Isolation of *Plasmodium* Sporozoites from Mosquito Salivary Glands

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A. Protocol

1. Dissect infected female *Anopheles stephensi* mosquitoes to obtain salivary glands as described in **Mosquito Salivary Gland Dissection** protocol.

    • Number of mosquitoes to dissect depends upon the number of sporozoites needed for the experiment to be performed and the species of *Plasmodium*.

        → ~ 10,000 *P. berghei* sporozoites/mosquito (depending on infection, range of: 8,000 – 20,000 sporozoites/mosquito)
        → ~ 35,000 *P. yoelii* sporozoites/mosquito (depending on infection, range of: 30,000 – 40,000 sporozoites/mosquito)

2. Keep salivary glands on ice in dissection medium until dissection is complete.

    • Dissection medium is determined by the experiment to be performed!

3. Grind mosquito salivary glands on ice to release sporozoites. To release sporozoites, dounce 3X while rotating the homogenizer.

        → If salivary glands are in a small volume of dissection medium (<500 μl), grind in 1.5 ml tube using blue homogenizer. A greater number of sporozoites can be counted if tube left on ice a few minutes after homogenization.

        → If salivary glands are in a large volume of dissection medium, grind in 1 ml Dounce homogenizer.

        → Homogenizer can be rinsed with dissection medium into the sporozoite-containing tube to obtain any sporozoites attached to it. However, this is not necessary and will increase the volume of medium in the tube.

4. If mosquito debris has to be removed, first spin sporozoite-containing medium at ~100 ×*g* (1000 rpm using Eppendorf 5415c; 900 rpm using Eppendorf 5417c) for 4 min at 4°C. Transfer sporozoite-containing supernatant to a new tube, add 250 – 500 μl fresh dissection medium to pellet in old tube, grind 1X, and spin again as above. Add the wash supernatant to the tube containing the sporozoites.

5. Using a 1000 µl tip, pipet the sporozoites gently 1X to mix and determine the volume of sporozoite-containing medium (from step 3 or 4) using the same tip.

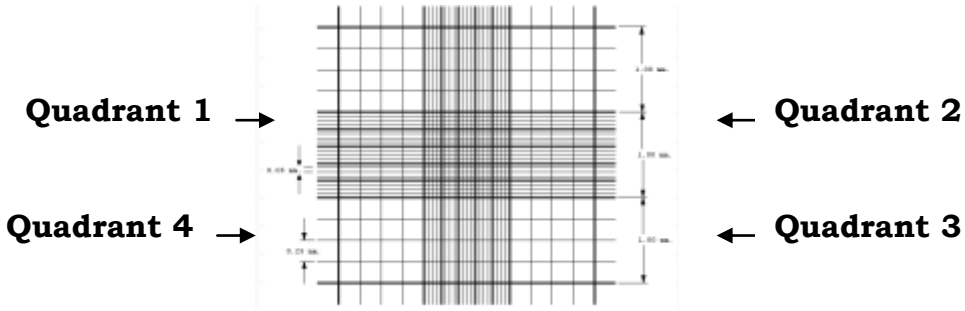
6. Determine number of sporozoites:

   a. place 10 µl of dissection medium containing sporozoites (step 3) on <u>one</u> counting area of a clean Hausser Levy hemacytometer. Hemacytometer should be cleaned with ddH_2O!

      → If a large number of sporozoites is expected, a dilution (1:5, 1:10, or 1:20) can first be made.

   b. Place hemacytometer on a wet Kimwipe in a covered Petri dish and allow sporozoites to settle for 7 – 10 min.

   c. Count the number of sporozoites in <u>two</u> of the four quadrants (see diagram below).



      → Count <u>diagonal</u> quadrants.

      For instance: **Quadrant 1 + Quadrant 3**
      <u>or</u>
      Quadrant 2 + Quadrant 4.

   d. Calculate total number of sporozoites:

$$\frac{(1^{st}\ \text{quadrant} + 2^{nd}\ \text{quadrant})}{2} = \#\ \text{of sporozoites} \times 10^4/\text{ml}$$

(# of spz × 10^4/ml) × volume of dissecting medium containing sporozoites (in mls) (from step 5) × dilution factor = total # sporozoites

7. If you need to resuspend the sporozoites in different medium or want them more concentrated, you can spin at 12000 – 15000 ×*g* (12000 - 14000 rpm using Eppendorf 5415c; 11000 – 13000 rpm using Eppendorf 5417c) for 4 min at 4°C to pellet*.

- If the number of sporozoites is great, they can be seen at a white pellet; if mosquito material is also present, the pellet will appear brown.

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*NOTE: The less the sporozoites are abused, the better!
Avoid excessive vortexing, pipetting, and centrifuging!