Electroelution of 31 kDa Immunogenic Protein Fraction from the Salivary Gland of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae)

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Abstract. Dengue Hemorrhagic Fever (DHF) is a disease generated by dengue virus infection. The dengue virus is transmitted to the host by mosquito vectors. The main and secondary vectors are *Aedes aegypti* and *Aedes albopictus*. Dengue virus transmission is mediated by salivary gland proteins that facilitate the blood-feeding process. Previous studies have shown that the molecular weight of 31 kDa is an immunogenic protein of *Ae. aegypti* belonging to the D7 family based on the result of Mass Spectrometry. This immunogenic protein can be used for the development of vector-based dengue vaccines. The purpose of this study is to purify the 31 kDa protein fraction from the salivary gland of *Ae. aegypti* and *Ae. albopictus* using the electroelution method. The study is conducted by collecting the salivary glands of *Ae. aegypti* and *Ae. albopictus*, SDS-PAGE, electroelution using the Bio-Rad 422 electroeluter, Dot Blot, and Western Blot. Both protein samples resulting from electroelution confirmed with a single band appeared in SDS-PAGE. The optimal conditions for electroelution are 3 h running time, 100 voltage, volume ±1 mL, and the concentration obtained are 1.789 mg/mL (*Ae. aegypti*) and 1.81 mg/mL (*Ae. albopictus*). These results are supported by the other result of the dark dots shown in Dot Blot and a single band of 31 kDa shown in Western Blot when it reacted with the serum of dengue patients, endemic healthy people, and neonates. These results indicate that the purified 31 kDa immunogenic protein fraction can be recognized by specific antibodies.

Keywords: Dengue · Electroelution · Immunogenic protein · Purification

1 Introduction

Dengue Hemorrhagic Fever (DHF) is a disease caused by dengue virus infection and has caused a high number of serious cases in the world [1]. The number of dengue cases in the world reaches 390 million per year, with 96 million of them experiencing various acute severities [2]. Indonesia has also high dengue cases per year, especially in 2020. From early 2020 until July, Indonesia has reached 71,633 cases with a death rate of 459. Several provinces have the highest number of dengue cases, one of which is East Java

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with 5,948 cases [3]. Jember Regency is one of the dengue-endemic area in East Java with 389 cases of DHF [4]. The high number of dengue cases both in the world and in Indonesia is inseparable from the existence of *Aedes* sp. mosquito as a vector that mediates the transmission of the dengue virus [1].

Aedes sp. mosquitoes that act as the main vectors of the dengue virus are *Ae. aegypti* (primary vector) and *Ae. Albopictus* (secondary vector) [5, 6]. *Ae. aegypti* is the primary vector because it is anthropophilic while *Ae. albopictus* is zoophilic [7, 8]. These properties can change due to the adaptation process, especially for *Ae. albopictus* was originally a forest species but can adapt to human settlements because it was caused by habitat loss [7]. The vector *Ae. albopictus* has also high tolerance to temperatures, especially cold temperatures, even though its natural habitat has an optimal temperature ranging from 28–32 °C as another adaptation [9]. Anthropophilic traits in *Ae. aegypti* can increase the risk of transmission pathogens [10]. Transmission of the dengue virus to the host's body occurs through the process of blood-feeding which is assisted by proteins in the salivary glands of the vector [11].

Proteins in the salivary glands of *Ae. aegypti* and *Ae. albopictus* are important keys in the process of virus transmission to the host because it contains anti-hemostatic components that can prevent vasoconstriction, platelet aggregation, and coagulation [11, 12]. These components can prevent blood clots to facilitate the process of blood-feeding by vectors [13]. In addition, the salivary glands of these mosquitoes also contain an immunomodulatory component, which can stimulate the host's immunity when there is repeated exposure to saliva [14]. These salivary gland proteins can modulate the host immune system to be categorized as immunogenic proteins [15].

Based on the research of Oktarianti et al. [16] found that in the salivary glands of *Ae. aegypti* contains two immunogenic proteins, 31 and 56 kDa based on Mass Spectrometry analysis, which can modulate the immune response of the population living in dengue-endemic areas. The 31 kDa protein fraction is one of the candidates for the development of a vector-based DHF vaccine. The main component of the 31 kDa protein is the D7 family protein [17]. The D7 family protein in the salivary glands of arthropods has the most abundance [18]. This protein can inhibit the activity of biogenic amines that cause the host's vasoconstriction and platelet aggregation process to fail [19]. The immunogenic protein fraction needs to be analyzed further to develop a DHF vaccine. Further analysis related to the activity of the protein fraction it is necessary to perform protein purification [20].

The aim of this research is to purify the 31 kDa protein fraction of *Ae. aegypti* and *Ae. albopictus* with electroelution method. Pure protein from a protein fraction of 31 kDa can be obtained from electroelution process. Electroelution is a method that can separate and collect protein samples from SDS-PAGE gel bands with the help of an electric current. The tool used for electroelution is called electroeluter. The advantage of this tool is can minimize the protein samples loss, so the pure protein can be collected maximally [21].

2 Materials and Methods

This research was conducted from December 2020–June 2021. Located at the Biotechnology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember. Mosquito rearing activities were carried out at the Animal

Care Biology Unit, Faculty of Mathematics and Natural Sciences, University of Jember. Landing collection activities of *Ae. aegypti* and *Ae. albopictus* was carried out around the Jember University campus area and Jember Regency.

2.1 The Salivary Glands Isolation

Ae. aegypti and *Ae. albopictus* that had been collected, each was placed in a plastic cup and put in a freezer or ice box for 30 s until 1 min to immobilize mosquitoes. Subsequently, the mosquitoes were transferred into a petri dish, and the salivary glands were isolated using the microdissection method under a stereomicroscope [22]. The object glass is dripped with 0.5% NaCl then the mosquito is placed on it. The dissection needle is placed between the head and the thorax and then the thorax is pressed while the head is pulled out of the thorax. Salivary glands will be attached to the head or thorax [22]. The isolated salivary glands were collected in sterile microtubes containing 10 μL 1 mM PMSF in PBS pH 7.4 and stored at −20 °C. Storage of salivary glands was collected in as many as 10 pairs in one microtube.

2.2 Electroelution 31 kDa Fraction of *Ae. aegypti* and *Ae. albopictus*

Salivary gland proteins of *Ae. aegypti* and *Ae. albopictus* went through two SDS-PAGE steps, the first was to isolate the 31 kDa protein fraction and the second was to confirm the appearance of a single band of 31 kDa protein fraction after the electroelution stage. The SDS-PAGE method was used for collecting the 31 kDa protein fraction which consisted of 12% separating gel and 4% stacking gel. The SDS-PAGE analysis was using a 20 μL sample containing 10 μL PMSF 1 mM in PBS pH 7.4, 10 mosquito salivary glands, and 10 μL loading buffer. The sample was heated with a thermoshaker at 95 °C for 4 min, and loaded into a gel well and run for 60 min at 150 V in a buffer electrode pH 8.3.

The 31 kDa protein fractions which were isolated and collected by the SDS-PAGE method were purified by the electroelution method. The electroelution method used an electroeluter model 422 (Bio-Rad, USA). This method used membrane previously washed in elution buffer containing NaN_3 (Sodium azide) for 1 h at 60 °C. The silicone adapter was attached to the base of the glass tube and the bubbles were removed. The wet membrane was placed on the base of the silicone adapter and filled with ±400 mL elution buffer. The glass tube was inserted into the gromet and placed on the elution tube and then dripped with elution buffer gradually. Each glass tube was filled with pieces of protein ribbon to the brim. The lower chamber was filled with 600 mL of elution buffer which is more than the surface of the adapter while the upper chamber was filled with 100 mL.

The step of confirming the molecular weight of the target protein after electroelution was using SDS-PAGE The SDS-PAGE analysis was performed by inserting 25 μL of sample and 5 μL loading buffer into the gel well and running for 1 h 30 min 100 V in electrode buffer 1 x pH 8.3. The SDS-PAGE gel then was soaked in Coomassie Brilliant Blue staining solution overnight and continued with destaining for 15 min 3 times. A single band of the visible target protein with a molecular weight of 31 kDa indicates that the electroelution step was successful.

2.3 Dot Blot Analysis

Detection of the electroeluted 31 kDa immunogenic protein was carried out using the Dot Blot method by reacting the protein with specific antibodies. Dot Blot analysis is the first step or rapid screening to examine the presence of immunogenic proteins through the presence of bonds between antigens and antibodies [23]. The dot blot analysis was started by cutting the PVDF membrane with the size of 2.5 cm × 2.5 cm. The PVDF membrane was immersed in methanol for 1 min, followed by TBS pH 7.4 for 3 min, additionally, the membrane was air-dried. The membrane was dripped with 5 µL of the sample. The samples used were purified protein, positive control (total protein extract of *Ae. aegypti* and *Ae. albopictus* salivary glands), and negative control (PMSF in PBS pH 7.4). The membrane that had been dripped with samples was air-dried, then incubated in 5% skim milk in TBS pH 7.4 for 1 h on a shaker. The membranes were washed with 3 10 ml TBS pH 7.4 for 5 min each. After that, the membrane was incubated in a 5% skim milk solution in TBS pH 7.4 which had been added with the primary antibody in a ratio of 1:500 for 2 h at 4 °C in a dark room.

The primary antibodies used were pool serum from DHF patients, healthy people living in dengue-endemic areas, and neonates. Then the membrane was washed again with 3 × 10 ml TBS pH 7.4 for 5 min each. The membranes were incubated in a 5% skim milk solution in TBS pH 7.4 and added with a secondary antibody (anti-human IgG) in a ratio of 1:5000 for 2 h at room temperature on a shaker. The membrane was then washed again with 3 × 10 ml of TBS pH 7.4 for 5 min each. Visualization was carried out by giving 1 ml of NBT/BCIP for 5 min in a dark room to see the bond between purified protein and antibodies [20]. The results of the dot blot visualization were then analyzed using *ImageJ* software to determine the difference in the color density of the resulting dots.

2.4 Western Blot Analysis

Western Blot in this study was used to further confirm the molecular weight of the electroeluted immunogenic protein carried out by transferring proteins from polyacrylamide gels to PVDF membranes [24]. Western Blot analysis needs to be done because the Dot Blot analysis does not provide information on the size of the molecular weight of the protein fraction detected for its immunogenic character [25]. The Western Blot stage begins with separating proteins based on molecular weight using the SDS-PAGE method, protein transfer to the PVDF membrane, membrane blocking, primary antibody incubation, secondary antibody incubation, and detection [26].

The Western Blot process begins will immersing the PVDF membrane in methanol solution for 1 min, and then immersed in transfer buffer. After that, the tissue paper and filter paper were soaked in transfer buffer for ±10–15 min. Then a sandwich-like arrangement from the upside to the bottom side was made consisting of three sheets of tissue, one sheet of filter paper, polyacrylamide gel, PVDF membrane, one sheet of filter paper, and three sheets of tissue. Stacks between components should be tight and ensure that there are no bubbles. The Western Blot method in this study modified the composition of the western blot components by replacing the fiber pad or blotting paper with three sheets of tissue. This modification is based on the optimization that has been done previously.

The protein in the polyacrylamide gel resulting from SDS-PAGE was transferred to the PVDF membrane through an electric current of 100 mA (constant current) for 30 min. The membrane from the running Western Blot has then washed 3 × 10 ml with TBS pH 7.4 for 5 min each. The membrane was then soaked with 5% skim milk dissolved in TBS pH 7.4 for 60 min on a shaker. The membranes were washed with 3 × 10 ml of TBS pH 7.4 for 5 min each. Next, the membrane was immersed in a 5% skim milk solution in TBS pH 7.4 to which a primary antibody (human serum) was added in a ratio (1:250) and incubated overnight at 4 °C in the dark. In the next process, the membrane was washed with 3 × 10 ml TBS pH 7.4 for 5 min each. The membrane was then immersed in 5% skim milk solution in TBS pH 7.4 to which a secondary antibody (Human Anti-IgG) was added in a ratio (1:2500) and incubated for 2 h at room temperature on a shaker. The membranes were washed with 3 × 10 ml TBS pH 7.4 for 5 min each. Membrane staining was carried out by giving 1 mL of NBT/BCIP solution for 10 min in the dark. The membrane is then soaked with distilled water to stop the reaction. The next process is the observation of the appearance of immunogenic protein bands on the surface of the PVDF membrane.

3 Result

3.1 Salivary Gland of *Ae. aegypti* and *Ae. albopictus*

The mosquito salivary gland is a pair of organs located in the thorax [27]. Mosquito salivary gland of *Ae. aegypti* and *Ae. albopictus* was obtained through the isolation process using the microdissection method [22]. The mosquito's salivary gland was isolated from the female mosquito, which the salivary gland of the female mosquito consists of immunogenic proteins that help the process of blood-feeding and transmission of pathogens by vectors [28]. The salivary glands of *Aedes* sp. it has two parts, each part consists of a median lobe and two lateral lobes. The two lobes are connected by two salivary ducts [29] as shown in Fig. 1 and also, can be seen the comparison between *Ae. aegypti* and *Ae. albopictus* salivary gland with references. The salivary duct consists of a common duct, two main ducts, and an internal duct [30]. Each lobe consists of two parts, namely proximal (close to the duct) and distal (at the end of the lobe).

Each lobe of the salivary glands of *Aedes* sp. secretes a variety of proteins. Proteins secreted by the salivary glands play a role in the blood-feeding process. The lateral lobes secrete apyrase while the proximal lateral lobes secrete alpha-glucosidase. The median lobe secretes important proteins such as the D7 family, lectin-binding protein, and apyrase [29, 31].

3.2 Salivary Gland from *Ae. aegypti* and *Ae. albopictus*

The salivary gland of *Ae. aegypti* that have been isolated were analyzed using the SDS-PAGE method. This method is for separating proteins based on their molecular weight [32]. The results of the analysis of the salivary glands of *Ae. aegypti* showed the presence

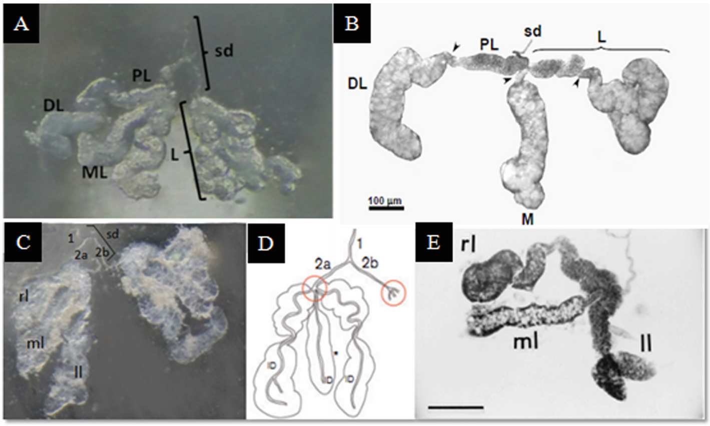


Fig. 1. (A) The salivary gland of *Ae. aegypti*; (B) the salivary gland of *Ae. aegypti* [29] (C) the salivary gland of *Ae. albopictus*; (D) the salivary glands of *Ae. albopictus* [30]; (E) the salivary gland of *Ae. albopictus* [31]; salivary duct (sd), common duct (1), main duct (2a and 2b), right lateral lobe (rl), median lobe (ml), left lateral lobe (ll), proximal lobe (pl), distal lobe (dl), and lateral lobe (l). (A) and (C) are the salivary gland pictures from this research; (B), (D), and (E) are the salivary gland pictures from the references.

of several protein bands with molecular weights around 103, 91, 88, 75, 71, 68, 64, 60, 58, 53, 48, 46, 44, 32, 31, 29, 27, 25 and 14 kDa in Fig. 2. The purpose of this protein separation is to facilitate the process of isolating the target protein, 31 kDa. The 31 kDa protein isolation process was carried out aseptically using a disposable blade. Based on the research of Oktarianti et al. [16] 31 and 56 kDa proteins from the salivary glands of *Ae. aegypti* are immunogenic protein because it is able to modulate the immune response of people living in dengue-endemic areas.

Protein with a molecular weight of 31 kDa was identified as containing 13 types of protein and protein D7 was the main component of this protein fraction [17]. The D7 protein in the Arthropod salivary glands was the most abundant compared to other proteins [33] and in the salivary glands of *Ae. aegypti* protein D7 is located in the distal lateral and medial lobes [27]. The D7 protein can bind to biogenic amines such as serotonin, histamine, and norepinephrine. Biogenic amines play an important role in host hemostasis [18], so when biogenic amines are bound, platelet aggregation and vasoconstriction are inhibited which makes mosquitoes easy to do blood-feeding and transmit pathogens [34].

Salivary gland proteins *Ae. albopictus* were separated based on their molecular weight by SDS-PAGE analysis. Some of the proteins that were separated were 16 bands with molecular weights of 119, 107, 96, 74, 67, 57, 54, 48, 31, 30, 28, 27, 23, 14, 13, and 12 kDa in Fig. 2. The 31 kDa protein band was collected aseptically with a disposable blade in a laminar airflow cabinet (LAF) to avoid contamination to the gel [35].

Salivary gland protein fraction *Ae. albopictus* with a molecular weight of 31 kDa is suspected that the D7 family protein. The D7 family is the most abundant in the salivary glands of hematophagous arthropods, including mosquitoes [18]. The D7 family protein has a function as an antihemostatic and anti-inflammatory which will inhibit platelet

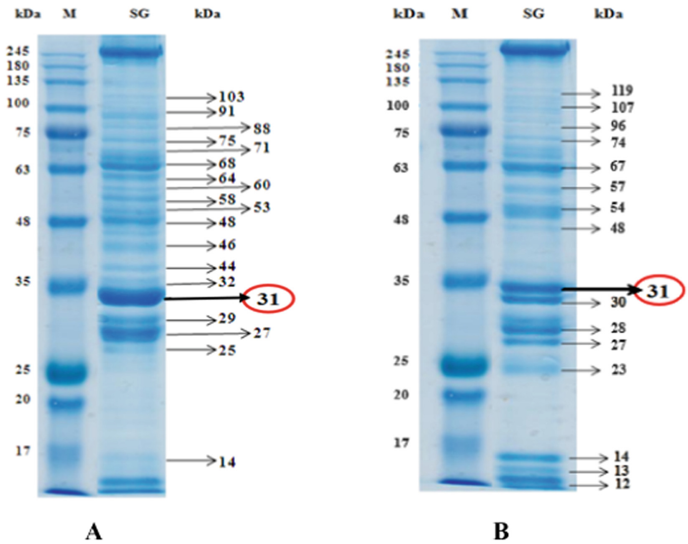


Fig. 2. The SDS-PAGE protein separation results of the total salivary gland extract (A) *Ae. aegypti* and (B) *Ae. albopictus* (Scanner Canon MP287). (M) Marker blueEye prestained protein ladder, and (SG) salivary gland.

aggregation from the host's body and facilitate the mosquito blood-feeding process. The function of the D7 family protein is for ligand binding to the N-terminal and C-terminal domains [18]. The binding of this ligand has functioned as antihemostatic with the mechanism that the N-terminal domain binds to cysteinyl leukotrienes and the C-terminal domain binds to biogenic amines and eicosanoids [18, 34].

3.3 Electroelution 31 kDa Salivary Gland of *Ae. aegypti* and *Ae. albopictus*

Purification of the 31 kDa protein fraction was carried out by the electroelution method which aims to remove proteins from polyacrylamide gels with the help of electric voltage [36, 37]. The electroelution process in this study was carried out with help an electroeluter.

This tool has several advantages, a shorter time, avoids contaminants, does not require expensive reagents, and produces a good recovery rate of protein [21, 38]. Protein recovery is affected by several factors such as the physicochemical properties of the protein, time and temperature, and the amount of purified protein [39].

The electroelution process was carried out by inserting 100 pieces of 31 kDa protein bands. Firstly, a glass tube had been assembled with frit, silicon adapter, and membrane. The protein pieces pun into the inside of a glass tube with elution buffer. The protein in the glass tube will be eluted out of the gel through the frit and will be collected on the membrane. The membrane in the electroeluter has pores of 12–15 kDa so that proteins with a molecular weight larger than the pores cannot exit the membrane [38]. The confirmation of 31 kDa is in Fig. 3.

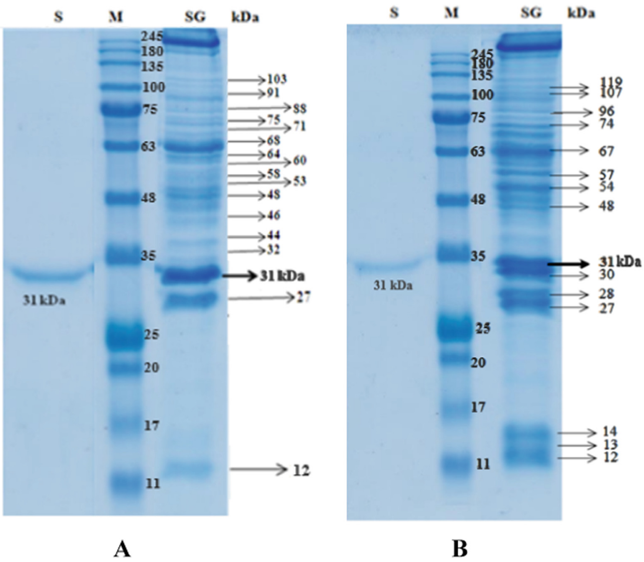


Fig. 3. The SDS-PAGE purification confirmation results of 31 kDa protein fraction (A) *Ae. aegypti* and (B) *Ae. albopictus* (Scanner Canon MP287). (S) protein sample, (M) marker blueEye prestained protein ladder, and (SG) salivary gland.

The results of electroelution optimization for the 31 kDa protein fraction from the salivary glands of *Ae. aegypti* and *Ae. albopictus* can be carried out at room temperature with a voltage of 100 V (constant volt) for 3 h. The final results were obtained ±1 mL of the target protein with a concentration of 1.789 mg/mL for *Ae. aegypti* and 1.81 mg/mL for *Ae. albopictus*. Purification of 31 kDa *Ae. aegypti* and *Ae. albopictus* protein fraction by electroelution with electroeluter requires ± 3 h.

3.4 Dot Blot Analysis

Dot Blot visualization showed that the purified 31 kDa protein sample was immunogenic because it could be recognized by antibodies from the pooled serum of DHF patients, the serum pool of endemic healthy people, and the pool of neonate serum which was marked by the appearance of dark-colored spots on the membrane. Dark spots on the membrane indicate the presence of bonds between antigen and antibody [20]. The intensity of the dot color on the membrane shows how strong the binding of the antigen and antibody [40]. Positive control in the form of total protein extract of *Ae. aegypti* and *Ae. albopictus* showed a positive reaction, the results obtained by following the research of Oktarianti et al. [16] showed that the salivary glands of *Ae. aegypti* contains immunogenic proteins. The negative control (PMSF in PBS pH 7.4) showed a negative reaction. The results of the positive and negative controls prove that the work method is correct. The result of Dot Blot analysis is shown in Table 1.

Based on the visualization of the Dot Blot results, it can be seen that the purified 31 kDa *Ae. aegypti* sample reacted with the serum pool of DHF patients, endemic healthy people, and neonates had different color densities. The purified 31 kDa sample reacted with the serum pool of endemic healthy people and had the highest dot density as indicated by the largest percentage value of 27.48%. The 31 kDa sample reacted with the serum pool of DHF patients and showed a percentage value of 23.31%. The 31 kDa sample which was reacted with the neonatal serum pool had the lowest dot density compared to the sample reacted by the serum pool of DHF patients and endemic healthy people with a percentage value of 12.44%. The difference in color density of the dot indicates the difference in the strength of the bond between the antigen and antibody. According to Sukarjati et al. [41] and Wathon et al. [20] the thicker color of the dot is the greater concentration in the reaction between antigen and antibody.

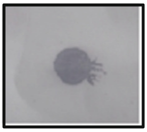
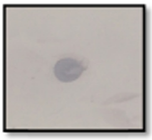
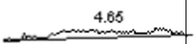
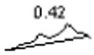
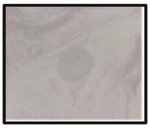
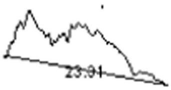
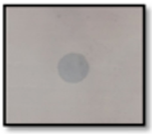
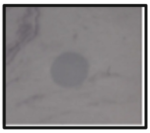
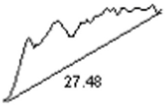
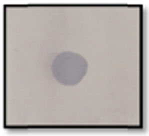
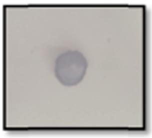
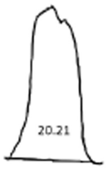
The results of the *Ae. albopictus* Dot Blot analysis showed qualitative visualization with the appearance of black dots on the PVDF membrane. The density of the spot's blackness on the PVDF membrane indicates the high or low bond between antigen and antibody which can be measured by image processing software using *ImageJ* as shown in Table 1. The data obtained are semi-quantitative in the form of graphs and numbers [42]. The *ImageJ* graph of the purified 31 kDa protein fraction in the positive control had a percentage of 27.61%, while the negative control was 0.42%. The graphic results of the purified 31 kDa protein fraction reacted with the serum of endemic healthy people were the widest among the other serum sample with a percentage of 28.54%. The graph of the 31 kDa protein fraction reacted with serum from DHF patients was 23.22% and the lowest was reacted with neonate serum, which was 20.21%.

3.5 Western Blot Analysis

Based on the dot blot and western blot analysis that has been done, it is known that the purified 31 kDa protein can be recognized by the serum of endemic healthy people, the serum of DHF patients, and the serum of neonates. The purified 31 kDa protein can be recognized by the serum pool of endemic healthy people because these people who live in dengue-endemic areas have specific antibodies to the salivary protein *Ae. aegypti*. Based on research conducted by Doucoure et al. [43] and Londono-Renteria et al. [44] has proven that people who live in dengue-endemic areas have natural antibodies against *Ae. Aegypti*. The study also showed the endemic people had high concentrations of IgG against the salivary protein of *Ae. aegypti*. The antibodies were probably due to frequent exposure to vector saliva. This agrees with the statement of Zabriskie [45] that repeated exposure to the same antigen will trigger the formation of a secondary immune response and can produce higher concentrations of IgG in a short time. A positive result of a purified sample of 31 kDa *Ae. aegypti* from endemic healthy serum according to research by Oktarianti et al. [16] showed that proteins with a molecular weight of 31 kDa and 56 kDa from the salivary glands of *Ae. aegypti* is immunogenic and can modulate the immune response of people living in endemic areas. The figure of Western Blot analysis results is shown in Fig. 4.

The purified 31 kDa protein can also be recognized by antibodies from the serum pool of DHF patients, but the band in Western Blot results are very thin. According to Mahmood and Yang [46], the band on the PVDF membrane that is less clear in the Western Blot results can be caused by the concentration of used primary and secondary antibodies being too low and the antibody conditions being deficient in quality. This

Table 1. Dot blot visualization results of the purified 31 kDa fraction of immunogenic protein from the salivary gland of *Ae. aegypti* and *Ae. albopictus*

Sample/ Treatment	Visualization of *Dot Bot** (*Ae. aegypti*)	*ImageJ* (*Ae. aegypti*)	Visualization of *Dot Blot*** (*Ae. albopictus*)	*ImageJ* (*Ae. albopictus*)
Positive control (total protein extract of *Ae. aegypti* salivary glands) reacted with the serum pool of endemic healthy people and *Ae. albopictus* reacted with DHF patient				
Negative control (PMSF in PBS) reacted with the serum pool of endemic healthy people (*Ae. aegypti*) and with DHF patients (*Ae. albopictus*)				
Purified 31 kDa reacted with a pool of DHF patient serum				
Purified 31 kDa reacted with a pool of endemic healthy people's serum				
Purified 31 kDa reacted with a pool of neonate serum				

*Canon EOS 1300D camera **Sony ILCE-5100 camera

agrees with the statement of Ghosh et al. [47] that the concentration of primary antibodies has an influence on the signal intensity of western blot results.

Positive results from the neonate serum pool in dot blot and western blot analysis were caused by the presence of antibodies that the baby had passively obtained from the mother (maternal antibodies) who had been exposed to *Ae. aegypti* previously in endemic areas [48]. The results of the positive control (crude salivary gland extract *Ae. aegypti*) in western blot analysis showed 2 immunogenic protein bands 31 and 56 kDa.

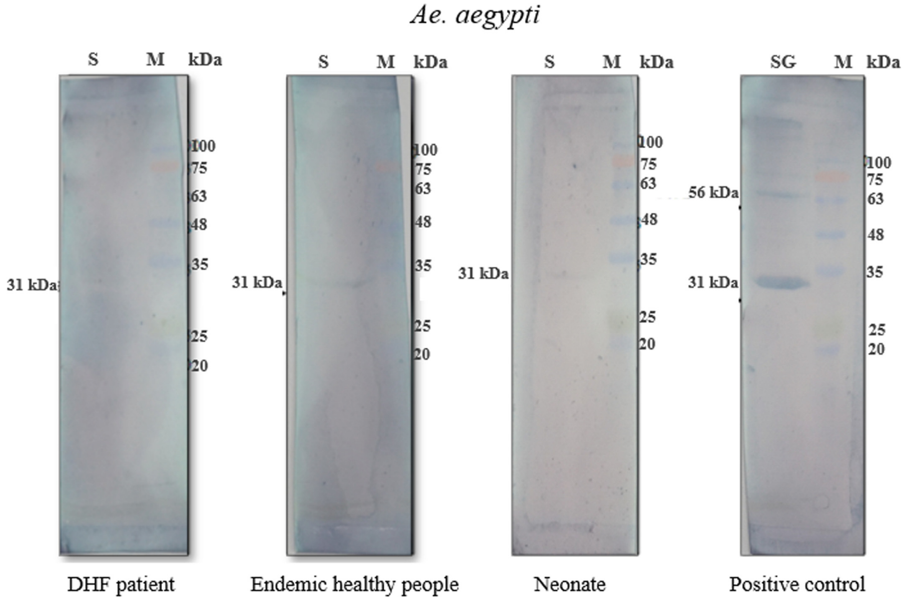


Fig. 4. Western blot analysis visualization results of *Ae. aegypti* (Canon EOS 1300D camera). (S) Protein sample, and (M) marker bluEye prestained protein ladder.

This is in accordance with the research results of Oktarianti et al. [16] that 31 and 56 kDa proteins are immunogenic proteins.

Western Blot result on purified 31 kDa of *Ae. albopictus* did not show any band. A possible factor that can affect this result is the efficiency of protein transfer from the polyacrylamide gel to the PVDF membrane. The protein transfer that occurs has the possibility that only a portion of the purified 31 kDa protein fraction is transferred from the polyacrylamide gel to the PVDF membrane, causing the blood serum antibodies of DHF patients and endemic healthy people unable to recognize the antigen. According to Wathon et al. [49], several factors can influence the success of Western Blot, one of which is the transfer efficiency of polyacrylamide gel proteins to the PVDF membrane.

The 31 kDa protein fraction from the salivary glands of *Ae. aegypti* and *Ae. albopictus* was successfully purified by the electroelution method using an electroeluter at a voltage of 100 V for 3 h. Electroelution results obtained pure protein ±1 ml with a concentration of 1.789 mg/mL (*Ae. aegypti*) and 1,81 mg/mL (*Ae. albopictus*). The success of purification was confirmed using the SDS-PAGE method and showed the appearance of a single band of purified 31 kDa protein. The results of dot blot and western blot analysis showed a cross-reaction between the purified 31 kDa protein and antibodies from the serum of endemic residents, DHF patients, and neonates. This indicated the immunogenic characteristics of the target protein.

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