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Title: Tick Blood Feeding Through an Artificial Membrane on a 3D-printed Chamber in Ixodid tick research

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes , stereo microscope only**

No

Our protocol does not involve light microscopy, stereo microscope only.

Our protocol requires visuals under a stereo microscope for only a few steps. Our stereo microscopes are below

Nikon SMZ-745 &

Amscope SF-2TRA

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

About 100 yards away, the lab and the interview office are in different rooms. But the protocol will be all in the lab (same room), and only the interview portion is in a different room.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 29

Number of Shots: 53

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Videographer's NOTE: Shots 1.1.1 - 1.5.1 were filmed after the entire demo, so those will appear on the end of the roll. The interview location was extremely limited, so while the visual is nice and the audio is clean from the MKE 600. But there could be a benefit of using "deEcho" on the audio due to the space that was available to us.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Paulina Maldonado-Riuz:** My research focuses on reducing the incidence of tick-borne diseases by understanding the molecular and microbial factors that influence tick vector competence.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Paulina Maldonado-Ruiz:** Existing artificial tick feeding systems lack flexibility, require large blood volumes, and complex setups; this protocol introduces a modular, reusable system enabling small-scale, customizable experiments.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.13.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Paulina Maldonado-Ruiz:** This protocol enables researchers to study tick feeding behavior, physiology, and molecular responses under controlled in vitro conditions.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1*

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Paulina Maldonado-Ruiz:** A key challenge is variable feeding success, requiring precise optimization of environmental conditions, membrane properties, and careful handling to maintain integrity and prevent contamination.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 4.3.1, 4.3.2*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Paulina Maldonado-Ruiz:** Future studies will optimize feeding across species, enable full life cycle maintenance, and integrate molecular tools to study pathogen transmission and develop targeted control strategies.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Tick Feeding Chambers

Demonstrator: Omayma Jaddou Najma

- 2.1. To begin, using gloves, wash the parts of the feeding chambers to ensure there are no resin residues before assembly [1]. If needed, gently sand the parts with fine 180 to 200 grit sandpaper and ensure that the bottom part is flat and smooth [2-TXT].
 - 2.1.1. WIDE: Talent washing the chamber parts while wearing gloves.
 - 2.1.2. Talent gently sanding the chamber parts with fine 180 to 200 grit sandpaper.
- 2.2. Expose all dry pieces to ultraviolet light for 10 minutes to ensure complete resin polymerization [1].
 - 2.2.1. Talent placing all dry pieces under ultraviolet light.
- 2.3. Then, glue the 3 millimeter by 2 millimeter round magnets into the slits on the top of the chambers and lid rings with clear epoxy glue [1] and allow it to dry overnight [2]. Ensure that the poles of the magnets face one direction to match the attraction of the poles on the magnets in the upper part of the lid ring [3].
 - 2.3.1. Talent gluing the round magnets into the slits on the top of the chambers and lid rings with clear epoxy glue.
 - 2.3.2. Talent places the assembly aside for drying.
 - 2.3.3. Talent aligning the magnet poles in one direction on the chamber and lid ring parts.
- 2.4. Assemble all pieces of the model to ensure that all components fit properly [1].
 - 2.4.1. Talent assembles all chamber components to check the fit.

3. Membrane Preparation for Feeding Chambers

- 3.1. Place a 30 centimeter by 30 centimeter piece of plastic wrap on a clean surface [1]. Stretch the plastic wrap on a flat surface to remove wrinkles and secure the edges with tape [2].
 - 3.1.1. Talent placing a 30 centimeter by 30 centimeter piece of plastic wrap on a clean surface.
 - 3.1.2. Talent stretching the plastic wrap and securing the edges of the plastic wrap with tape.

- 3.2. Place 4–5 individual sheets of lens cleaning paper over the extended plastic wrap, spacing each sheet approximately 5 centimeters apart [1].
 - 3.2.1. Talent placing single sheets of lens cleaning paper onto the plastic wrap.
- 3.3. To prepare the silicon membrane, add 15 grams of silicone clear caulk, 4.5 grams of silicone oil, and 2.9 grams of hexane into a plastic weighing boat [1]. Using a spatula, gently mix the ingredients until combined [2].
 - 3.3.1. Talent adding silicone clear caulk into a plastic weighing boat. Authors: Please keep labelled bottle of silicon oil and hexane in the frame.
 - 3.3.2. Talent gently mixing the mixture with a spatula.
- 3.4. Then, place 2 to 3 grams of silicone mixture along the smaller edge of the lens cleaning paper to secure it to the plastic wrap [1]. Allow the silicone to seep through the lens paper for 5 to 10 seconds [2].
 - 3.4.1. Talent applying a small amount of silicone mixture along the edge of the lens cleaning paper on the plastic wrap.
 - 3.4.2. Shot of the silicone seeping through the lens paper.
- 3.5. Next, using a straight-edge instrument such as a lid, ruler, or spatula, evenly spread the silicone mixture across the lens cleaning paper [1]. Ensure the tool covers the entire area of the lens cleaning paper in a single stroke to prevent membrane ruffling and edge ripples [2].
 - 3.5.1. Talent spreading the silicone mixture across the lens cleaning paper using a straight-edge tool.
 - 3.5.2. Shot of the silicone forming a smooth, even layer across the entire lens cleaning paper.
- 3.6. Return the spreading tool to the starting position to collect any remaining silicone mixture on the tool [1]. Spread the collected silicone across the membrane to ensure all the mixture is used and to avoid gaps [2]. Allow the membranes to air-dry overnight at room temperature [3].
 - 3.6.1. Talent moving the spreading tool back to the starting position to collect residual silicone.
 - 3.6.2. Talent redistributing the collected silicone evenly across the membrane surface.
 - 3.6.3. Talent placing the prepared membranes aside on a flat surface to dry.

- 3.7. Now, measure the membrane thickness using a digital outside micrometer measuring tool [1]. Select appropriate membrane thicknesses for different adult tick species, ranging from short mouthparts to longer mouthparts [2].
 - 3.7.1. Talent measuring the thickness of a dried membrane using a digital outside micrometer.
 - 3.7.2. Shot of different membranes labeled or grouped by thickness.
- 3.8. Then, remove all parts from the previously assembled chambers [1]. To apply silicone glue to the bottom rim of the chambers, fill a 1-milliliter syringe with silicone glue [2].
 - 3.8.1. Talent disassembling the previously assembled chambers.
 - 3.8.2. Talent filling a 1 milliliter syringe with silicone glue.
- 3.9. Place a 200-microliter pipette tip onto the tip of the syringe [1]. Screw the pipette tip securely onto the syringe end as with a needle tip [2]. Use this setup to enable fine application of glue to the bottom rim of each chamber [3].
 - 3.9.1. Talent attaching a 200 microliter pipette tip to the syringe. NOTE: 3.9.1 and 3.9.2 are filmed in a single shot
 - 3.9.2. Talent securing the pipette tip onto the syringe end.
 - 3.9.3. Talent applying glue precisely along the bottom rim of a chamber using the modified syringe.
- 3.10. Now, place the chamber over the membrane and position a weighted object on top to ensure proper settling [1-TXT]. Allow the assembly to sit overnight at room temperature [2]
 - 3.10.1. Talent placing the chamber onto the membrane and positioning a weighted object on top.
 - 3.10.2. Talent places the assembly aside.
- 3.11. Using curved scissors, cut away the excess membrane from around the chambers [1].
 - 3.11.1. Talent trimming excess membrane around the chamber using curved scissors.
- 3.12. Remove any remaining plastic wrap from the membrane before using the chamber for tick feeding [1].

3.12.1. Talent peeling away remaining plastic wrap from the membrane.

3.13. Assemble the chamber parts and include a 35 millimeter by 10 millimeter petri dish at the bottom [1]. Invert the petri dish lids and place them between the magnets to serve as chamber lids [2].

3.13.1. Talent assembling the chamber components with a petri dish at the bottom.

3.13.2. Talent inverting the petri dish lid and placing it between the magnets as a chamber lid.

3.14. Place the chamber membrane into the bottom portion of a 35 millimeter by 10 millimeter petri dish filled with distilled water and allow it to soak overnight [1].

3.14.1. Talent placing the chamber membrane into a petri dish containing distilled water.

4. Tick Feeding and Blood Preparation

Demonstrator: Kara Golden

4.1. Use a heated metal scalpel or a thin metal spatula to make 2 to 3 small breathing holes in the lids of each chamber [1].

4.1.1. WIDE: Talent creating small breathing holes in a petri dish lid using a heated metal scalpel.

4.2. Supplement the blood with gentamicin sulfate to a final concentration of 50 micrograms per milliliter [1]. Store the supplemented blood at 4 degrees Celsius and use it within one week [2-TXT].

4.2.1. Talent adding gentamicin sulfate to a blood sample.

4.2.2. Talent placing the supplemented blood into cold storage.

4.3. Place 2 to 3 milliliters of blood into a clean 35 millimeter by 10 millimeter petri dish [1]. Position the chamber on top of the petri dish [2] and adjust the chamber height by rotating the adjustment ring to increase or decrease the volume of blood used per chamber [3]. Adjust the chamber to maintain a 1 to 2 millimeter gap between the membrane and the bottom of the petri dish [4].

4.3.1. Talent pipetting blood into a petri dish.

4.3.2. Talent placing the chamber onto the petri dish.

4.3.3. Talent rotating the adjustment ring on the chamber.

4.3.4. Talent adjusting the chamber height to maintain a small gap above the petri dish.

4.4. Now, place unfed ticks into the chambers with equal numbers of males and females. Ensure both sexes are included to support feeding [1].

4.4.1. Talent placing ticks into the chamber.

4.5. Then, place the chambers in covered dry baths with a humidity source such as water cups under a 12-hour light and 12-hour dark photoperiod [1-TXT].

4.5.1. WIDE: Talent placing chambers into a covered dry bath with water cups for humidity. **TXT: Temperature: 37 °C ± 1 °C; Relative humidity: 63 % ± 2 %**

5. Blood Replacement and Chamber Maintenance

Demonstrator: Sydney Upton

5.1. Prepare two squirt bottles for rinsing solutions, one with 0.005 percent nystatin solution in distilled water and another with only distilled water [1].

5.1.1. Talent filling one squirt bottle with 0.005 percent nystatin solution while keeping the other bottle in the frame.

5.2. After 12 to 16 hours, prepare new petri dishes with fresh blood [1]. Place the dishes in the dry bath at 37 degrees Celsius before positioning the chamber [2].

5.2.1. Talent adding fresh blood into clean petri dishes.

5.2.2. Talent placing the petri dishes into the dry bath.

5.3. In the meantime, remove the chamber from the used blood [1]. Briefly rinse the membrane underside with distilled water for 3–5 seconds [2], followed by a 3–5 second rinse in 0.005% nystatin to prevent fungal contamination, and finish with a final distilled water rinse to remove any residual nystatin.[3].

5.3.1. Talent lifting the chamber from a petri dish containing used blood.

5.3.2. Talent rinsing the underside of the membrane with distilled water using a squirt bottle.

5.3.3. Talent rinsing the membrane with nystatin solution.

5.5. After rinsing, place the chamber into a new petri dish containing fresh blood [1]. **NOTE: This step is placed here as per videographer's note**

5.5.1. Talent placing the rinsed chamber onto a petri dish with fresh blood.

5.4. Discard all used blood and rinsing water into a container containing 0.5 percent sodium

hypochlorite solution according to institutional safety protocol [1].

5.4.1. Talent pouring used blood and rinsing water into a waste container with sodium hypochlorite solution. NOTE: 5.4.1 is filmed after 5.5.1

~~5.5. After rinsing, place the chamber into a new petri dish containing fresh blood [1].~~

~~5.5.1. Talent placing the rinsed chamber onto a petri dish with fresh blood. NOTE: This step is moved before 5.4~~

5.6. Place the chambers in 0.5 percent sodium hypochlorite solution for up to 30 minutes to reuse them [1]. Wash the chambers with dish soap after bleach treatment [2].

5.6.1. Talent placing chambers into a container with sodium hypochlorite solution.

5.6.2. Talent washing chambers with dish soap after bleach treatment.

Results

6. Results

- 6.4. Optimal feeding rates were achieved at membrane thicknesses of 100–200 micrometers for *Amblyomma americanum* [1] and 100–250 micrometers for *Amblyomma maculatum* [2].
 - 6.4.1. LAB MEDIA: Table 1. *Video editor: Highlight the membrane thickness value “100–200” in the row for A. americanum.*
 - 6.4.2. LAB MEDIA: Table 1. *Video editor: Highlight the membrane thickness value “100–250” in the row for A. maculatum.*
- 6.5. *Rhipicephalus sanguineus* showed the highest feeding rates at 70–100 micrometer membrane thickness [1]. The highest feeding rates for *Ixodes scapularis* [2] and *Dermacentor variabilis* were observed at a membrane thickness of less than 150 micrometers [3].
 - 6.5.1. LAB MEDIA: Table 1. *Video editor: Highlight the membrane thickness value “70–100” in the row for R. sanguineus.*
 - 6.5.2. LAB MEDIA: Table 1. *Video editor: Highlight the membrane thickness value “<150” in the row for I. scapularis.*
 - 6.5.3. LAB MEDIA: Table 1. *Video editor: Highlight the membrane thickness value “<150” in the row for D. variabilis.*
- 6.6. Partially fed female *Amblyomma americanum* were classified into unfed, 2–3 days partially fed, 4–5 days partially fed, and engorged stages [1].
 - 6.6.1. LAB MEDIA: Figure 5. *Video editor: Show the sequence of ticks labeled “Unfed,” “Partially fed 2–3 days,” “Partially fed 4–5 days,” and “Engorged >6 days”.*
- 6.7. The engorged feeding stage included ticks at the onset of rapid engorgement with weights ranging from 48.9 milligrams to 109 milligrams [1], and fully engorged ticks with weights ranging from 272.7 milligrams to 348.7 milligrams [2].
 - 6.7.1. LAB MEDIA: Figure 5. *Video editor: Highlight the largest tick under “Engorged >6 days”.*
 - 6.7.2. LAB MEDIA: Figure 5. *Video editor: Emphasize the size difference between the engorged tick and the smaller earlier-stage ticks.*

1. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /ˌʌl.trəˈvaɪ.ə.lət/
Phonetic Spelling: uhl·truh·VY·uh·luht
2. Polymerization
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerization>
IPA: /pəˌlɪm.ər.əˈzeɪ.ʃən/
Phonetic Spelling: puh·LIM·uh·ruh·ZAY·shuhn
3. Epoxy
Pronunciation link: <https://www.merriam-webster.com/dictionary/epoxy>
IPA: /ɪˈpɑːk.si/
Phonetic Spelling: ih·POK·see
4. Silicone
Pronunciation link: <https://www.merriam-webster.com/dictionary/silicone>
IPA: /ˈsɪl.əˌkoʊn/
Phonetic Spelling: SIL·uh·kohn
5. Hexane
Pronunciation link: <https://www.merriam-webster.com/dictionary/hexane>
IPA: /ˈhekˌseɪn/
Phonetic Spelling: HEK·sayn
6. Micrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
IPA: /maɪˈkraː.məˌtər/
Phonetic Spelling: my·KRAA·muh·tur
7. Amblyomma
Pronunciation link: <https://www.howtopronounce.com/mblyomma>
IPA: /æmˈbliːɑːmə/
Phonetic Spelling: am·BLEE·ah·muh
8. Rhipicephalus
Pronunciation link: <https://www.howtopronounce.com/rhipicephalus>
IPA: /ˌrɪpɪˈsefələs/
Phonetic Spelling: rip·ih·SEF·uh·luhs
9. Ixodes
Pronunciation link: <https://www.howtopronounce.com/ixodes>
IPA: /ɪkˈsoʊdiːz/
Phonetic Spelling: ik·SOH·deez
10. Dermacentor
Pronunciation link: <https://www.howtopronounce.com/dermacentor>
IPA: /dɜːˈmæsəntɔːr/
Phonetic Spelling: dur·MAS·uhn·tor
11. Gentamicin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gentamicin>

- IPA: /ˌdʒen.təˈmaɪ.sɪn/
Phonetic Spelling: jen·tuh·MY·suhn
12. Nystatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/nystatin>
IPA: /naɪˈstætɪn/
Phonetic Spelling: ny·STAT·in
13. Sodium hypochlorite
Pronunciation link: <https://www.merriam-webster.com/dictionary/hypochlorite>
IPA: /ˌsoʊ.di.əm ˌhaɪ.pooˈklɔːr.aɪt/
Phonetic Spelling: SOH·dee·uhm hy·poh·KLOR·yt
14. Luria-Bertani
Pronunciation link: <https://www.merriam-webster.com/dictionary/Luria-Bertani>
IPA: /ˈlʊr.i.ə bɜːrˈtɑːni/
Phonetic Spelling: LOOR·ee·uh ber·TAA·nee
15. Optical density
Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>
IPA: /ˈɑːp.tɪ.kəl denˈsɪti/
Phonetic Spelling: AAP·tih·kuhl den·SUH·tee
16. Neutralisation
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralization>
IPA: /ˌnuː.trə.ləˈzeɪ.ʃən/
Phonetic Spelling: noo·truh·luh·ZAY·shuhn
17. Fluorometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>
IPA: /flʊˈrɑː.mə.tər/
Phonetic Spelling: floor·RAA·muh·tur
18. Thermal cyclers
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermal>
IPA: /ˈθɜː.məl ˈsaɪ.klɜː/
Phonetic Spelling: THUR·muhl SY·klur
19. Barcoded
Pronunciation link: <https://www.merriam-webster.com/dictionary/barcode>
IPA: /ˈbɑːr.koʊd/
Phonetic Spelling: BAR·kohd
20. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /ˈeθ.ə.nəl/
Phonetic Spelling: ETH·uh·naal
21. Elution
Pronunciation link: <https://www.merriam-webster.com/dictionary/elution>
IPA: /iˈluː.ʃən/
Phonetic Spelling: ee·LOO·shuhn
22. Nanopore
Pronunciation link: <https://www.howtopronounce.com/nanopore>

- IPA: /'nænə.pɔːr/
Phonetic Spelling: NAN·uh·por
23. Priming
Pronunciation link: <https://www.merriam-webster.com/dictionary/priming>
IPA: /'praɪ.mɪŋ/
Phonetic Spelling: PRY·ming
24. Basecalling
Pronunciation link: No confirmed link found
IPA: /'beɪs.kɔː.lɪŋ/
Phonetic Spelling: bays·kaw·ling
25. Circularisation
Pronunciation link: <https://www.merriam-webster.com/dictionary/circularization>
IPA: /,sɜː.kjə.lə.ə'zeɪ.ʃən/
Phonetic Spelling: sur·kyuh·lur·uh·ZAY·shuhn
26. Chimeric
Pronunciation link: <https://www.merriam-webster.com/dictionary/chimeric>
IPA: /kaɪ'mer.ɪk/
Phonetic Spelling: ky·MER·ik
27. Consensus
Pronunciation link: <https://www.merriam-webster.com/dictionary/consensus>
IPA: /kən'sen.səs/
Phonetic Spelling: kuhn·SEN·suhs
28. Medaka
Pronunciation link: <https://www.howtopronounce.com/medaka>
IPA: /mɛ'dɑːkɑː/
Phonetic Spelling: meh·DAA·kah
29. Transconjugants
Pronunciation link: No confirmed link found
IPA: /,trænz'kɑːn.dʒə.gənts/
Phonetic Spelling: tranz·KON·juh·guhnts
30. Electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>
IPA: /ɪ.lek.troʊ.fə'riː.sɪs/
Phonetic Spelling: ih·LEK·troh·fuh·REE·suhs