Video Article

Protocol for Dengue Infections in Mosquitoes (A. aegypti) and Infection Phenotype Determination

Suchismita Das[1], Lindsey Garver[1], Jose Ruiz Ramirez[1], Zhiyong Xi[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: https://www.jove.com/video/220
DOI: doi:10.3791/220

Keywords: Cellular Biology, Issue 5, mosquito, dengue, fever, infectious disease

Date Published: 7/4/2007

Citation: Das, S., Garver, L., Ramirez, J.R., Xi, Z., Dimopoulos, G. Protocol for Dengue Infections in Mosquitoes (A. aegypti) and Infection Phenotype Determination. *J. Vis. Exp.* (5), e220, doi:10.3791/220 (2007).

Abstract

The purpose of this procedure is to infect the Aedes mosquito with dengue virus in a laboratory condition and examine the infection level and dynamic of the virus in the mosquito tissues. This protocol is routinely used for studying mosquito-virus interactions, especially for identification of novel host factors that are able to determine vector competence. The entire experiment must be conducted in a BSL2 laboratory. Similar to Plasmodium falciparum infections, proper attire including gloves and lab coat must be worn at all times. After the experiment, all the materials that came in contact with the virus need to be treated with 75% ethanol and bleached before proceeding with normal washing. All other materials need to be autoclaved before discarding them.

Video Link

The video component of this article can be found at https://www.jove.com/video/220/

Protocol

A. Propagate the virus in the C6/36 cell line.

1. Cells grow to 80% confluency in 75 cm^2 flask;
2. Remove the media with 3-4 ml remaining in the flask;
3. Take 0.5 ml stock virus and add into the above cell with the multiplicity of infection of 1.5 virus particles per cell. Shaking the flask for 15 minutes slowly at the room temperature.
4. Incubate for 45 minutes at 5% CO_2 and 37°C
5. Add 30 ml media, and incubate for 5 days.

B. Prepare the mixture of virus and blood

1. Detach the cell with the scraper and transfer the cell and media to the 50 mL conical tubes;
2. Centrifugate at 800g for 10 minutes; collect the supernatant, but leave 1ml of supernatant with the cell pellet;
3. Freeze the above cell pellet in dry CO_2 and then thaw in 37°C water bath, repeat two to three times;
4. Centrifugate at 800g for 10 minutes, take the supernatant, and combine with the supernatant collected from step 3;
5. Collect whole human blood with the same procedure (step 3) for preparation of gametocyte culture to infect Anopheles mosquito with Plasmodium falciparum;
6. Combine the equal amount of the above whole human blood with virus supernatant from step 4, and add human serum (10% of the whole volume).
7. Incubate the above mixture of human blood and dengue virus for 30 minutes at 37°C water bath

C. Feeding mosquitoes

This procedure is almost identical to what have been described in the section for *Plasmodium falciparum* infections in mosquitoes. We assay the midgut infection at day 7, and the salivary infection at day 14 after blood-feeding. All material that came in contact with the virus are treated first with 75% ethanol and then with 10% bleach.

D. Assay the virus titer in mosquito tissue

1. Two or three days before dissection of mosquito tissue, grow C6/36 cell in a 24 well plate such that the cell reach 80% confluency at the day when the assay is conducted;
2. Mosquitoes are anesthetized at 4°C, sacrificed and surfaced sterile by soaking them in 75% ethanol for 1 minute. Then mosquitoes were washed with sterile water twice before dissection of midgut or salivary gland under the dissecting microscope. From this step, all the

procedure below needs to be conducted in a sterile environment such as a biological safety cabinet. These tissues are transferred to a tube containing 150 µl media and homogenized with a Kontes pellet pestle motor for 90 seconds;

3. Make a 1:100 dilution by adding 10 µl of the homogenate into 990 µl media;
4. Make further 1: 10, 1:100 and 1:1000 dilution with the medium in the 96 well plate;
5. Remove the media in the 24 well plate, and add 100 µl of each of the above four dilutions to each corresponding well;
6. Shake the plate slowly for 15 minutes at the room temperature, then incubate for 45 minutes at 5% CO_2 and 37°C;
7. Add 1ml of methycellulose overlay (1%) to each well;
8. Incubate plates at 37°C with 5% CO_2 for 5 days;
9. The steps from here on do not require a sterile environment but as with any other pathogen care should be taken at all times. Discard methycellulose overlay from each plate and blot to remove excess media
10. Add 1ml of methanol/acetone fixative (1:1) to each well. Keep at 4°C for 1 hr;
11. Pour off the fixative, and wash once with 1X PBS;
12. Make 1:1000 dilution to the primary antibody against the virus with 5% blotto;
13. Add 200 µl of diluted hyperimmune fluid to each well, incubate at 37°C for 1 hr;
14. Remove hyperimmune fluid and wash plate with 1 X PBS
15. Prepare a 1:1000 dilution of goat anti-mouse conjugate (KPL Cat# 074-1806) in 5% blotto (5g powdered skim milk in 100 ml of 1 X PBS), and then add 200 µl to each well, and incubate at 37°C for 1 hr;
16. Prepare substrate as follows: add 1 tablet of 3'-Diaminobenzidine terrahydrochloride (Sigma Cat# D5905) in 20 ml of 1 X PBS, after dissolved, and then add 8 µl of 30% hydrogen peroxidase
17. Add 200 µl of the above substrate to each well. Let stand at room temperature for 10 minutes
18. Remove substrate and stop reaction by adding 1ml of distilled water to each well
19. Pour off water and blot plates to dry
20. Count the virus particle