Video Article

Protocol for Plasmodium falciparum Infections in Mosquitoes and Infection Phenotype Determination

Zhiyong Xi[1], Suchismita Das[1], Lindsey Garver[1], George Dimopoulos[1]

[1]Malaria Research Institute, Bloomberg School of Public Health, Johns Hopkins University

Correspondence to: George Dimopoulos at gdimopou@jhsph.edu

URL: http://www.jove.com/video/222
DOI: doi:10.3791/222

Keywords: Cellular Biology, Issue 5, mosquito, malaria, genetics, injection, RNAi, Plasmodium, TIssue Culture, Cell Culture, Insect

Date Published: 7/4/2007

Citation: Xi, Z., Das, S., Garver, L., Dimopoulos, G. Protocol for Plasmodium falciparum Infections in Mosquitoes and Infection Phenotype Determination. *J. Vis. Exp.* (5), e222, doi:10.3791/222 (2007).

Abstract

Once a gene is identified as potentially refractory for malaria, it must be evaluated for its role in preventing Plasmodium infections within the mosquito. This protocol illustrates how the extent of plasmodium infections of mosquitoes can be assayed. The techniques for preparing the gametocyte culture, membrane feeding mosquitoes human blood, and assaying viral titers in the mosquito midgut are demonstrated.

Video Link

The video component of this article can be found at http://www.jove.com/video/222/

Protocol

A. Preparation of gametocyte culture:

1. Bring fresh human blood and serum to 37^{o}C in a water bath.
2. In a fume hood, under sterile conditions, pipet 1 mL of warm blood into an eppendorf tube. Spin the blood in a centrifuge for 5 minutes at 2200 rpm. Erythrocytes should be pelleted; remove and discard the clear serum. Wash erythocytes with an equal volume of new serum 3 times to obtain the whole blood for the gametocytes.
3. Retrieve the gametocyte culture from the CO_2 incubator or candle jar. This culture should be maintained on RPMI media and fed to the mosquitoes on day 16 of the culture. To determine the gametocytemia % (on day 16 of the culture) make a thin smear on a glass slide with one drop blood and perform a Geimsa stain. Determine the number of gametes in a field of the parasite culture. It is usually in the range of 2 - 4%. Calculate the volume of whole blood that you will need to achieve the desired final % gametocytemia (0.3 -1 %).
4. Remove most of the media from your gametocyte culture using a pipet, taking care not to disturb or suck up the parasites (parasites will appear as black while the media will be light pink/red). Pipet the rest of the media and parasites into a 15 ml falcon tube. Spin in a centrifuge for 5 minutes at 2200 rpm. Parasites and erythrocytes will pellet, remove, and discard media supernatant without disturbing parasites.
5. Combine parasites with the volume of prepared whole blood (from step A2) to give you the desired final gametocytemia according to previous calculations. Pipet to mix thoroughly. (~1 minute)

This is now the sample you will feed to your mosquitoes. Keep at 37^{o}C in a water-bath.

B. Feeding mosquitoes:

1. Put your *Anopheles* mosquitoes (~30-50) into wax-lined cardboard cups covered with mesh netting secured by a rubber band or cardboard lid supplied with cup. We use a battery-operated aspirator for this step.
2. Prepare the circulation water-bath by inserting nozzles on either side of glass feeders to rubber tubing. Connect as many feeders as you need in a series; one end of the series should be connected by tubing to the circulator of the water bath (the part that pushes water through the feeders), the other end of the series should consist of rubber tubing submerged in the bath to allow the circulated water to empty back into bath. Turn on the bath until the water is about 37^{o}C and the water is circulating through the feeders and back to the bath. This is essential to keep the blood warm and keep the parasites alive.
3. Prepare the membrane (we use small squares of stretched parafilm) by stretching around the glass membrane feeder, using pressure to seal tightly to the feeder opening. This membrane will mimic the skin.
4. Place one feeder membrane-side down onto each mosquito cup and secure feeders using clamps or tape. The membrane should sit flush with the net so mosquitoes can access it from inside the cup.
5. Carefully pipette parasite-blood meal (from step A5) into the neck of the feeder, making sure the blood goes all the way through the neck into the reservoir, contacting the membrane so the mosquitoes can access the blood. We use about 200 µl of blood per feeder, but this can vary.
6. Allow mosquitoes to feed. We allow them to feed for ≈30 minutes (variable, depending on theexperiment; for most experiments, occasionally monitoring how many have already taken a meal by looking at the blood content in the abdomen.)
7. After feeding, remove feeders from the mosquito cups. Turn off the water bath and disconnect feeders from tubing.

8. Remove the membrane and soak feeders in 10% bleach to decontaminate and rinse with water. Place contaminated material (membranes, paper towels, gloves, etc) into a small biohazard bag and deposit in a large biohazard receptacle.
9. Anesthetize mosquitoes by placing cups in refrigerator or cold room. Once mosquitoes are fully anesthetized, transfer them on a petri dish embedded in an ice bucket. Sort mosquitoes, looking carefully at the abdomens for any sign of a blood meal, and discard those that did not feed. Some mosquitoes will be fully engorged while others will show a narrow band of ingested blood; all are acceptable as "fed". Put the fed mosquitoes back into the cardboard cups.
10. Provide a sugar meal (a piece of cotton soaked in 10% sucrose) to mosquitoes, and incubate under normal insectary conditions for 7 days. Everyday, the mosquitoes should be monitored for the mortality rate. Also, the cotton should be made wet with the sucrose, so that they are not deprived of food. It is better, yet,to add a paper towel soaked in distilled water, to keep the mosquitoes moist. These mosquitoes are *P. falcipraum* infected, so all care must be taken so that no mosquitoes can escape from the cup. It is best to put the cups in a small cagefor double protection.

C. Dissecting mosquito midguts and estimating oocyst loads:

1. Aspirate mosquitoes (from step B) from cardboard cups using a battery-operated aspirator. Anesthetize mosquitoes, which are inside the removable aspirator reservoir, by moving to a cold room or burying the reservoir in ice for at least 5 minutes or until mosquitoes stop moving.
2. Transfer anesthetized mosquitoes from the reservoir to a glass petri dish embedded in ice. Spread the mosquitoes to a single layer and cover with a plastic petri dish lid. Mosquitoes that can not be accommodated on the dish remain in the reservoir, which is returned to cold room or ice.
3. Using fine-tipped forceps, transfer a single mosquito from the dish to 100 ul PBS contained on a glass slide mounted under a dissection microscope. Leave the others covered in the chilled petri dish.
4. Using fine-tipped forceps, hold mosquitoes at the thorax and the posterior abdomen. Pull the body apart until the midgut is exposed. Use forceps to remove excess tissue from gut.The gut looks like a white, sac-like body. Remove excess tissue from the gut.Mount the gut in one well of a 12-well glass slide and immerse in 5-10 µl of 0.2% mercurochrome. Squash the carcass and discard on damp paper towel.Continue for other mosquitoes until the 12-well slide is full. Cover the mounted guts with a glass coverslip.
5. Examine guts under a light microscope, looking for round oocysts that stain bright pink against the clear/faint pink gut tissue. Count oocysts, record and continue to the next sample. Continue for all of the mosquitoes from all experimental treatments.
6. When finished, wipe slide clean with a paper towel. Place the paper towel with guts, and paper towel with carcasses, into small biohazard bag and discard in the trash. Gloves are worn throughout procedure. Care is taken not to let any infected mosquitoes escape.

Discussion

There can be some variations in the Plasmodium infection and infection phenotype determination and different labs may follow different techniques.

References

1. Avila, S.L. and Ferreira, A.W. 1996. Malaria diagnosis: a review. Braz J Med Biol Res. 29(4):431-43. Review.

2. Bell, A.S. and Ranford-Cartwright, L.C. 2004. A real-time PCR assay for quantifying Plasmodium falciparum infections in the mosquito vector. Int J Parasitol. 34(7):795-802.

3. Collins, K.M., Hochberg, L.P., Ryan, P.R., Collins, W.E, Wirtz, R.A and Ryan, J.R. 2004. Quantification of Plasmodium malariae infection in mosquito vectors. Ann Trop Med Parasitol. 98(5):469-72.

4. Czeher, C., Labbo, R., Djibrilla, A., Here, L., Arzika, I. and Duchemin, J.B. 2006. ELISA study of oocyst-sporozoite transition in malaria vectors. Parasite. 13(3):257-61.

5. Haghdoost, A.A., Mazhari, S. and Bahadini, K. 2006. Comparing the results of light microscopy with the results of PCR method in the diagnosis of Plasmodium vivax. J Vector Borne Dis. 43(2):53-7.

6. Patsoula, E., Spanakos, G., Sofianatou, D., Parara, M. and Vakalis, N.C. 2003. A single-step, PCR-based method for the detection and differentiation of Plasmodium vivax and P. falciparum. Ann Trop Med Parasitol. 97(1):15-21.

7. Osta, M.A., Christophides, G.K. and Kafatos, F.C. 2004. Effects of mosquito genes on Plasmodium development. Science. 303(5666):2030-2.

8. Srinivasan, P., Abraham, E.G., Ghosh, A.K., Valenzuela, J., Ribeiro, J.M., Dimopoulos, G., Kafatos, F.C., Adams, J.H., Fujioka, H. and Jacobs-Lorena, M. 2004. Analysis of the Plasmodium and Anopheles transcriptomes during oocyst differentiation. J Biol Chem. 279(7):5581-7.