
Research Article,

Morphological Description of Den-3 Virus Infection Cells through EDTA Blood Feed In *Aedes Aegypti* Mosquitoe

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Abstract:

Mosquito *Ae. aegypti* is the main dengue virus vector which causes the virus to grow and develop properly. The Artificial Membrane Feeding (AMF) method is a method of indirectly transmitting the dengue virus in the *Ae. aegypti*. This method uses blood feed with EDTA anticoagulant and DEN-3 virus in the insectarium. One way to detect the dengue virus found in *Ae* mosquito cells. *aegypti* is an immunohistochemical Streptavidin Biotin Peroxidase Complex (SBPC). In this method, the morphological picture of DEN-3 virus infection cells will be seen through the heparin anticoagulant blood feed using the AMF method. Anticoagulants function to slow the blood clotting to feed the *Ae* mosquitoes. *Aegypti*. The aim of this study was to determine the Positive Infection Rate of DEN-3 virus and the morphological picture of DEN-3 virus infection cells using the AMF method of EDTA anticoagulant blood feed through SBPC immunocytochemical examination of *Ae* mosquitoes. *aegypti*. The design method of this study was experimental, infected *Ae.aegypti* mosquitoes using anticoagulant blood containing DEN-3 virus orally as an infectious sample. Negative control used *Culex* Sp mosquitoes and positive control used adult *Ae.aegypti* mosquitoes which were infected with DEN-3 virus by injection. Detection of DEN-3 virus in *Ae.aegypti* mosquitoes through SBPC immunocytochemical examination. Results of Positive Infection Rate for *Ae.aegypti* mosquitoes with EDTA anticoagulant as much as 23.20% through SBPC immunocytochemical examination with a picture of cell morphology of DEN-3 virus infection. The conclusion of SBPC immunocytochemical examination showed positive cell morphology of DEN-3 virus infection with EDTA anticoagulant in *Ae.aegypti* mosquitoes fed by human blood orally through the AMF method.

Keywords: EDTA, AMF, Imunositokimia SBPC, blood feeding, *Ae. aegypti*.

Introduction:

Dengue Haemorrhagic Fever (DHF) is a disease caused by dengue virus consisting of 4 virus serotypes, DENV-1, DENV-2, DENV-3 and DENV-4. Dengue virus is derivate from *Arthropod-Borne Virus*, *Flavivirus* genus, and *Flaviviridae* family, and is transmitted through the bite of *Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*).¹

The World Health Organization (WHO) noted as Indonesia the country with the highest dengue fever case in Southeast Asia since 1968 until 2009 and has increased the number of DHF cases from 58 cases in 1968 to 126,675 cases in 2015^{2,3}. The high rate of dengue fever mortality causes interest

for researchers to know the role of *Ae. aegypti* mosquitoes as a research model and can be reared in the.⁴

At present, research using *Direct Feeding Assay* (DFA) method in some countries cannot longer be used anymore because of the difficulty of obtaining research permits that have conflict with the ethical code of research. *Artificial Membrane Feeding* Method (AMF) is a method of dengue virus transmission in *Ae. aegypti* indirectly. This AMF method can be used instead of *Direct Feeding Assay* (DFA) method to determine the dengue virus infection in the *Ae. aegypti* mosquito body⁵. Blood feeding of female adult mosquitoes is useful as a source of energy, colonization and

maintenance that are often used for research on vector-borne disease, insecticide resistance testing and mosquito colonization in the laboratory. This test requires blood feed as a substitute for direct feed by using AMF method^{6,7,8}

Blood feeding on infected mosquitoes can cause mosquitoes to produce viruses that can be transmitted to the host⁽⁹⁾. Blood used for the study by the AMF method requires heat that has been set at 37° C according to human body temperature and anticoagulant which serves to slow the occurrence of blood spotting so that mosquitoes can suck blood as feed⁽¹⁰⁾. Anticoagulant heparin is the best anticoagulant compared to other anticoagulant groups and can be used to maintain *Ae. Aegypti* colonies in the insectarium⁽¹²⁾. Anticoagulant EDTA show no difference in termination from survival, fertility in productivity and hatching rates on adult females *Ae. aegypti* who have been infected with dengue virus until the 8th generation⁽¹³⁾.

In this study, the researchers wanted to determine the Positive Infection Rate of the DEN-3 virus and the morphology of the DEN-3 virus infection cells using the AMF method of blood feed with anticoagulant EDTA through SBPC Immunocytochemical examination of *Ae. aegypti*.

Material and Method:

This research was an experimental research. The population was *Ae. aegypti* results of colonization in the insectarium of the Department of Parasitology Faculty of Medicine of Public Health and Nursing, Universitas Gadjah Mada. This study had been approved by the Research Ethics Committee of Medicine and Health, The Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada Number KE / FK / 1161 / EC / 2017.

Preparation of females *Ae. Aegypti* infected with DEN-3 through the Artificial Membrane Feeding (AMF) method.

The sample of the study was adult female *Ae. aegypti* F1050 generation 7-9 days which was the result of colonization that will be infected by DEN-3 virus using anticoagulant EDTA via oral AMF method. In the control group I, mosquitoes were suspended with DEN-3 virus mixed with O human blood group which did not contain IgM dengue antibody and anticoagulant EDTA. The suspension is fed into a small mosquito feeder

and then flows at 37 ° C from the waterbath.

Preparation of adult female *Ae. aegypti* infected with DEN-3 virus as a positive control and *Culex sp* as a negative control

Negative control used *Culex Sp* mosquito and positive control used 3-day adult female *Ae. aegypti* infected by DEN-3 virus through piston injection. This test used supernatant DEN-3 virus cultured on C6 / 36 cells culture (Namru isolates). In the treatment group and control group, DEN-3 virus was detected by SBPC Immunocytochemistry

SBPC Immunocytochemical Method:

Head squash preparation on *Ae. aegypti* had undergone incubation period of DEN-3 virus infected after day 12. Kaput is separated by mosquito syringe needles on *poly-L-Lysine* coated glass. The preparations were fixed with absolute cold methanol and dried at room temperature. After drying, the preparations were painted with the SBPC Immunocytochemical Method. The primary antibody used was the DSSE10 monoclonal antibody (1:10). The result was then observed under a light microscope. The results of SBPC immunocytochemical tests on head squash preparations were stated positive if there were brown cells and spread among the brain tissue, while negative if the brain cell cell's cytoplasm was blue. Positive rate infection was obtained by counting the positive cells of the antigen divided by the total number of positive and negative cells in each field and then multiplied by 100¹⁴

Analisis statistic:

The unpaired t test from statistical analysis in this study was conducted to determine the value of the positive infection rate using the SBPC Immunocytochemistry method. The total number of mosquitoes used was 100 with 4 replications which had a capacity of 25 individuals per paper cup. In this study, the normal distribution of the data was tested by Saphiro-Wilk and if the distribution of the data was not normally distributed, then the analysis used the Mann-Whitney test.

Result:

This research used a sample of colonized *Ae. aegypti* infected by dengue virus using anticoagulant EDTA via oral AMF method. This

research has obtained approval from the Ethical Commission of FKMK UGM with letter number KE / FK / 1161 / EC / 2017.

Positive Infection Rate (PIR) of Ae. aegypti mosquito by administering anticoagulant EDTA and heparin through SBPC immunocytochemical examination

Positive results of antigens are indicated by the presence of brown color on the granules and cytoplasm in the hematocytes in the head squash preparations whereas the negative results appear purplish blue as presented in figures 1.

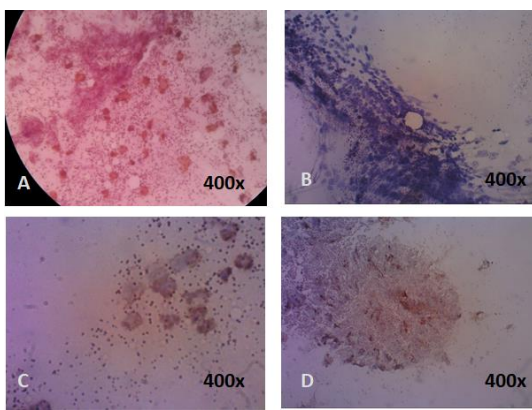


Figure 1. Microscopic photo of the Immunocytochemical preparation of SBPC head squash Ae. aegypti mosquito infected with Dengue-3 virus by intrathoracic injection as a positive control (brown color cells) (A). Immunocytochemical preparation of SBPC head squash for Culex Sp mosquitoes as a negative control (purple blue cells) (B). Positive result (brown color) on adipose tissue Immunocytochemical preparation of SBPC head squash mosquito Ae. aegypti infected with DEN-3 virus orally with EDTA anticoagulant and incubation period of 12 days using monoclonal antibody DSSC10 (1:10) as primary antibody (C). Positive results (brown color) on brain tissue Immunocytochemical preparation of SBPC head squash mosquito Ae. aegypti infected with DEN-3 virus orally with EDTA anticoagulant and incubation period of 12 days using monoclonal antibody DSSC10 (1:10) as primary antibody (D).

Result of examination of positive infection rate (PIR) number of Ae. aegypti mosquitoes with anticoagulant EDTA and heparin on SBPC Immunocytochemical methods are presented in Table 1.

Table 1: Positive Infection Rate (PIR) of Ae. aegypti with anticoagulant EDTA on SBPC Immunocytochemical Method

Treatment	Ae. Aegypti mosquito	Positive Infection Rate
Repetition EDTA 1	1	41,04
	2	6,67
	3	43,86
	4	0,00
	5	0,00
Repetition EDTA 2	1	0,00
	2	31,37
	3	66,67
	4	27,27
	5	4,08
Repetition EDTA 3	1	0,00
	2	16,48
	3	60,33
	4	0,00
	5	0,00
Repetition EDTA 4	1	75,00
	2	80,47
	3	57,45
	4	59,59
	5	36,80

Average and standard deviation EDTA = 30, 35(28,68)

The Mann-Whitney test was used to analyze the results of the number of Positive Infection Rate (PIR) of Ae. aegypti with EDTA anticoagulant in the immunocytochemical method shown in Table 1, consisting of the number of subjects, median, mean rank and p-value of each group.

Table 2. Statistical Analysis of Positive Infection Rate (PIR) of Ae. aegypti with EDTA anticoagulant administration on SBPC Immunocytochemical Method

	Median (Minimum-Maksimum)	Mean Rank	Nilai p
EDTA	29,32(0,00-80,47)	23,20	0,068

In the Mann-Whitney analysis test results, p value = 0.068 (one-tailed), it is clinically proven that there is a significant difference in the number of Positive Infection Rate (PIR) of Ae. aegypti by giving EDTA anticoagulant through SBPC Immunocytochemistry examination. These results prove that the EDTA anticoagulant produces a Positive Infection Rate (PIR) of Ae. aegypti through SBPC Immunocytochemistry examination.

Discussion:

The results of clinical analysis tests on the Positive Infection Rate (PIR) of *Ae. aegypti* with EDTA anticoagulant using the SBPC Immunocytochemistry method, there was a significant difference. These results can be seen from the average ranking between groups so that it is proven that EDTA can be used as an anticoagulant in developing the DEN-3 virus in the body cells of *Ae. aegypti*. EDTA anticoagulant has hyperosmolar properties that can cause changes in the structure of erythrocyte membranes, hemolysis and a decrease in blood pH. Live in an acidic environment and do not inhibit the replication of the dengue virus in the body of *Ae. aegypti*.^{16,17,18}

Immunocytochemical methods are capable of detecting low levels of antigen. This method has several advantages, including not requiring special equipment, using only a light microscope, being able to identify sub-cellular compartments containing antigens and using antibodies that are specific to antigenic proteins expressed on epitopes. SBPC immunocytochemistry with primary antibody DSSE10 was able to detect DEN-3 virus infection starting from the 2nd day of incubation. This shows that SBPC Immunocytochemistry with DSSE10 antibody can detect dengue antigen in *Ae. aegypti* mosquitoes earlier before the virus life cycle in the mosquito body is complete.²⁰

Conclusion and Suggestion:

Based on the research that has been carried out, it can be concluded that the positive result of dengue virus antigen is indicated by the presence of brown color in the granules and cytoplasm of the hematocytes in head squash preparations found in adipose tissue and brain tissue, while negative results show a purplish blue color in each cell in head squash preparations. EDTA can be used as an anticoagulant in developing the DEN-3 virus in the body cells of *Ae. aegypti* orally through the AMF method and on the Positive Infection Rate of DEN-3 virus with morphological features of DEN-3 virus infection cells through SBPC Immunocytochemistry examination, but research can be continued by using different anticoagulants.

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