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Advances in Biological Sciences Research

Asmoro Lelono · Muhammad Akbar Bahar · Syubanul Wathon · Kartika Senjarini · Asep Ginanjar Arip · Ramdhan Putrasetya · Beny Andika · Nadhea Ayu Sukma · Bambang Sugiharto *Editors*

# Proceedings of the 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021) · Volume 27

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# Preface ICOLIB 2021

I am pleased to welcome all of the speakers and participants to the 2021 International Conference on Life Sciences and Biotechnology (4th ICOLIB), which is held from 15 to 16 November 2021, virtually on Zoom meeting. The conference is organized by the Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Jember.

This year the conference's theme is "Towards Sustainable Development: Application of Biosciences to Improve Welfare and Quality of Life". Along with the theme, we have four conference topics; there are Applied Sciences (Agriculture, Biotechnology and Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity and Bio-conservation, and Health and Medicine (Pharmacy and Medical Sciences). This scientific event provides a platform for researchers, academics, professionals, industries, and policymakers to exchange ideas, share the recent advances and development in life sciences, and can be a valuable place for starting fruitful collaboration, especially in uncovering the potential of biodiversity at the molecular level to biosphere.

This year's conference is also the first time held online due to the global pandemic situation. However, it becomes a blessing in disguise, because the conference becomes accessible to a wider audience and participants from all over the world. The number of participants registered is 223, among them, 170 participants will present their research.

Most of the participants are from Indonesia but also we have participants from Malaysia, Czech Republic, China, Philippines, Pakistan, and India. Besides the participants, the online of this conference also allows us to invite speakers from Netherlands, Germany, USA, and Australia. There will be Prof. A.G.G (Ton) Groothuis from GELIFES Institute, Groningen University Netherlands, Prof Antonius Suwanto from IPB Indonesia, Prof Simon Griffith from Department of Biological Sciences at Macquarie University, Sydney, Dr. Jorge A. Santiago-Blay from the Department of Paleobiology MRC-121 National Museum of Natural History Smithsonian Institution, USA, Prof. Elvira Hoerandl from George-August, Goothingen University Germany, Dr. Kahar Muzakar from Biology Department, Jember University, and Dr. Christina Bauch from Instituto Universitario in Lisbon, Portugal and Groningen University, Netherlands.

The output of this conference will be published in the Atlantis Press Proceeding: Part of Nature in series of "Advances in Biological Sciences Research", the Journal of ILMU Dasar MIPA, and the Journal of Tropical Biodiversity and Biotechnology. Finally, I would like to acknowledge the Board of Jember University which supported this conference. And I also like to thank to Vanadia and DAAD as one of the main sponsors which make this conference possible.

I hope this conference will be fruitful for everyone. We look forward to seeing you all at the next ICOLIB conference.

Asmoro Lelono
Chairman of 4th ICOLIB 2021

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# Peer-Review Statements

Bambang Sugiharto[1,2(✉)], Asmoro Lelono[1,3], Muhammad Akbar Bahar[4,5], Syubanul Wathon[1], Asep Ginanjar Arip[6], Kartika Senjarini[1], Ramdhan Putrasetya[1], Beny Andika[1], and Nadhea Ayu Sukma[1]

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AQ1 All of the articles in this proceedings volume have been presented at the International Conference on Life Science and Biotechnology (ICOLIB) on November 15–16, 2021 at Jember University Indonesia. These articles have been peer-reviewed by the members of the Scientific Committee of ICOLIB and approved by the Editor-in-Chief, who affirms AQ2 that this document is a truthful description of the conference's review process.

## 1 Review Procedure

The reviews were double-blind. Each submission was examined by two reviewer(s) independently. The conference submission management system was easy chair.

We divided the submission of the ICOLIB participant into two categories, the first is abstract and the second the full manuscript submission. The submissions of the abstract were first screened for generic quality, relatedness to the main topic and suitableness by the editorial team. Based on this initial screening, all of the abstracts would be classified by the main theme, i.e. Applied Sciences (Agriculture, Biotechnology & Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity & Bioconservation, Health & Medicine (Pharmacy & Medical Sciences). All of the selected abstracts would be presented by their author during the ICOLIB conferences. The committee gave an option to the authors to publish their manuscripts or just presented their work in the ICOLIB conference.

All of the complete manuscripts then follow the review process, the first step was to evaluate the relatedness to the proceedings series "Advances in Biological Sciences Research", scientific quality, novelty and contribution to the science. The second step is checking the similarity using Turnitin to evaluate the textual overlap and detect the possible sign of plagiarism. The third step was to send for peer review by matching each

B. Sugiharto—Editors-in-Chief of the ICOLIB.


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paper's topic with the reviewers' expertise, taking into account any competing interests. However, in some case, we also sent the manuscript to the third reviewer to consider another opinion if the first two reviewers has an opposite decision. A paper could only be considered for acceptance if it had received favourable comments and suggestions from the two reviewers. The recommendations then sent back to the author to address the reviewer's comment. The acceptance or rejection of a revised manuscript was final. In the final steps, all of the manuscripts were adjusted in their layout and some of the technical editing for the pre-print version. This preprint document would be sent to the author for clarification. They also should be sent a statement of the novelty and originality of the study.

## 2 Quality Criteria

Reviewers were instructed to assess the quality of submissions solely based on the academic merit of their content along the following dimensions. The editorial gave a rubric for a guideline which contains some important aspect related to the quality of the manuscript such as:

1. Pertinence of the article's content to the scope and themes of the conference;
2. Clear demonstration of originality, novelty, and timeliness of the research;
3. Soundness of the methods, analyses, and results;
4. Adherence to the ethical standards and codes of conduct relevant to the research field;
5. Clarity, style, cohesion, and accuracy in language and other modes of expression, including figures and tables.

We have a policy that each manuscript should be reviewed by two reviewers and each reviewer only reviews two manuscripts. The consequences of this policy is that we contact more reviewers, in total we ask 61 reviewer for completing the review process.

## 3 Key Metrics

| | |
|---|---|
| Total submissions | 118 |
| Number of articles sent for peer review | 67 |
| Number of accepted articles | 61. |
| Acceptance rate | 51.5% |
| Number of reviewers | 61 |

## 4 Competing Interests

Neither the Editor-in-Chief nor any member of the Scientific Committee declares any competing interest.

# In Vitro Analysis of Human IgG Immune Response Against 31 kDa and 67 kDa Immunogenic Protein from Aedes albopictus Salivary Glands

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**Abstract.** Mosquito salivary glands contain protein substances that can facilitate dengue virus transmission. Those proteins have abilities to induce an immune response and specific antibody production. Previous studies showed that 31 kDa and 67 kDa protein fractions from *Aedes albopictus* salivary glands are immunogenic protein. They can be recognized by the human IgG antibodies of endemic residents from Jember Regency by the Western Blot analysis. However, the IgG levels are not known quantitatively. Therefore, this study is expected to decide the level of IgG as a human humoral immune response against 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands. We separated salivary gland proteins using SDS-PAGE and continued using the electroelution method to get purified protein fraction. These proteins then cross-reacted with human blood serum using Indirect ELISA. The results showed that the IgG level against 31 kDa protein fraction based on the absorbance value in the healthy human population, DHF patients, and neonates were 0.229, 0.198, 0.151 respectively in individual serum and 0.229, 0.198, 0.151 in pool serum. While the IgG level against 67 kDa protein fraction based on the absorbance values in the individual serum of healthy human population, DHF patients, and neonates were 0.4293, 0.3159, 0.2997 respectively and 0.410, 0.096, 0.037 in pool serum. Those results indicate that the IgG antibodies of each population group can well recognize the 31 kDa and 67 kDa protein fractions. The highest levels of IgG found in healthy residents, followed by DHF patients, and neonates.

**Keywords:** Absorbance · IgG · Immunogenic · Protein

## 1 Introduction

Dengue Haemorrhagic Fever (DHF) is an infectious disease which is brought about by 4 serotypes of dengue virus (DENV) [1]. This infection in humans occurs through transmission cycle between humans and *Aedes aegypti* mosquito as the primary vector or *Aedes albopictus* mosquito as the secondary vector [2, 3]. *Ae. Albopictus* is known


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has ability to transmit 4 dengue virus serotypes (DENV-1 to DENV-4) [4]. Recent study has shown that ability of *Ae. Albopictus* to transmit dengue virus is as high as *Ae. Aegypti* [5]. It can be a dangerous health threat for human in the world because this species is invasive. They can adapt to tropical and subtropical climates which make them spread to several continents including Asia, America, Africa, and Europe [6]. The cases of this disease in various countries usually arise due to the invasion of *Ae. Albopictus* and the entry of people infected with the dengue virus [3].

Transmission of dengue virus by vectors into the human body occurs through the salivary glands in the blood feeding process [7]. Mosquito salivary glands contain protein substances which has several functions for blood feeding process and also facilitate the DENV transmission. These functions are anticoagulation, anti-inflammatory, and vasodilation [8]. They can also induce host cellular immunity and production of specific antibodies [9].

Previous research showed that there were several immunogenic protein fractions from *Ae. Albopictus* salivary glands. Those fractions are 31 kDa, 47 kDa, and 67 kDa [10]. 31 kDa protein fraction from *Ae. Aegypti* salivary glands has D7 protein as the main component, while the 67 kDa protein in previous study which has similar molecular weight range has Apyrase as the main component [11]. D7 is one of the Odorant-binding Protein (ODP) superfamily member that is found in the salivary glands of hematophagous arthropods [12]. This protein is able to inhibit the activity of biogenic amines such as leukotrienes, histamine, and serotonin that play a role in vasoconstriction process [13] and fight host inflammatory response by reducing monocytes and macrophages activation [12]. Apyrase protein plays a role in the antiplatelet aggregation process [14, 15].

Immunogenic proteins can elicit an adaptive immune response that triggers the production of antibodies against salivary components [16]. Exposure to salivary immunogenic proteins will change the host immune response from a subset of Th1 cells to a subset of Th2 cells so the Th2 cells can induce B cells to differentiate into plasma B cells and produce specific antibodies such as IgG [17]. 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands can be recognized by human serum IgG of endemic residents from Jember Regency [10]. However, the level of protein fraction immunogenicity is not yet known quantitatively through analysis of IgG levels. Therefore, this study aims to analyze IgG levels in vitro as human humoral immune response in recognizing 31 kDa and 67 kDa protein fractions from of *Ae. Albopictus* salivary glands.

## 2 Materials and Methods

### 2.1 Species Identification and Salivary Gland Dissection

The mosquitoes used in this study were female *Ae. Albopictus* who has done blood feeding process [8]. Morphological identification of *Ae. Albopictus* was based on Zootaxa 598 book [19]. *Ae. Albopictus* has morphological characteristics of a thorax with a straight white scale line called medial longitudinal. Mesepimeron forming an inseparable V letter scale, and the middle femur of *Ae. Albopictus* has dark color without a white line scale. Identification of male and female mosquitoes can be seen from their palps and

antennae the structure. The female mosquito has an antennae structure with few hairs and the mosquito palps are shorter than the proboscis.

Mosquito salivary glands of female *Ae. Albopictus* were performed using microdissection method [20]. Mosquitoes were placed in a refrigerator at 20 °C for 30 s to weaken their movement. ± 50 l of 0.5% NaCl solution was dropped on a glass object of stereo microscope. The salivary glands obtained were then put into a microtube containing a solution of Phenyl Methyl Sulfonyl Fluoride (PMSF) (Sigma, USA) in PBS and then stored at 20 °C until it is used.

### 2.2 Protein Extraction and Separation by SDS-PAGE Analysis

10 µl of salivary gland sample was added with 10 µl of loading buffer (Tris HCl, 50% glycerol, 10% SDS, β-mercaptoethanol, and 1% bromophenol blue). It was incubated in a thermoshaker at 95 °C for 4 min. 20 µl sample and 5 µl protein marker (GangNam-STAIN Prestained Protein Leader, iNtRON, Korea) were put into acrylamide gel (12% separating, 4% stacking) in SDS-PAGE running machine. The electrophoresis process was running at a constant voltage at 150 V for 60 min at room temperature in a running buffer (Tris base, Glycin, SDS 0.1%). The electrophoresis gel was stained using a staining dye solution (Comassie Brilliant Blue, methanol, glacial acetic acid, aquadest) for 60 min and continued with the destaining process using destaining dye solution (methanol, glacial acetic acid, aquadest) 3 times for 15 min each step. The protein bands of 31 kDa and 67 kDa in the SDS-PAGE gel were cut and store into the electrode buffer (Tris base, Glycin, SDS 0.1%) pH 8.3 at 4 °C.

### 2.3 Purification of 31 kDa and 67 kDa Protein Fraction

Purification of 31 kDa and 67 kDa protein fractions was carried out using an Electroeluter 422 (Biorad, USA). The cap membrane was soaked with elution buffer (Tris base, Glycin, SDS 0.1%) for 1 h at 60 °C. Fritz membrane, glass tube, and silicon adapter were immersed in elution buffer at room temperature. The component was attached to the adapter then the adapter is filled with elution buffer. The gel piece was inserted into the tube containing the elution buffer and the module is inserted into buffer chamber. Proteins were electroeluted for 3 h at a constant voltage 100 V at room temperature. Protein concentration was measured by Bradford (Sigma, USA) Microplate Assay method at a wavelength of 595 nm.

### 2.4 Blood Sera Collection

30 sera samples were taken from healthy people (20–30 years old), infant (neonates) and DHF patients living in Jember, East Java, a region within the endemic level for dengue. We used neonates because they had not received exposure from salivary glands protein of *Ae. Albopictus*. So, this is for comparison to adult serum (DHF patients and Healthy resident) which was assumed that the IgG levels of neonates group were low. All participants gave informed consent and were asked for an agreement to take part in the study. The serum sample collection protocol was approved by the Ethical Committee Number 1034/UN25.8/KEPK/DL/2020 from the Medical Faculty of the University of Jember-Indonesia.

### 2.5 IgG Quantification by ELISA Analysis

The IgG levels were measured by indirect ELISA method. We used this method because this method has high sensitivity and specificity in recognizing antigens. Elisa indirect also uses signal-specific secondary antibodies to detect the presence of the desired specific antibody in the sample being tested. 31 kDa and 67 kDa protein fraction extracts from *Ae. Albopictus* salivary gland were diluted in 0.1 M bicarbonate buffer (pH 9.6). The 96-well ELISA plates well (Corning, USA) were coated with 4 µg/ml 31 kDa and 67 kDa protein (50 µL/well) overnight at 4 °C. The microplate wells were washed 3 times with 250 µl of PBST (PBS, pH 7.4; 0.05% Tween-20S). The wells were blocked with 1% BSA in PBST for 2 h at 37 °C then washed it 3 times with 250 µl PBST. Serum was diluted 1:1000 and coated 50 µl dilution serum into microplate wells with 2 h incubation at 37 °C. It washed for 3 times with 250 µl PBST. The wells then coated with 50 µl Horse Radish Peroxidase (HRP) enzyme and conjugated Rabbit anti-Human IgG (Rockland, USA) (1:5000) for 1 h at 37 °C. It washed 3 times with 250 µl PBST. 50 µl TMB substrate (Rockland, USA) were coated into wells in the dark and incubated for 30 min. The enzymatic activity was stopped by 50 µl H2SO4 1 M. The sample absorbances were read with ELISA reader at 450 nm.

### 2.6 Statistical Analysis

We tested the normality and homogeneity of the data using SPSS16 as preliminary test. The absorbance value then analyzed using Independent T Test to determine the significant difference in IgG levels from each group. The absorbance value was interpreted to be same as IgG level that was successfully bound to the 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands.

## 3 Result and Discussion

### 3.1 Morphology of Ae. Albopictus and Salivary Gland Structure

The morphology of *Ae. Albopictus* can be seen in Fig. 1A. *Ae. Albopictus* adults have a black body with white spots on the legs. It has black with white basal scales on each tarsal segment in front, midle, and hind legs. Macroscopically the morphology looks similar to *Ae. Aegypti.* These similar morphological differences often lead people to incorrect identification and make wrong assumption about those two species. However, their differences from *Ae. Aegypti* can be seen in the pattern of the scutum on the dorsal side of the thorax, the mesepimeron scales on the lateral side of the thorax, and the scales on the femur of the middle leg. Scutum morphology of *Ae. Albopictus* shown in Fig. 1B. *Ae. Albopictus* has scales resembling a straight white line in the middle of the scutum. The scale pattern in the mesepimeron part of Fig. 1C has a white colour and it connected each other like the V letter. In addition, the scales on the femur of the middle leg of *Ae. Albopictus* in Fig. 1D shows that it is entirely black.

Morphological differences by sex can be seen in Fig. 2A. Both male and female *Ae. Albopictus* has some different of characteristics. The observations result showed that male *Ae. Albopictus* has long antennae with many hairs and the palps are almost the

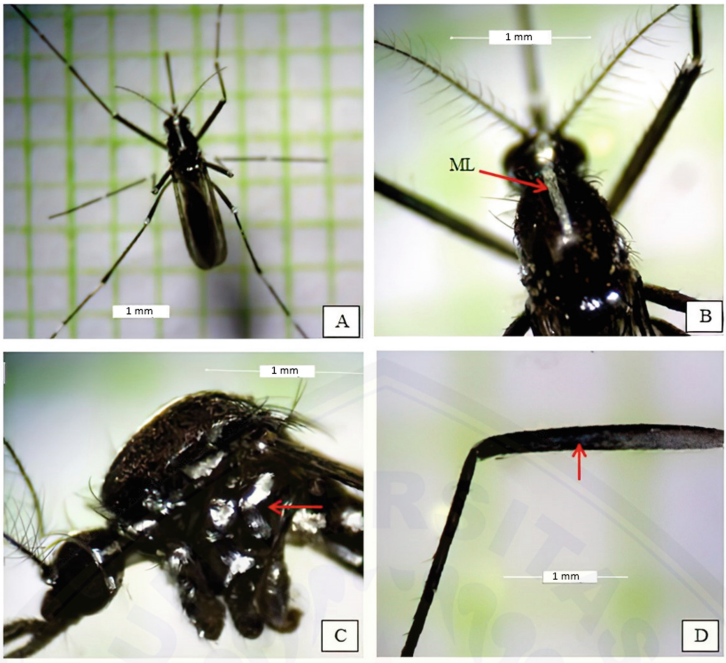


**Fig. 1.** Morphology of *Ae. Albopictus.* (A) The whole body at 10x magnification; (B) Scutum, ML (Median Longitudinal) line, 30x magnification; (C) Mesepimerone 40x magnification; (D) Anterior midfoot femur,30x magnification (Nikon SMZ735 Microscope. Optilab Camera).

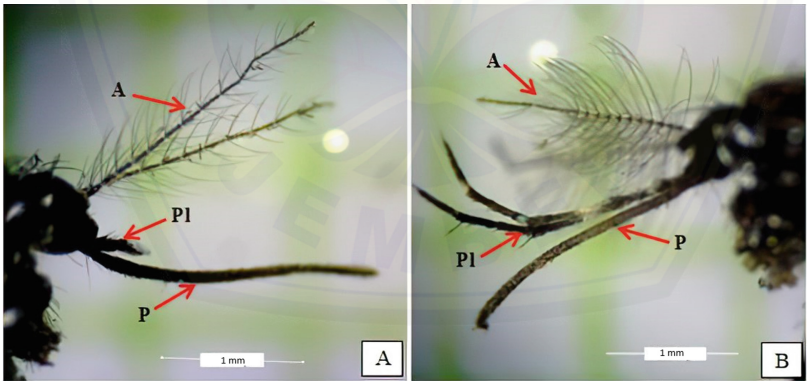


**Fig. 2.** The difference between female (A) and male (B) *Ae. Albopictus.* Antenna (A), Proboscis (P), and Palpus (Pl) (30x Magnification Nikon SMZ735 Microscope. Camera Optilab).

same length as the proboscis. While the female mosquito of *Ae. Albopictus* in Fig. 2B has shorter and less dense antenna hairs and the palps is shorter than the proboscis.

*Ae. Albopictus* has a pair of salivary glands. Each part consists of three transparent white lobes, one in the middle and two on the sides (Fig. 3). This result is supported by

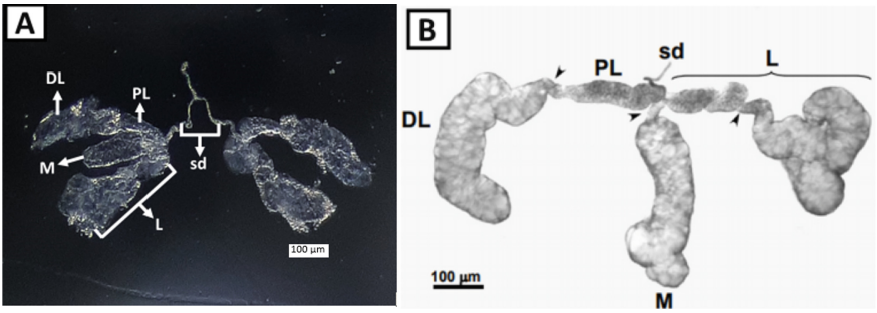


**Fig. 3.** The salivary glands of female *Ae. Albopictus* (A); The salivary glands of female *Ae. Aegypti* according to literature [22] (B). They are composed of the salivary duct (SD), Lateral lobe (L), Medial lobe (M), Proximal-lateral lobe (PL), and Distal-Lateral lobe (DL).

other research which states that each part of the salivary gland has three lobes, one lobe in the middle and the other two lobes located on the sides. The middle lobes are called the single medial lobes and the other two lobes on the sides are called the lateral lobes [21]. The lateral lobe is divided into two types (proximal lateral lobe and distal lateral lobe) [22]. Each lobe may contain a different type of protein [21, 22]. The medial lobe contains several proteins such as Angiopoetin, and Sialokinin. While the lateral lobe contains D7, Lysozyme, and Apyrase [22].

### 3.2 Protein Profile of Ae. Albopictus Salivary Gland

The protein fractions from *Ae. Albopictus* salivary gland have been showed in Fig. 4. The result shows that there are 17 protein bands with molecular weights in the range of 13–96 kDa. Those molecular weights are 13, 14, 15, 23, 26, 27, 28, 30, 31, 47, 51, 53, 61, 67, 72, 77, and 96 kDa. Based on previous research, the protein fraction with molecular weights of 31 kDa, 47 kDa, and 67 kDa are immunogenic [10]. Immunogenic proteins can induce the induction of cellular immune responses and the production of specific antibodies [9].

Several proteins contained in the 31 kDa and 67 kDa fractions have an important role in the blood feeding process. D7 protein is able to resist vasoconstriction and platelet aggregation and modulate the human immune response [16]. It also has ability to inhibit the activation of host innate immune cells, such as neutrophils, monocytes, and macrophages [12]. While Apyrase protein plays a role in the antiplatelet aggregation process [14]. Another protein fraction component, serpine, plays a role in anti-inflammatory and antiplatelet aggregation processes [13]. In addition, the protein angiopoietin causes inflammation in the human body, causing increased endothelial permeability and stretching between cells. It will then make it easier for neutrophils to migrate to damaged cell.

### 3.3 Human IgG Immune Response Against 31 kDa and 67 kDa Protein Fractions

The absorbance value for each individual sample sera can be seen in Fig. 5. The absorbance value indicates that the IgG level of each individual varies in the group

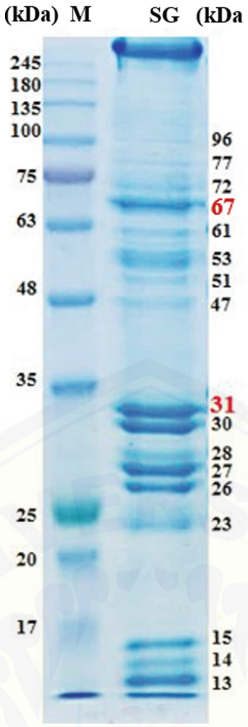


**Fig. 4.** Protein profile *Ae. Albopictus* salivary gland. (M) Gangnam-stained protein ladder markers. (SG) *Ae. Albopictus* salivary glands protein fraction (HP Desktop Scanner GT 5810).

of neonates, healthy residents, and DHF patients. The differences of absorbance value for each individual sample sera can be influenced by individual physiology factors and environment factors. Physiology factors that can affect antibody levels are genetic factors, age, gender, nutritional status, and diseases that affect the immune system [23, 24]. While environment factors are mosquito population density [25] and intensity of mosquito exposure [26]. Individuals exposed with saliva containing 31 kDa and 67 kDa protein *Ae. Albopictus* with different intensities can have different concentrations of IgG. Several studies showed that mosquito salivary proteins ability which induce antibody response is significantly related to the amount of exposure in a person [27]. The human humoral immune response to arthropods varies depending on the level of exposure [26].

Human IgG antibodies are firmly connected to the intensity of exposure to mosquitoes and proteins of salivary gland. The people who frequently exposed to mosquitoes, they will have a higher levels of salivary anti-protein IgG compared to people who are rarely exposed to mosquitoes [29]. Primary exposure of antigen has IgM levels which tend to be higher than IgG levels, while when there is secondary exposure, the presence of IgG will tend to be higher than IgM. Secondary exposure of antigen in the host, will increase the proliferation of memory B cells and plasma B cells. The memory B cells will respond more quickly when there were exposures from the same

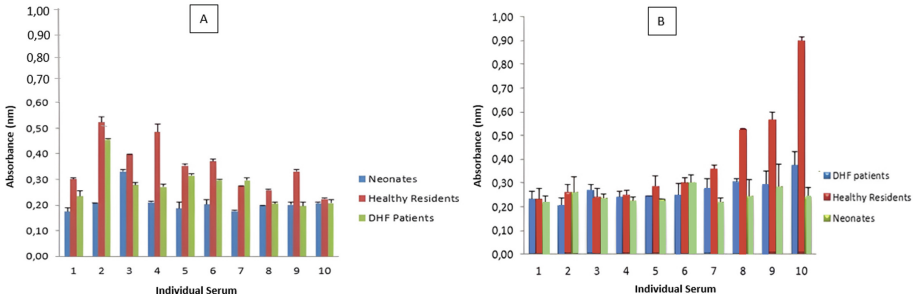


**Fig. 5.** Individual Human Immune Response (IgG) against 31 kDa (A) and 67 kDa (B) protein fraction.

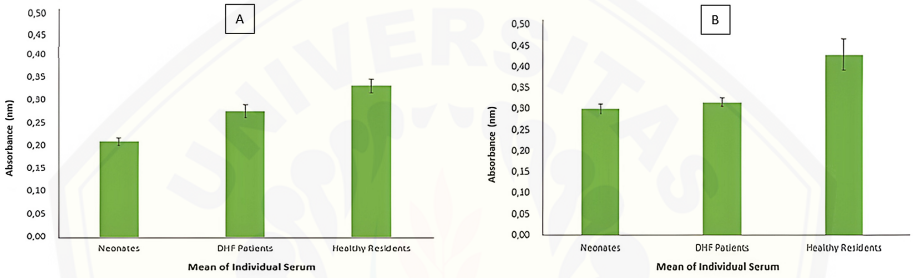


**Fig. 6.** Mean of Individual Human Immune Response (IgG) against 31 kDa (A) and 67 kDa (B) protein fraction.

antigen, while plasma B cells will produce larger amounts of IgG to fight the antigen [30]. Therefore, many exposures will cause IgG production in greater quantities [31].

The average absorbance value of those individual sera which can bind to 31 kDa and 67 kDa protein fractions are shown in Fig. 6. The average absorbance value of IgG which can bind to 31 kDa protein fraction in the healthy resident group, DHF patients, and neonates sample sera were 0.331, 0.276, and 0.209, respectively. The results of statistical analysis showed that the significance value was ($P > 0.05$), therefore there was a significant difference of the average absorbance value in each group of healthy residents-humans, healthy residents-DHF patients, and neonates-DHF patients. Meanwhile, the average absorbance value of IgG level which can bind to 67 kDa protein fractions in the healthy resident group, DHF patients, and neonates sample sera were 0.429, 0.315, and 0.299, respectively. Statistical analysis result showed that there was a significant difference between the group of DHF patients-healthy residents and neonates-healthy residents. While, there was no significant difference in neonates-DHF patient group in this research.

We also analysis IgG levels from the pool sera to support the results of the average individual serum absorbance values and to identify the group with the highest IgG levels based on the pool sera. The IgG levels in pool sera from neonates, healthy residents, and

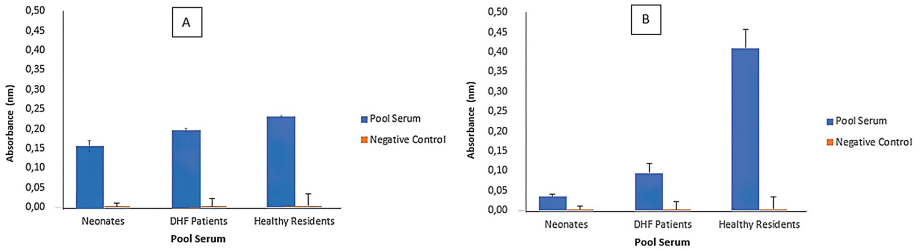


**Fig. 7.** The levels of Human Immune Response (IgG) from pool serum sample against 31 kDa (A) and 67 kDa (B) protein fraction.

DHF patients are shown in Fig. 7. The average absorbance values of healthy residents, DHF patients, and neonates which can bind to 31 kDa protein fractions were 0.229, 0.198, and 0.151, respectively. While average absorbance values of healthy humans, DHF patients, and neonates which can bind to 67 kDa protein fractions were 0.410, 0.096, and 0.037 respectively. Based on the average absorbance value of individual serum and pool serum. It can be seen that in this research both IgG levels of individual serum and pool serum have the same pattern. The highest IgG levels is in healthy residents, DHF patients, and neonates, respectively. This absorbance values showed that the healthy resident group had the highest humoral immune response to 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands compared to DHF patients and neonates. This result is also supported by other research which showed that the level of IgG antibodies to arthropods saliva were significantly higher in healthy resident individuals than in the group of patients with vector-related diseases [32].

The absorbance value of DHF patients is higher than neonates but lower than healthy residents. The results of this study were strengthened by other research who has same result that no relationship was found between antibody levels to mosquito salivary gland proteins and vector-mediated disease infection [25]. Low levels of IgG can be caused by the intensity of exposure to salivary gland proteins which is quite low. In addition, germinal center damage that occurs in DHF patients can also result in low IgG antibody responses with low affinity [33]. Dengue virus can cause fatal germinal center damage in humans [34]. The germinal center is located in the splenic parenchyma and gathering place for lymphocyte cells. The increasing specific immunity response is in line with the diameter of the germinal center which is influenced by the level of B lymphocyte cell proliferation. If the germinal center is damaged, there will be fewer B lymphocytes which are proliferates. So, the fewer antibodiy levels which will be produced [35].

The absorbance value of the neonate group was the lowest compare with healthy residents and DHF patients. Neonates were assumed to have not been exposed to 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands because they were infant. IgG that was successfully recognized by 31 kDa protein in the neonate sample sera was IgG obtained from the mother through transfer from the placenta [36]. It has been known that only IgG immunoglobulins which is capable to crossing the placenta and reaching the fetal circulation in the womb. Transplacental transfer of IgG in neonates begins at 13 weeks of gestation and at 17–22 weeks of gestation the concentration is relatively low, i.e., 5–10% of maternal IgG levels. This IgG concentrations will continue

to increase a at 32 weeks of gestation until birth [37]. Transfer of antibodies across the placenta allows new born babies to acquire identical antigen-recognition patterns between mother and baby [38]. Therefore, serum from neonates has specific IgG levels which are capable to recognize 31 kDa and 67 kDa protein fractions from *Ae. Albopictus* salivary glands. Although receiving IgG transfer from the mother, the IgG levels of neonates are quite low because they have an underdeveloped immune system [18]. In addition, the level of IgG transferred from the mother is lower. It is reported that the total IgG concentration in umbilical cord serum tends to be lower than the total maternal IgG concentration [28]. This is known that transplacental can reduce the ratio of IgG transfer, although the amount of the transferred IgG is still high [36].

Based on those results, it is concluded that IgG level which can recognize the 31 kDa protein fraction based on the absorbance value in the healthy human population, DHF patients, and neonates were 0.229, 0.198, 0.151 respectively based on individual serum sample and 0.229, 0.198, 0.151 in pool serum sample. While the IgG level which can recognize the 67 kDa protein fraction based on the absorbance values in the individual serum of healthy human population, DHF patients, and neonates were 0.4293, 0.3159, 0.2997 in individual serum sample and 0.410, 0.096, 0.037 in pool serum sample. These results indicate that the IgG antibody of each population group can well recognize the 31 kDa and 67 kDa protein fraction. There is same pattern of highest IgG level of each group in individual serum and pool serum samples. The highest IgG levels are found respectively in healthy population group, followed by DHF patients, and neonates.

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