Variations in Dilution of DSSE 10 Antibody in Immunocytochemistry Technique to Detect Dengue-3 Virus in *Aedes aegypti* Mosquitoes

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ABSTRACT

Introduction: Dengue viruses, globally the most prevalent arboviruses, are transmitted to humans by persistently infected Aedes mosquitoes. The most important vector of Dengue virus is the mosquito *Ae. aegypti*, which should be the main target of surveillance and control activities. Virologic surveillance for Dengue viruses in its vector has been used as an early warning system to predict outbreaks. Detection of Dengue virus antigen in mosquito head squash using immunocytochemical streptavidin biotin peroxidase complex (SBPC) assay is an alternative method for Dengue vector surveillance.

Objective: The study was aimed to compare several variations of MAb DDSE10 dilutions used in immunocytochemical SBPC assay to detect Dengue virus infection in head squash of *Ae. aegypti*.

Methods: The study design was experimental. Artificially-infected adult *Ae. aegypti* mosquitoes of DENV 3 were used as infectious samples and uninfected adult *Ae. aegypti* mosquitoes were used as normal ones. The immunocytochemical SBPC assay using monoclonal antibody DSSE10 with 4 variations of dilution (1:5, 1:10, 1:20, and 1:50) was applied on mosquito head squash to detect Dengue virus antigen. The results were analyzed descriptively.

Results: All variants of MAb DSSE10 dilutions in immunocytochemical SBPC assay showed positive imunoreaction in infected mosquito head squash. All variants of MAb DSSE10 dilutions in immunocytochemical SBPC assay showed negative immunoreaction in uninfected mosquito head squash.

Conclusion: Monoclonal antibody DSSE10 could be used in immunochemistry technique to detect Dengue-3 virus antigen in *Aedes aegypti* infected intrathoracally, with 1:50 dilution.

Keywords: *Aedes aegypti*, Dengue virus, Immunocytochemical, SPBC, Monoclonal Antibody DSSE-10

INTISARI

Pendahuluan: Virus Dengue, yang secara global merupakan arbovirus yang paling banyak, ditransmisikan ke manusia melalui nyamuk Aedes yang terinfeksi secara persisten. Vektor virus Dengue yang paling penting ialah nyamuk Ae. Aegypti, yang seharusnya menjadi target utama pada kegiatan surveilans dan pengontrolan. Surveilans virus Dengue pada vektornya telah digunakan sebagai sistem peringatan dini untuk memperkirakan wabah. Deteksi antigen virus Dengue pada pencet kepala nyamuk memakai *immunocytochemical streptavidin biotin peroxidase complex (SBPC) assay* merupakan metode alternative untuk surveilans Dengue.

Tujuan: Penelitian ini bertujuan untuk membandingkan beberapa variasi pengenceran MAb DDSE10 yang digunakan pada imunositokimia SBPC untuk mendeteksi infeksi virus Dengue pada pencet kepala *Ae. aegypti*.

Metode: Rancangan penelitian ini adalah penelitian eksperimental. Nyamuk *Ae. aegypti* dewasa yang diinfeksi buatan dengan DENV3 digunakan sebagai sampel infeksius dan untuk sampel normal digunakan nyamuk dewasa tak terinfeksi. Uji imunositokimia SBPC memakai antibodi monoklonal DSSE10 dengan 4 variasi pengenceran (1:5, 1:10, 1:20, dan 1:50) diaplikasikan pada pencet kepala nyamuk untuk mendeteksi

antigen virus Dengue. Hasil dianalisis secara deskriptif.
Hasil: Semua variasi pengenceran MAb DSSE10 pada uji imunositokimia SBPC menunjukkan imunoreaksi positif pada sediaan pencet kepala nyamuk terinfeksi. Semua variasi pengenceran MAb DSSE10 pada imunositokimia SBPC menunjukkan imunoreaksi negatif pada sediaan pencet kepala nyamuk tak terinfeksi.
Simpulan: Antibodi monoklonal DSSE10 dapat dipakai pada teknik imunositokimia untuk mendeteksi antigen virus Dengue-3 pada *Aedes aegypti* terinfeksi secara intratoraks, dengan pengenceran 1:50.

Kata kunci: *Aedes aegypti*, virus Dengue, Imunositokimia, *SPBC*, antibodi monoklonal DSSE-10

INTRODUCTION

Dengue haemorhagic fever (DHF) is one of the infectious diseases which still become health problems in Indonesia. It is caused by Dengue virus, which consists of four serotypes, those are, Dengue-1, Dengue-2, Dengue-3, and Dengue-4. Dengue-3 is known as the most dominant serotype in Indonesia. Based on survey conducted in 1985 in six cities in Indonesia (Medan, Jakarta, Yogyakarta, Pontianak, Ujung Pandang, and Manado), in the period of 1984-1985, Dengue virus was isolated from 59 patients who showed positive hemagglutination inhibition (H.I.), consisted of 50% Dengue-3, 30% Dengue-2, and 20% Dengue-1. Dengue-4 was not isolated in this period. Furthermore, Dengue-3 was associated with the more severe cases[1].

The main vector of DHF is *Aedes aegypti* and *Ae. albopictus* mosquitoes, but the one which has significant role in the transmission is *Ae. aegypti*, because of its living preference inside and around the house, so that they have more contact with humans. Meanwhile, *Ae. albopictus* mosquitoes prefer to stay outside of the house, causing them to have less contact with human.

Surveillance of DHF vector is important as one of the efforts to control DHF. However, vector surveillance is still emphasized on larva survey. Survey of adult mosquitoes and Dengue virus examination in the body of mosquitoes is rarely conducted. Finding the virus in the vector body may be the basis for effective Early Warning System (EWS) to prevent outbreaks of DHF[2].

Immunocytochemistry is one of the methods used to detect Dengue virus in mosquito. Immunocytochemistry is a visualization of the location of antigen reacted with specific antibody, which in turn is bound with antibody labelled with marker, such as peroxidase or alkaline phosphatase enzyme, in light microscope specimens. Recently, immunocytochemistry method using streptavidin biotin, avidin biotin complex, alkaline phosphatase-anti-alkaline phosphatase, or peroxidase-antiperoxidase has been available commercially. Immunocytochemical streptavidin biotin peroxidase complex (SBPC) assay uses secondary antibody labelled with biotin, which recognizes primary antibody. Furthermore, the use of streptavidin conjugate labelled with horseradish peroxidase enzyme and mixed chromogen substrate is also needed to detect antigen in cells and tissues with very high sensitivity[3].

Immunocytochemical SBPC assay is known to have high sensitivity, thus, low level antigen may be detected. This assay has advantages, because it does not need special instruments (it only needs light microscope commonly available in laboratories), and does not need particular skill. With this method, researcher may find subcellular compartment which contains antigen. The method uses specific antibody to particular antigen protein expressed in specific epitope[3].

Dengue Team at Universitas Gadjah Mada has been succeeded in producing monoclonal

antibody to Dengue virus, one of them is antibody secreted by hybrid (clonal) cell DSSE10. This antibody ischaracterized as IgG1, and does not exhibit any cross reaction to Japanese Encephalitis and Chikungunya antigens[4].

Development of monoclonal antibody DSSE10 is still ongoing, to find new breakthrough for effective, simple, and fast diagnostic test for Dengue infection. One effort that may facilitate the development is the characterization of the monoclonal antibody. In this study, optimization of DSSE10 antibody dilution in immunocytochemistry to detect Dengue-3 virus in *Aedes aegypty* mosquito was conducted.

MATERIALS AND METHODS

Population of *Ae. aegypti* mosquitoes infected intrathoracally[5] with Dengue-3 virus and incubated for 5 days, and for this purpose, laboratory colonies of *Ae. aegypti* were used. Dengue-3 virus in both groups were detected with immunocytochemistry assay of the head of each mosquitoes.

Head squash Smears were made from each head of the mosquito, and fixated with cold absolute methanol, and dried in room temperature. Dengue virus was identified by using immunocytochemical SBPC assay. Primary antibody used was monoclonal antibody DSSE10 with various dilution: 1:5, 1:10, 1:20, and 1:50. Control was made with mosquitoes infected with Dengue-3 intrathoracally with incubation period of 5 days, and Dengue-3 was detected with RT-PCR technique. This control smears was not given primary antibody. Result were examined with light microscope. Result of immunocytochemical SBPC assay in head squash was Dengue antigen positive when the smears had brown granules scattered between brain tissues; and the result was negative when the cytoplasm of brain cells was blue or pale, and the brown granules were not found around the brain cells.

RESULT AND DISCUSSION

Detection of Dengue antigen in head squash smears was initially developed by Rosen and Gubler (1974)[5] using Direct Fluorescence Antibody test (DFAT). Dengue antigen positive result was shown by fluorescent granules and greenish fluorescence in the cytoplasm of brain cells. Optimization test of antibody DSSE10 dilution to detect Dengue-3 virus in *Aedes aegypti* mosquito was conducted with immunocytochemical SBPC assay.

Figure 1-4 showed positive reaction in Dengue-3-infected *Ae. aegypti* smears with all monoclonal antibody concentrations. It suggested that Dengue virus-3 antigen in *Ae. aegypti* infected intrathoracally may be detected with immunocytochemistry using monoclonal antibody DSSE10, starting from 1:50 dilution. Meanwhile, there were no positive reactions in head squash Smears of *Ae. aegypti* not infected by Dengue-3 virus with all concentrations of monoclonal antibody DSSE10. Cell cytoplasms of brain tissue in uninfected mosquitoes were blue, and had no brown granules between cells.

Positive reaction in head squash Smears stained with immunocytochemical SBPC assay was shown by brown granules scattered around the infected cells. The more antigen in the head squash, the wider the brownish granules were scattered. These brown granules were appear because of a reaction between peroxidase enzyme which hydrolyzed chromogen substrate (DAB).

Immunocytochemistry was one of the methods used to detect antigen in cells. In immunocytochemical SBPC assay, the primary antibody was specific monoclonal antibody to the detected antigen. After the antigen was bound by primary antibody, monoclonal antibody would be bound by secondary antibody which recognized primary antibody. Biotin was used to label the secondary antibody. When

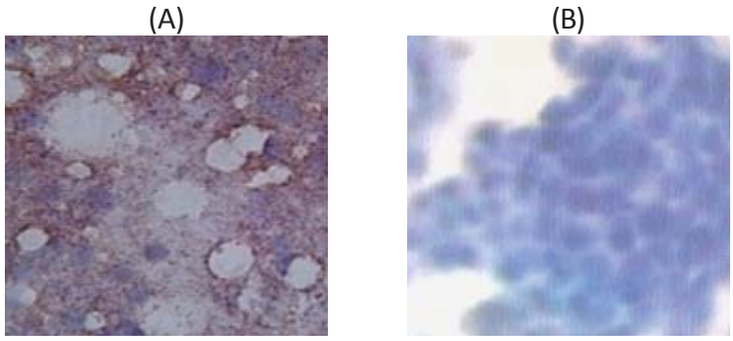

Figure 1. Immunocytochemistry result using DSSE10 antibody with 1:50 dilution. Positive reaction was shown in infectious mosquito (A) and negative reaction was shown in non-infectious mosquito (B).

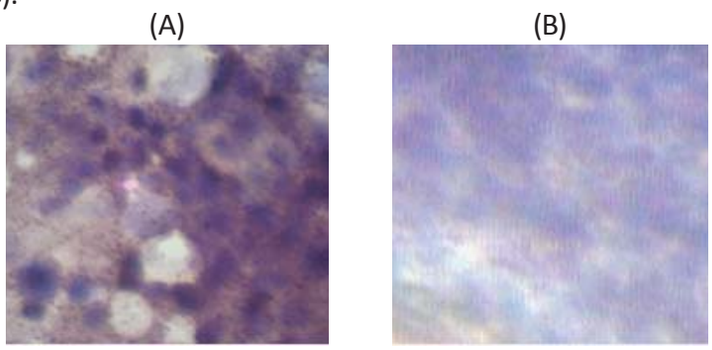

Figure 2. Immunocytochemistry result using DSSE10 antibody with 1:10 dilution. Positive reaction was shown in infectious mosquito (A) and negative reaction was shown in non-infectious mosquito (B).

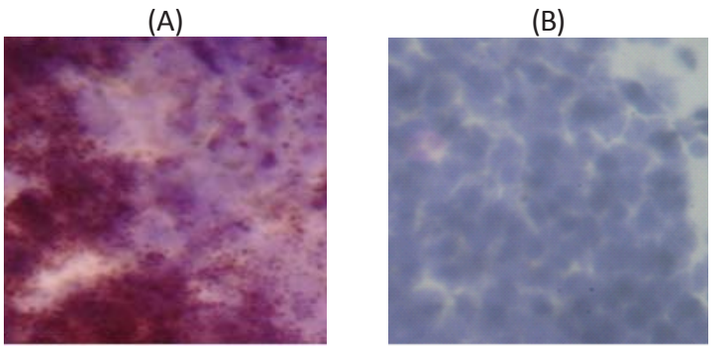

Figure 3. Immunocytochemistry result using DSSE10 antibody with 1:20 dilution. Positive reaction was shown in infectious mosquito (A) and negative reaction was shown in non-infectious mosquito (B).

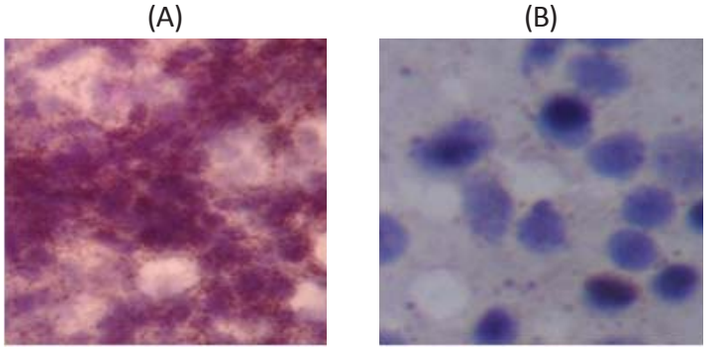

Figure 4. Immunocytochemistry result using DSSE10 antibody with 1:5 dilution. Positive reaction was shown in infectious mosquito (A) and negative reaction was shown in non-infectious mosquito (B).

streptavidin conjugate was added, it wil be bound by biotin. Streptavidin was labelled with horseradish peroxidase enzyme. This enzyme would hydrolyze the chromogen substrate (DAB). Therefore, smears which contained antigen would be stained brown[3].

In this study, Dengue-3 virus was detected with monoclonal antibody DSSE10 as primary antibody. Antibody DSSE10 is one of the specific monoclonal antibodies of Dengue produced by Dengue Team of UGM. Classification test with ELISA showed that it is included in Class IgG1 and IgM, and after several recloning of hibridoma cells, DSSE10 was included in class IgG1[5].

Monoclonal antibody DSSE10 in previous studies showed strong immunoreactivity to Dengue-1, Dengue-2, Dengue-3, and Dengue-4 viruses, and has shown no cross-reaction to Japanese Encephalitis and Chikungunya antigens, based on ELISA[5]. The result of this study showed that antibody DSSE10 might be used to detect mosquitoes infected with Dengue-3 virus intrathoracally with incubation period of 5 days with 1:50 dilution. Therefore, detection of Dengue-3 virus antigen in head squash smears of *Ae. aegypti* infected thoracally may be conducted with immunocytochemistry technique using primary antibody DSSE10, starting from 1:50 dilution.

Head squash smears of *Ae. aegypti* not infected with Dengue-3 virus showed pale cytoplasm, and there were no brown granules around the brain cells. It indicated that monoclonal antibody DSSE10 could not recognize the brain cells of the mosquitoes, so there were no possibility of false positive reaction in uninfected mosquitoes.

Examination with immunocytochemistry technique may produce false positive reaction, where the smears contains no antigens showes positive immunoreactivity. It may be caused by various factors, and one of them is endogenous peroxidase compounds, which produced by each cells in normal tissues[7]. In this study, effort has been made to minimize the reaction by adding peroxidase blocking solution to diminish the activity of endogenous peroxidase.

CONCLUSION

Monoclonal antibody DSSE10 may be used in immunochemistry technique to detect Dengue-3 virus antigen in *Aedes aegypti* infected intrathoracally, with 1:50 dilution. Monoclonal antibody DSSE10 did not recognize cells in brain tissues of uninfected mosquitoes.

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