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Title: Extraction of Saliva, Haemolymph, Salivary Glands, and Midgut from Individual Ticks (*Acari: Ixodidae*)

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

XCorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No.**

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 20.10.2025

When you are ready to submit your video LAB MEDIAs, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 11
Number of Shots: 20

Introduction

INTRODUCTION:

1.1. **Rua Khogali:** My research investigates tissue-specific microbiomes and pathogens in camel ticks to understand their role in pathogen transmission and vector competence.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.1*

What are the most recent developments in your field of research?

1.2. **Rua Khogali:** This protocol enabled the first molecular comparison of tick-borne pathogens and microbiomes across different fluids/tissues collected from an individual tick.

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

CONCLUSION:

What significant findings have you established in your field?

1.3. **Rua Khogali:** My research showed tissue-specific microbiomes and pathogens, identified haemolymph as a marker of vector competence, and revealed key microbial interactions.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What research gap are you addressing with your protocol?

1.4. **Rua Khogali:** This protocol enables screening and tracking pathogens in individual tick tissues, instead of relying on whole-tick homogenates.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

What advantage does your protocol offer compared to other techniques?

1.5. **Rua Khogali:** Collecting multiple tissues from a single tick, preserves tissue-level resolution, providing more accurate insights into vector competence and pathogen transmission mechanism.

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

Ethics Title Card

This research has been approved by Pwani University Ethics Review Committee and the Institutional Animal Care and Use Committee (IACUC) at *icipe*

Protocol

NOTE: SCRIPT DRAFTED FROM FOOTAGE

2. Saliva and Hemolymph Collection from Ticks Following Pilocarpine Stimulation

Demonstrator: Rua Khogali

2.1. To begin, collect specimens of *Hyalomma dromedarii*, *Hyalomma rufipes*, *Amblyomma gemma*, and *Rhipicephalus pulchellus* from camels **[1].** Sterilize the tick exterior carefully by wiping the whole tick with a bleach-soaked paper towel and ethanol **[2].**

2.1.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 01:55-01:58

2.1.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 02:36 – 02:50

2.2. When the tick has dried, gently clean the mouth parts with the forceps by holding a small piece of paper towel soaked in 70 percent ethanol then dry again **[1]**.

2.2.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 02:51 – 03:01

2.3. Place the tick on a clean glass slide under the microscope with the ventral surface of the tick facing upward **[1]**, and hold it securely between the thumb and index finger **[2]**.

2.3.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:02 – 03:09

2.3.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:10– 03:15

2.4. Inject 20 microliters of a mixture consisting of 2 percent Pilocarpine hydrochloride and PBS in a 1 to 1 ratio directly behind coxa 4 using a 31-gauge syringe **[1]**. Rotate the tick so that the dorsal surface is exposed, and fix it in place on one end of the slide by using transparent tape **[2]**.

2.4.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:16 – 03:23

2.4.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:26 – 03:35

2.5. Place a small piece of non-toxic modeling clay at the opposite end of the slide to provide a fixed surface for support **[1]**. Wait for the tick to produce saliva **[2]**. Insert the 0.5 to 10 microliter tip between the palps to ensure it covers the hypostome **[3]**. Draw the saliva into the pipette tip **[4-TXT]**.

2.5.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:36 – 03:49

2.5.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:50 – 03:53

2.5.3. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 03:53 – 04:02

2.5.4. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:03 – 04:08
TXT: Create pressure for 5 - 10 s to stimulate saliva release

2.6. To collect the hemolymph, cut off one of the tick's legs using a size 11 scalpel blade **[1]**. Gently press the body, taking care not to apply too much pressure as this may rupture the tick or internal organs and release the midgut contents **[2]**.

2.6.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:09 – 04:16

2.6.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:17 – 04:25

2.7. Collect the hemolymph droplet using a pipette with a 0.5 to 10 microliter tip placed on the leg **[1]**.

2.7.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:26 – 04:32

3. Tick Dissection and Tissue Collection

3.1. To dissect the tick, first heat paraffin wax using a soldering iron to create a melted spot in the Petri dish where the tick will be placed **[1]**.

3.1.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:36 – 04:45

3.2. Place the tick in the melted wax cavity, ensuring that the ventral part of the tick's body is submerged and held firmly in the wax **[1]**.

3.2.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:46 – 04:56

3.3. Then cut the dorsal edge of the body carefully using the scalpel blade **[1]**, and remove the dorsal cover of the tick body **[2]**. Extract the salivary glands, midgut, and other tissues using forceps **[3-TXT]**.

3.3.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 04:57 – 05:03

3.3.2. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 05:04 – 05:06

3.3.3. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 05:07 – 05:12
TXT: Transfer each to a separate slide or Petri dish

3.4. Thoroughly wash each of the extracted tissue types in 5 drops or 50 microliters of PBS in separate slides **[1]**.

3.4.1. LAB MEDIA: JoVE68952_Khogali et al._Footage.mp4 05:11 – 05:26

Results

4. Results

4.1. DNA concentration was higher in hemolymph than in saliva across all four tick species **[1]**.

4.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight all four boxplots under the "Haemolymph" group*

4.2. Among the four tick species, *Rhipicephalus pulchellus (RIP-ih-SEF-uh-lus- pull-KELL-us)* hemolymph showed the highest DNA concentration compared to the others **[1]**.

4.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the green boxplot under "Haemolymph" for Rh. pulchellus*

Pronunciation Guide

1. **Haemolymph**
   Pronunciation link: https://www.merriam-webster.com/dictionary/haemolymph
   IPA: /ˈhiːməˌlɪmf/
   Phonetic Spelling: HEE-muh-limf
2. **Ixodidae**
   Pronunciation link: No confirmed link found (common texts use "ixodidae" pronounced as /ˌaɪkɒˈdaɪiː/)
   IPA (American): /ˌaɪkəˈdaɪi/
   Phonetic Spelling: eye-kuh-DY-ee
3. **Pilocarpine**
   Pronunciation link: https://www.merriam-webster.com/dictionary/pilocarpine
   IPA: /ˌpaɪləˈkɑːrpin/
   Phonetic Spelling: pie-luh-KAR-peen
4. **Paraffin**
   Pronunciation link: https://www.merriam-webster.com/dictionary/paraffin
   IPA: /ˈpærəfɪn/
   Phonetic Spelling: PAR-uh-fin
5. **Hyalomma**
   Pronunciation link: No confirmed link found
   IPA (American): /haɪˈæləmə/
   Phonetic Spelling: hy-AL-uh-muh
6. **Rhipicephalus**
   Pronunciation link: No confirmed link found
   IPA (American): /ˌraɪpɪˈsɛfələs/
   Phonetic Spelling: ry-pi-SEF-uh-luhs
7. **Midgut**
   Pronunciation link: https://www.merriam-webster.com/dictionary/midgut
   IPA: /ˈmɪdˌgʌt/
   Phonetic Spelling: MID-gut