COMPATIBILITY TEST DIAGNOSTIC KITS OF NONSTRUCTURAL-1 DENGUE ANTIGEN IMMUNOCROMATOGRAPHY METHOD OF PATIENT'S SERUM WITH SUSPECT DENGUE HAEMORRHAGIC FEVER

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ABSTRACT

This study aims to determine the comparison of diagnostic kit test results of the Nonstructural-1 dengue antigen which is currently being developed with Nonstructural-1 dengue antigen commercial kit used in hospitals using methods Immunocromatography of patient's serum withDHF suspect. The study design used a cross-sectional method with a total one hundred and three samples that met the inclusion criteria. Collection and examination of samples were carried out at the Pathology Laboratory of the Education Hospital of Hasanuddin University Makassar during March to June 2018. The results showed that the examination using the development NS1 kit obtained positive results as many as 56 samples and negative results as many as 47 samples, while the examination using a commercial NS1 kit obtained positive results as many as 51 samples and negative results as many as 52 samples. The Cohen's Kappa test results obtained a value of p=0,000 (<0.05) indicating that there was a significant match between the development NS1 kit and the commercial NS1 kit used in the hospital and had a very good degree of suitability because of the Cohen's Kappa value obtained in the research is equal to K=0.903 (>0.75). In this study, the sensitivity level of NS1 kit development was 100%, specificity was 90.4%, positive predictive value was 91.1%, negative predictive value was 100%, and the accuracy value was 95.1%.

Keywords: NS1 Dengue Kit, Immunochromatography Method, Dengue Hemorrhagic Fever

BACKGROUND

Dengue infection is currently one of the major health problems in the world (WHO, 2011). The World Health Organization (WHO) estimates that around 50-100 million cases of dengue virus infection occur with 24,000 deaths each year (Soedarto,2012). Data from all over the world shows that Asia ranks first in the number of people with Dengue Hemorrhagic Fever (DHF). Meanwhile, from 1968 to 2009, WHO noted that Indonesia was the country with the highest DHF cases in Southeast Asia (Center for Epidemiological Data & Surveillance of the Indonesian Ministry of Health, 2010).

This disease is caused by infection with dengue virus which is transmitted through the *Aedes aegypti* and *Aedes albopictus* mosquitoes which are infected with the virus and are widespread in the tropics and subtropics (RI Ministry of Health, 2011). Dengue infection can cause clinical manifestations that range from relatively mild symptoms of dengue fever to hemorrhagic symptoms in the form of Dengue Shock Syndrome (SSD) that can cause death, so a fast, precise and accurate diagnosis is needed to immediately treat the patient so that he does not fall into more weight (Young et al., 2000).

Early diagnosis is needed for patient management and monitoring of mortality and morbidity rates (Ramirez et al., 2009). The diagnosis of dengue is difficult to enforce on the first few days of illness because the symptoms that appear are not specific and difficult to distinguish from other infectious diseases so that it can cause a delay in diagnosis. Diagnosis of dengue disease in addition to assessing clinical symptoms also requires laboratory testing to confirm the diagnosis (Muhamad, 2017). Laboratory diagnosis of dengue infection can be established by detecting specific viruses, genome sequences, antibodies and viral antigens (Pei-Yun & Jyh-Hsiung, 2004). Currently, two DHF diagnosis approaches are being developed, namely the use of anti-dengue virus antibodies as antigen capture or by finding

the viral antigen that causes dengue infection in serum or plasma of DHF patients. Both have advantages and disadvantages that affect the time and accuracy of the diagnosis (Harly & Basundari, 2009).

Some serological tests of DHF have low sensitivity due to one of them due to the inefficiency of antigens used in detecting antibodies. Dengue virus consists of single-stranded positive-sense RNA (ssRNA sense +). Inside the genome there is a single Open Reading Frame (ORF) that encodes 2 kinds of proteins, namely structural proteins, and non-structural proteins. Structural proteins consist of C (coreprotein/capsid/core), M (membrane protein, including preMembran/prM) and E (envelope protein) and 7 types of non-structural proteins namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5. In stimulating the formation of antibodies among the structural proteins the highest immunogenicity sequence is protein E and followed by prM and C, while the non-structural proteins that are most important are NS1 proteins. The NS1 protein is a glycoprotein with a molecular weight of 46-50 kDa, this protein is excreted by cells infected with dengue virus and has an important role in the diagnosis and severity of the patient. (Alcon et al., 2002).

Dengue NS1 antigen is an abundant non-structural glycoprotein produced by the virus during the early stages of infection and found in infected cells in the cell membrane and secreted into the extracellular space, which plays an important role in viral replication and maturation (Ni Luh, 2011). Dengue NS1 antigen can be detected in patients with dengue virus serotypes 1,2,3 and 4 and can be detected on days 1 to 9 onset of fever. Protein NS1 Dengue virus is the only non-structural protein found in dissolved form released and circulating in the bloodstream. The protein has the potential as a basis for developing diagnostic tools, because dengue NS1 antigens have high immunogenicity and are able to induce

antibodies through binding activities of the complement system and are in high concentration during the initial clinical phase in the serum of patients who have primary or secondary infections (Alcon et al., 2002).

Diagnosis using anti-dengue virus antibodies that have been developed to date include hemaglutination inhibition (HI), neutralization, immune fluorescent antibody test, immunosorbent assay (ELISA) enzyme link, complement fixation, dot blotting, and western blotting but the test still has limited time and costs (Yondri, 2013). Other laboratory tests that can be done to support the enforcement of diagnoses of dengue virus infection are viral culture and isolation, Reverse Transcription Polymerase Chain Reaction (RT-PCR), serological tests (examination of anti-dengue IgM and IgG) and routine hematological examinations. Virus isolation and PCR are still the gold standards for detecting dengue virus, but there are limitations to these checks, especially the cost, time and processing techniques. Serological examination of anti-dengue IgM and IgG which is routinely and relatively easy to do, but still has limitations, namely the inability to detect the infection process earlier (Ni Luh, 2011). At present, a new examination has been developed, namely the development of a diagnosis of DHF with a detection approach to Non Structural-1 antigens that can detect or diagnose dengue virus infection earlier, even on the first day of fever onset, because Non Structural-1 proteins circulate in high concentrations within blood of the patient during the initial acute phase, both during primary and secondary infections without the need to wait for antibodies to form (Alcon et al.,2002). The presence of the NS1 antigen examination is very important because supportive therapy and patient monitoring can be done immediately and can reduce the risk of complications and death.

In line with the background above, there are now many dengue Non Structural-

1 Antigen diagnostic tools in circulation and have varying levels of specificity and sensitivity. Currently the reagent kits circulating in Indonesia are imported import reagents in the manufacture using recombinant antigens originating from strains outside Indonesia. The use of imported reagent kits in Indonesia with antigens that do not match local strains can cause a lack of sensitivity and specificity of diagnostic test kits. In addition, commercial reagents have a relatively expensive sale value. Various studies have been conducted to test the accuracy of the dengue NonStructural-1 commercial antigen diagnostic kits that are used in various health service locations and have examination results with varying degrees of accuracy. The purpose of this study was to determine the suitability of the results of the dengue Non Structural-1 antigen diagnostic kit test that is currently being developed with a commercial Non Structural-1 antigen kit used in hospitals by using immunochromatographic methods from the serum of patients suspected of DHF.

MATERIALS AND METHODS
Types and Design of Research

This study was an observational analytic study with a cross-sectional design (cross-section). The research subjects were suspected DHF patients who underwent outpatient and inpatient care at the Hasanuddin University Higher Education Hospital (UH RSPTN) Makassar and conducted NS1 examinations.

Research Place and Research Time

Collection of suspected DHF serum samples and examinations will be carried out at the Clinical Pathology Laboratory of UH Makassar RSPTN during March to June 2018.

Population and Sample Techniques

The population in this study was suspected DHF patients who underwent outpatient and inpatient care at UH Makassar Hospital. The sample in this study were all affordable populations that met the research criteria. The sample collection in this study used a purposive sampling method.

Research Tools and Materials

Equipment: The tools used in this study include: phlebotomy device in the form of Holder, Tourniquet, 70% alcohol cotton, Plastering, Flashback or Wing needle, 3 mL red lid plant tube, 3 mL K2EDTA tube, Micropipette, and Yellow tip. Materials used: Serum, plasma EDTA or Whole Blood, Panbio Dengue Early Rapid kit (commercial reagent) and Non Structural-1 dengue Antigen kit that is being developed is in vitro diagnostic kit dengue Non Structural-1 Antigen with immunochromatographic testing methods using local strains from Indonesian isolates currently under development by Hasanuddin University Medical Research Center (HUMRC) in collaboration with the Mochtar Riady Institute for Nanotechnology (MRIN) to qualitatively detect Non Structural-1 Antigen dengue virus in human serum or plasma for early diagnosis of acute dengue infection (Yaumil, 2014).

Ways of working

The blood of patients with suspected DHF taken as much as 3 ml through the medianacubiti or cephalica vein was allowed to freezer ± 30 minutes, then centrifugation for 15 minutes at 3600 rpm. Plasma serum or EDTA obtained was used as a sample in the study to carry out a Non Structural-1 dengue antigen examination.

Remove the dengue strip from the wrapper and place it on a dry flat surface using a disposable pipette in the diagnostic kit, add 3 drops (about 100 µL) of the sample into the sample well, turn on the timer (timer set for 15-20 minutes), kit begins to detect, it will appear purple moving past the reading area of the results in the middle part of the diagnostic kit, the interpretation of the results of the inspection is read at 15-20 minutes, after the timer has

reached 15-20 minutes, the results can be interpreted (Dussart et al., 2006) .

Interpretation of the examination results, negative results: if there is only one color line on the control line "C" in the reading area of the results on the diagnostic kit, this indicates that the negative Non Structural Antigen-1 examination results are positive: if there are two color lines on the "T" test line and the "C" dick line in the reading area of the results on the diagnostic kit, this indicates that the results of the Non Structural Antigen examination are positive, invalid results: if in the reading area the results of the "C" control mark are not visible line, the result is considered invalid. This can be caused because the work is not in accordance with the instructions on work procedures or can be caused by the quality of the kit that deteriorates for example because the diagnostic kit has been expired. Recommended retesting specimens (Department of Parasitology FK UI, 2008).

Data Management and Analysis

The results of the examination data obtained are processed by tabulation and displayed in table form. The diagnostic test results are included in the 2x2 table. The 2x2 table is then calculated to find sensitivity, specificity, positive predictive value, negative predictive value, and accuracy then Cohen's Kappa statistical test is used to determine the degree of conformity between the dengue Non Structural-1 antigen diagnostic kit currently being developed with the commercial dengue Non-Structural antigen kit used in hospitals, while to see the relationship between the examination of dengue Non-Structural-1 antigen and the results of dengue IgGIgM examination immunochromatography method used the chi-square statistical test and to assess the results of Non-Structural-1 antigen examination dengue with leukocyte values, erythrocytes, hematocrit, hemoglobin and platelet values are used Independent t-test statistics. Calculation of analysis is done using a computer using SPSS 22 software for Windows.

RESEARCH RESULT

Subject characteristics in this study consisted of gender, age, and duration of fever (days). In table 1 shows that of 103 subjects in this study, the number of male patients was 62 people (60.2%) and women as many as 41 people (39.8%). For the age classification in this study,most patients were patients aged 21-30 years as many as 30 people (29.1%) and the least patients with age <1 year, only 1 person (1.0%). While for the duration of fever (days) of 103 patients suspected of dengue in this study, the highest duration of fever was fever on the third day as many as 37 people (35.9%) and the least amount was fever on the 7th day which was only as much as 2 people (1.9%).

Table 2 shows that of the 103 serum samples of patients with suspected DHF in this study, the results of the examination were obtained using the diagnostic kit of the Non Struktrural-1 dengue antigen which is currently being developed with 56 positive samples and 47 negative samples. while the examination using a Non Struktrural-1 commercial antigen-1 diagnostic kit used in the hospital obtained positive results of 51 samples and 52 negative results. To determine the suitability between the results of the dengue NonStructural-1 antigen diagnostic kit examination which is currently being developed with a commercial dengue NonStructural-1 antigen kit used in the hospital, the Cohen's Kappa test was conducted.

The Kappa coefficient value (K) obtained from the comparison between the dengue Non-Structural-1 antigen diagnostic kit currently being developed with the commercial dengue Non-Structural-1 antigen kit used in the hospital is used to determine the reliability of the diagnostic test. The Cohen's Kappa test results obtained a value of p=0,000 (<0.05) indicating that there was a significant match

between the NS1 kits being developed and the commercial NS1 kit used in the hospital and having a very good degree of suitability because of the value of Cohen's Kappa obtained in this study that is equal to K=0.903 (> 0.75).

In this study the determination of the level of sensitivity, specificity, positive predictive value, negative predictive value and accuracy to prove the ability of dengue NonStructural-1 antigen diagnostic kits currently being developed in detecting dengue virus infection compared to commercial NonStructural-1 dengue antigen kits used in hospitals. Of the 103 samples in this study, obtained 51 pure positive samples (a), 5 false-positive samples (b), there were no false-negative samples (c) and 47 pure negative samples (d). From these results it can be calculated the sensitivity of the dengue NonStructural-1 antigen diagnostic kit that is currently being developed which is 100%, specificity is 90.4%, positive predictive value is 91.1%, negative predictive value is 100%, and accuracy value of 95, 1%.

DISCUSSION

This study involved 103 patients with suspected DHF who performed a Non Structural-1 dengue antigen immunochromatographic method and were willing to participate in the study by giving informed consent. This amount exceeds the minimum sample required in the study. The collection and examination of research samples were carried out at the Clinical Pathology Laboratory of UH Makassar Hospital in South Sulawesi Province.

Based on the characteristics of the research subjects, the respondents' gender in this study from 103 respondents had more male respondents, namely 62 people (60.2%) and women as many as 41 people (39.8%). This is in line with the research conducted by Yondri in 2014 in 69 out of 69 respondents, the sex of the most dengue sufferers, namely 39 men (56.5%), then 30 women (43.5%) (Yondri, 2013). Then another study conducted by Megariani et al

in 2014 in Padang from 50 respondents found that there were more men than women, namely 27 (54%) men and 23 (46%) women (Megariani et al., 2014). The same results were obtained by Libraty et al in 2002 who obtained more male sufferers than women with a ratio of 2: 1 (Libraty et al., 2002). Rothman et al. In 2007 stated that the low percentage of women with DHF compared to men was due to the female immune system being better than men. In women, more anti-inflammatory production is more so that women infected with DHF provide less clear clinical complaints and rarely experience hospitalization (Megariani et al., 2014). Soedarmo et al. In 2009 stated that XX chromosomes in girls have a role in managing immunoglobulin production quantitatively (Soedarmo, 2009). In contrast to the molecular research conducted by Kalayanarooj et al in 2005 in Timor Leste, there were more girls suspected of having dengue virus infection (Kalayanarooj et al., 2005). However, the Halstead et al 2004 study proved that there was no difference between the infection response of girls and boys (Megariani et al., 2014).

This is in line with the statement in the book "Dengue Fever in Children" by Soedarmo in 2009 stated that in Indonesia and the Philippines, there is no difference between girls and boys who suffer from dengue fever (Soedarmo, 2009). Another study conducted by NurKhakimatulFaizah in 2016 in Jakarta out of 91 respondents obtained the number of male dengue patients as many as 47 people (51.6%) and women as many as 44 (48.4%) people, from the results of these studies it appears that there is no significant difference between male and female DHF patients (Nur, 2016). This is also in accordance with data from the Ministry of Health of Indonesia in 2010 in "Window Epidemiology Bulletin, Main Topic: Dengue Hemorrhagic Fever" stated that the distribution of dengue cases by sex, the percentage of male and female sufferers is almost the same. The number of sufferers of the male sex is

10,463 people (53.78%) and women are 8,991 people (46.23%). This illustrates that the risk of dengue for men and women is almost the same, not dependent on gender (Epidemiological Data & Surveillance Center of the Indonesian Ministry of Health, 2010).

Furthermore, the characteristics of the research subject are based on age. Age is one of the characteristics that can affect the condition of a health problem or disease because age is very influential on the level of exposure, the magnitude of the risk, and certain resistance properties. Age is one of the important variables because many diseases are found with various frequencies related to age, some infectious diseases, for example, show that a young age has a higher risk than adult age (Rosa & M.Sabir, 2011).

In this study obtained from 103 respondents with the youngest age 8 months (<1 year) and the oldest 60 years, the most age is respondents 21-30 years as many as 30 people (29.1%), then respondents with ages 1-10 years as many as 28 people (27.2%) and respondents aged 11-20 years as many as 24 people (23.3%), respondents with age 31-40 years as many as 12 people (11.7%), respondents with ages 41-50 years and ages 51-60 years have the same value is as many as 4 people (3.9%) while the respondents with age <1 year at least are only as many as 1 person (1.0%). The results of this study are in line with the research of Rosa DwiWahyuni et al in 2011 in Makassar showing that the percentage of DHF cases according to the highest age group was in the 19-49 years age group, which were 89 people (43.4%) and followed by the 6-14 year age group of 48 people (23.4%), while the lowest age group in the 0-1 year group is as many as 5 people (2.4%). Other research that has the same results was carried out by Rosmila in Ex. TamamaungKec. Panakukang that dengue disease is more common in adults, out of 27 people with dengue there are 26 people (30.2%) who are classified as adults and 1

person (8.3%) who are classified as toddlers and children (Rosa & M.Sabir, 2011) However, the results of this study are not in line with the research conducted by Yondri in 2014 where the youngest respondents were 4 months and the oldest 61 years, the most aged between 11-20 years as many as 22 people (31.9%), then respondents with ages 2-10 year as many as 17 people (24.6%), respondents with age 21-30 years and ages> 41 years have the same value as many as 8 people (11.6%), while respondents with age ≤ 1 year and 31-40 years with the least amount and has the same value as many as 7 people (10.1%) (Yondri, 2013).

Furthermore, the characteristics of the research subject were based on the duration of fever, from 103 respondents of patients suspected of DHF in this study, based on the duration of the patient's fever when admitted to the shortest hospital was fever on the 2nd day and the longest was the 7th day fever with the most fever Day 3 fever was 37 people (35.9%), then day 4 fever was 25 people (24.4%), then day 5 fever as many as 17 people (16.5%) and 2nd day fever as many as 16 people (15.5%), 6th day fever as many as 6 people (5.8%) and 7th day fever with the lowest number, only 2 people (1.9%). This is in line with the research conducted by Cindy et al. In 2015 in Manado, with 37 of the highest number of patients based on the duration of the patient's fever when admitted to hospital, 21 people (56.8%), later in the day. 4 as many as 9 people (24.3%), and the fifth day as many as 7 people (18.9%) (Cindy et al., 2015). This result is in accordance with the theory that the peak increase in the level of dengue Non-Structural-1 antigen increased with the highest level on the third day of fever and the level will decrease after the fifth day (Mitayani, 2011). The results were not much different from the ones obtained by Megariani et al. In 2014 in Padang from 50 respondents based on the duration of the patient's fever when entering the hospital with the highest results, on the third day as

many as 28 people (56%), then the second day as many as 21 people (42%), and 1st day as many as 1 person (2%). In the 2014 study of Megariani et al. The percentage of positive NS1 was greater on day 3 of fever compared to day 2, Dussart et al. In 2008 studied 299 dengue fever patients in France, NS1 sensitivity was found on days of 0-4 days of fever at 87.6% and 43.5% in 5-10 day fever. Datta et al in India in 2010, comparing NS1 in the acute and convalescent phases, found 71.42% positive NS1 in the acute phase, whereas in the NS1 convalescent-phase there was only 6.38% positive. The sensitivity of NS1 is high in the early phase of fever because NS1 protein circulates in high concentrations in the patient's blood during the initial acute phase, both in primary and secondary infections. High levels of NS1 until the 5th day of fever are associated with the time of occurrence of viremia because it is a period of viral replication and the absence of antibodies to the virus. The levels of viremia and NS1 levels also depend on the intrinsic characteristics of the infecting virus strains and the immunity status of the patients themselves (Megariani et al., 2014).

In this study the researchers tested the level of suitability of the test results between the Non Struktrural-1 dengue antigen kit which is currently being developed with the Non Structural-1 commercial antigen kit used in hospitals obtained results from 103 serum samples of patients with suspected DHF in this study, obtained the results of the examination are the results of testing using a dengue Non Struktrural-1 antigen diagnostic kit which is currently being developed obtained 56 positive samples and 47 samples are negative, while the examination uses a commercial Non-Structural-1 dengue antigen diagnostic kit used at home pain obtained positive results of 51 samples and negative results of 52 samples. To determine the suitability between the results of the dengue Non-Structural-1 antigen diagnostic kit examination which is currently

being developed with a commercial dengue Non-Structural-1 antigen kit used in the hospital, the Cohen's Kappa test was conducted. The Cohen's Kappa test results obtained a value of p = 0,000 (<0.05) which indicates that there is a significant suitability of test results between NS1 kits being developed with commercial NS1 kits used in hospitals and having a very good degree of suitability because of the value of Cohen's Kappa obtained in this study that is equal to K = 0.903 (> 0.75).

In this study the determination of the sensitivity level, specificity, positive predictive value, negative predictive value and accuracy to prove the ability of the dengue Non-Structural-1 antigen diagnostic kit currently being developed in detecting dengue virus infection compared to Non-Structural-1 dengue antigen kits commercial used in hospitals. Of the 103 samples in this study, obtained 51 pure positive samples (a), 5 false-positive samples (b), there were no false-negative samples (c) and 47 pure negative samples (d). From these results it can be calculated the sensitivity of the dengue Non-Structural-1 antigen diagnostic kit that is currently being developed which is 100%, specificity 90.4%, positive predictive value 91.1%, negative predictive value 100%, and accuracy value of 95, 1%. In this study there were different examination results between the results of the NS1 kit test that was being developed and the commercial NS1 kit used in the hospital, which was 5 samples. Where on the examination using a nonStructural-1 commercial antigen kit used in the hospital negative results were obtained while the examination using a dengue Non-Struktrural-1 antigen diagnostic kit which is currently being developed is obtained by positive examination results. The five samples that gave different results (false positives) were examined using the PCR method as the gold standard to determine the detection results of dengue Non-Structural 1 antigen, in theory Virus isolation and PCR was still the gold standard for detecting dengue

virus. In this study, the researchers chose to determine the results of five samples that gave different results. Further testing was done using PCR instead of virus isolation because there were limitations to the examination, especially the time and processing techniques. The results of the examination using the PCR method as the gold standard gave negative results to these 5 samples, this is probably due to the storage process of the sample which is not in accordance with the standard because 5 samples that have different incubation at -20 ° C for 6 months RNA can be ascertained from dengue virus is likely to have been damaged, this is in line with the research conducted by EnnyNugraheni et al. 2016 in Bengkulu on "Molecular Diagnosis of Dengue Virus" in his study stated that for storage management of samples the PCR method was examined in detecting dengue virus RNA, a virus that will directly checked stored at -4 ° C or -8 ° C for inspection less than 24 hours, but if the inspection is carried out more than 24 hours the sample storage must be stored at -70°C and maintained so that no liquefaction occurs to prevent damage Dengue virus RNA due to dengue virus including virus which is labile to temperature and other chemical factors (Nugraheni & Sulistyowati, 2016). Five samples that gave different results had also been carried out with dengue IgM and IgG examination and gave results from these 5 samples. 4 samples gave positive dengue IgM immunochromatography method while 1 sample obtained negative IgM dengue immunochromatography method, whereas for dengue IgG immunochromatography method of 5 Different samples examined by 4 samples obtained negative results of dengue IgG immunochromatography method and 1 dengue positive IgG immunochromatography method, IgG and IgM degue immunochromatography method was performed using fresh serum (less than 24 hours) before the serum sample of patients suspected of dengue was frozen at temperature of -20 ° C.

The results of this study are in line with the research conducted by Megariani et al. In Padang in his study to determine sensitivity, specificity, positive and negative predictive values and determine the accuracy level to prove the ability of NS1 to diagnose dengue virus infection compared to PCR as the gold standard. In his research obtained 50 samples suspected of having dengue virus infection. 24 positive samples correctly suffered DHF (a), 1 false-positive sample (b), 2 false-negative samples (c), and 23 true negative samples (d). then the results of NS1 sensitivity were 92.3%, specificity 95.8%, positive predictive value 96%, negative predictive value 92% and NS1 accuracy for diagnosis of dengue virus infection by 94%. From these results there were 24 samples that gave positive results both with PCR and rapid test NS1 while using PCR 26 positive samples were obtained while using the NS1 rapid test only 25 samples were positive. From the results of the test carried out the Kappa test and obtained a value of 0.88 which shows the suitability of the results between PCR and rapid test NS1 for the diagnosis of dengue virus infection with a value of p = 0,000 (<0.05) (Megariani et al, 2014). However, the results of this study are different from the results obtained in a study conducted by Paisal et al. 2016 in Jakarta to examine the suitability of detection of RT PCR, NS1 RDT, and IgM RDT results in patients with dengue disease. The results obtained were the suitability of the test results between RT PCR examination and NS1 RDT and RT PCR examination with IgM RDT in diagnosing dengue disease, Cohen's Kappa test was performed. The results of Cohen's Kappa test obtained for RT PCR examination with RDT NS1 found that the suitability was only a moderate level of value (K = 0.404, p = 0,000). Whereas for RT PCR examination with IgM RDT only had a very small match (K = 0.139, p = 0.046). It can be concluded that the suitability of NS1 RDT detection against RT PCR examination is only a moderate level and the suitability of RT PCR detection

compared to IgM RDT only has very little suitability (Paisal et al., 2016).

CONCLUSIONS AND RECOMMENDATIONS

There is a conformity of the results of a significant examination between the Non-Structural-1 antigen kit which is currently being developed with the commercial Non-Structural-1 antigen kit used in hospitals using immunochromatography methods and has a very good level of suitability, and the level of sensitivity of the kit is obtained Dengue Non-structural antigen diagnostic currently being developed that is compared with commercial NS1 kits is 100%, specificity is 90.4%, positive predictive value is 91.1%, negative predictive value is 100% and accuracy value is 95.1%.

Based on these results it can be suggested that further research is needed in the form of a comparative test between dengue Non Structural-1 antigen diagnostic kits currently being developed with a gold standard examination to detect the presence of dengue viruses such as dengue virus isolation culture or PCR examination of all patient serum samples suspects of DHF, in order to be able to assess the determination of the level of sensitivity, specificity, positive predictive value, negative predictive value and accuracy compared to the gold standard examination to detect the presence of dengue virus to prove the ability of the Dengue Non-Structural-1 antigen diagnostic kit currently being developed in detecting presence of dengue virus infection.

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ATTACHMENT

Table 1. Characteristics of Research Subjects

Characteristics of Research Subjects		Total (n = 103)	Percentage (100 %)
Gender	Male	62	60,2
	Female	41	39,8
Age Classification (Year)	<1	1	1,0
	1 - 10	28	27,2
	11 - 20	24	23,3
	21 - 30	30	29,1
	31 - 40	12	11,7
	41 - 50	4	3,9
	51 - 60	4	3,9
Old Fever (Day)	≤ 2	16	15,5
	3	37	35,9
	4	25	24,3
	5	17	16,5
	6	6	5,8
	≥ 7	2	1,9

Tabel 2. Conformance Test Results Between NS1 Kits that are Being Developed with Commercial NS1 Kits

Developed NS 1	Commercial NS 1		Total	Value p*	Cohen's Kappa
	Positive	Negative			
Positive	51	5	56		
Negative	0	47	47	0,000	0,903
Total	51	52	103		

*Uji Cohen's Kappa
 - Sensitivity   = 100 %
 - Specificity   = 90,4 %
 - Value is positive   = 91,1%
 - Value is negative   = 100%
 - Accuracy   = 95,1%