Mosquito Salivary Gland Dissections

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A. Solutions

‣ **For dissection:**

1. 70% ethanol

	For 50 mls:
70% EtOH	35 ml 100% EtOH
	15 ml ddH_2O

* Use high quality 200 proof ethanol; for example Sigma E702-3 for molecular biology

2. 1× PBS pH 7.4

	For 1 L:
137.0 mM NaCl	8.00 g NaCl
2.7 mM KCl	0.20 g KCl
1.4 mM KH_2PO_4	0.24 g KH_2PO_4 (anhydrous)
10.0 mM Na_2HPO_4	1.44 g Na_2HPO_4 (anhydrous)
	↑ 1 L ddH_2O

* pH should be around 7.4. Autoclave or filter sterilize solution.

* This solution can be made as a 10× stock.

3. *Aedes* saline pH 7.00

	For 500 ml:
150.0 mM NaCl	4.40 g NaCl
4.0 mM KCl	0.15 g KCl
3.0 mM $CaCl_2$	0.22 g $CaCl_2 \bullet 2H_20$
1.8 mM $NaHCO_3$	0.08 g $NaHCO_3$
0.6 mM $MgCl_2$	0.06 g $MgCl_2 \bullet 6H_20$
25.0 mM HEPES	3.00 g HEPES
	↑ 500 ml ddH_2O

* pH should be around 7.0. Autoclave or filter sterilize solution.

B. Protocol

1. Obtain female mosquitoes from cages and transfer to 14 ml polypropylene round bottom tubes (Falcon #35-2059).

2. Place tube on ice to anaesthetize the mosquitoes (~ 10 – 30 min.)

3. While mosquitoes are on ice, prepare:
    a. dissecting microscope with a glass plate
    b. 2 × 25g needles (one slightly bent) attached to 1 cc syringes
    c. 2 small Petri dishes (polystyrene 60 × 15 mm Corning #25010):
        1 with 70% ethanol; 1 with 1× PBS pH 7.4
    d. *Aedes* saline (dissection medium depends upon experiment to be performed - can use RPMI or DMEM as well)

4. Place anaesthetized mosquitoes in Petri dish containing 70% ethanol and gently swirl the ethanol to mix (so to completely saturate the mosquitoes), about 20 – 30 sec.

    * Do not leave mosquitoes in ethanol for a long period of time as the mosquitoes become soft and difficult to dissect.

5. With curved serrated micro-dissecting forceps, transfer the mosquitoes to Petri dish containing 1× PBS pH 7.4.

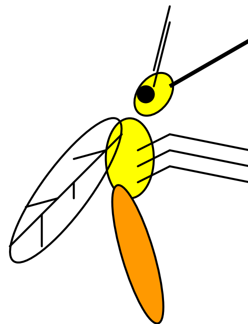
    * If the dissection will take a while, do not leave mosquitoes in saline for a long period of time as the mosquitoes become soft and difficult to dissect. Instead wet the mosquitoes in batches of no more than 50 at a time.

6. Place a small amount of *Aedes* saline on the glass slide (step 3a) (~ ≤100 μl).

7. With curved serrated micro-dissecting forceps line mosquitoes on the glass plate near the *Aedes* saline.

    NOTE: It is easier to dissect if mosquitoes are on their side with their heads upward.

    Example:



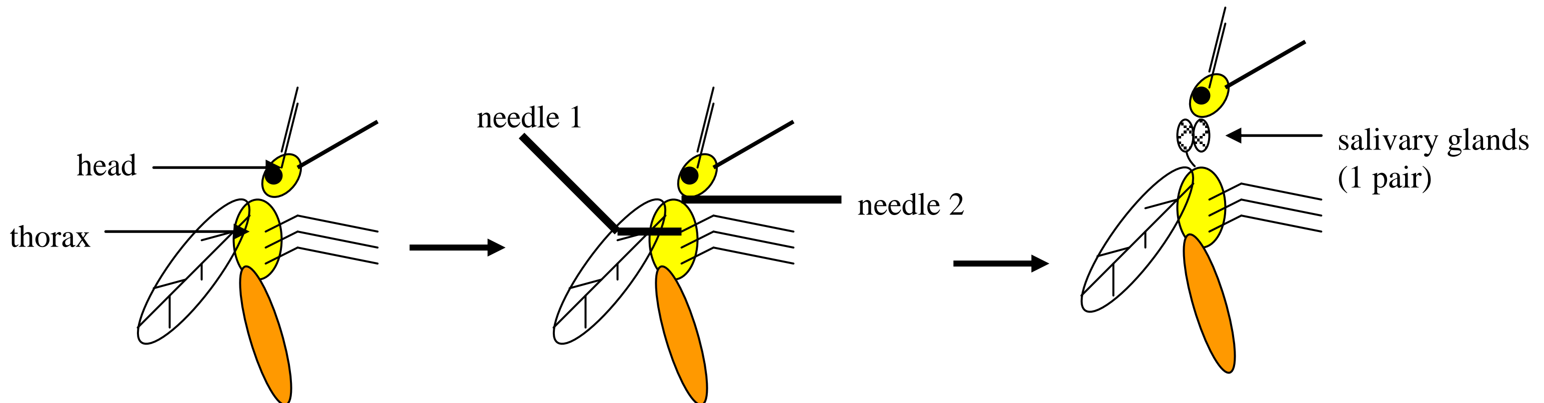
8. Using 2 syringes with 25g needles, dissect salivary glands (see diagram below):

    a. With the bent needle (in the left hand), press on the thorax.

b. Place the other needle (in the right hand) under the head of the mosquito and pull upward.

c. The salivary glands will be found between the head and thorax.

d. Cut the material above and below the salivary glands and move the glands to the *Aedes* saline. It is important to keep the salivary glands hydrated.



* If the number of dissections is great, the salivary glands will begin to stick to needles. Therefore, change the needles as is necessary to prevent loss of salivary glands.

9. Using a long Pasteur pipette transfer the medium containing the salivary glands to a tube and keep on ice until dissection is finished or the glands are ready to be used.

   * Do not let the glands enter the wide part of the Pasteur pipet because it is difficult to get them out.

   * A pulled Pasteur pipette can also be used to transfer the salivary glands. The salivary glands will stick to the sharp end of the pipette.

10. To pellet glands, spin for 5 sec at 16,000 $\times g$ (14000 rpm using Eppendorf 5415c) at 4°C. *Aedes* saline can be removed and replaced with fresh saline or other media.

11. Release sporozoites from glands by grinding with a small homogenizer

⇨ If salivary glands are in a large volume of dissection medium:
- grind glands in a Dounce homogenizer

  OR
- pellet glands by spinning for 5 sec at 16,000 $\times g$ (14000 rpm using Eppendorf 5415c) at 4°C; dissection medium can be removed for a smaller total volume.

⇨ A greater number of sporozoites can be counted if tube is left on ice a few minutes after homogenization.

For homogenization and counting sporozoites - see next protocol