick Rearing Facility and experimented with one temperature range and three relative humidities. This protocol can be modified for different species, life stages, temperatures, humidities, and other small organisms.

Set up

2h

1 Place a single tick with one wooden skewer in each tube and seal with a cap, labelling each tube with an individual identifier



PETG plastic tubes with wooden skewers prepared for ticks. Notice that we drilled holes in the caps to allow airflow between the tubes and the containers.

2 Place six tubes in each airtight container along with a humidity pack, labelling each container

Airtight container with six tubes, humidity pack, and data logger. Example container of *Dermacentor variabilis* (purple) at 58% RH; every container was replicated four times for a sample size of n=24 per RH group.

2.1 Confirm the humidity in one container of each RH level with the data logger

3 Program the climate chamber

> **Note**
>
> Reference your chamber's user's manual

3.1 To cycle between 20 °C to 30 °C, the temperature increments should be as follows:

- 3:00 - 25 °C

- 6:00 - 27.5 °C
- 9:00 - 30 °C
- 12:00 - 27.5 °C
- 15:00 - 25 °C
- 18:00 - 22.5 °C
- 21:00 - 20 °C
- 24:00 - 22.5 °C

3.2 To create a 12:12 light:dark photoperiod, lighting increments should be as follows:
- 9:00 - Lights on
- 21:00 - Lights off

Data collection

5w 5d

4 Place all of the airtight containers, filled with ticks and humidity packs, into the climate chamber and start the program

5 Each day thereafter, during the 9:00 to 12:00 or 30 °C increment, assess each tick for survivorship and questing behavior (see guidelines for qualifications of survivorship and questing)

> **Note**
>
> Only take one container out of the chamber at a time

> **Note**
>
> Collect a binary outcome for survivorship and questing, and measure the tick's height (to the nearest 0.5 cm) in the tube if it is found questing

5.1 Periodically move the data logger to a new bin to confirm that no unexpected changes to the climate inside the containers has occurred

6 Repeat steps 4 and 5 for 30 days or until all ticks have died

Spotlight video

7

h