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CONTENTS

Comparison of Immunochromatography Method and Immunocytochemistry Method in Rapid Detection of NS-1 Antigen in Dengue Infection

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ABSTRACT

Introduction: Rapid test kit based on immunochromatography test (ICT) in detecting dengue NS-1 antigen for early dengue infection is available in the market. Its availability allows earlier management for dengue infected patient but it remains costly to most people. Recently, Dengue Team of Universitas Gadjah Mada has developed monoclonal antibodies to detect the presence of dengue NS-1 antigen in leucocytes of infected patients based on Streptavidin Biotin Peroxidase Complex (SBPC) immunocytochemistry method.

Objectives: The objective of this study is to determine the validity of the immunochromatography (SD Dengue NS1 Ag) method by determining kappa agreement index between two observers, and to compare the diagnostic performances of ICT and immunocytochemistry methods in detecting dengue NS1 antigen in the blood samples.

Methods: A cross sectional study design is used. This study uses 35 blood plasma remains from a previous study conducted on RT-PCR method. Three drops of blood plasma were added into the well of SD Dengue Duo NS1 and results were read after 15-20 minutes. The diagnostic performances of ICT which defined by sensitivity, specificity, positive predictive value and negative predictive value were calculated and compared to secondary data of immunocytochemistry result from the same blood samples, with reference of RT-PCR as a gold standard. A McNemar's test was conducted and p value less than 0.05 was considered as significant different.

Result: Detection of dengue infection by using SD Dengue NS1 Ag has strong agreements between two observers with kappa value of 1, and the sensitivity of 50%, specificity of 91%, positive predictive value of 92% and negative predictive value of 45% with reference of RT-PCR as a gold standard. Meanwhile sensitivity and specificity value of the immunocytochemistry test were 88% and 100% respectively, and the positive and negative predictive values were 100,0% and 70,0% respectively with reference of RT-PCR as a gold standard. The immunocytochemistry assay showed overall accuracy of 91,0%.

Conclusion: Immunochromatography (SD Dengue NS1 Ag) method to detect NS-1 antigen has less sensitivity and specificity comparedto SBPC immunocytochemistry method.

Keyword: Immunocytochemistry, Immunochromatography, Streptavidin Biotin Peroxidase Complex (SBPC), NS-1 Ag, dengue

INTISARI

Pendahuluan: *Rapid test kit* untuk demam berdarah dengue berbasis prinsip immunochromatography (ICT) yang mendeteksi antigen NS-1telah tersedia. Walaupun demikian, biayanya tergolong tinggi. Baru-baru ini, Dengue Tim Universitas Gadjah Mada telah mengembangkan antibodi monoklonal untuk mendeteksi keberadaan dengue NS-1 antigen pada leukosit pada pasien yang terinfeksi berdasarkan metode imunohistokimia (IHC), Streptavidin Biotin Complex Peroxidase (SBPC).

Tujuan: Tujuan penelitian ini adalah untuk mengetahui validitas immunochromatography metode (*SD Dengue NS-1 Ag*) antara dua pengamat (nilai *kappa*), dan untuk membandingkan kinerja diagnostik antara metode ICT dan IHC dalam mendeteksi dengue NS1 antigen dalam darah sampel.

Metode: penelitian ini menggunakan desain studi cross sectional menggunakan 35 plasma darah yang telah dianalisa RT-PCR pada penelitian sebelumnya. Tiga tetes darah plasma ditambahkan ke dalam sumuran dari *SD Dengue Duo NS1* dan hasilnya dibaca setelah 15-20 menit. Kinerja diagnostik ICT yang didefinisikan oleh sensitivitas, spesifisitas, nilai prediksi positif dan nilai prediksi negatif dihitung dan dibandingkan dengan data sekunder dari hasil IHC dari sampel darah yang sama, dengan mengacu dari RT-PCR sebagai standar emas. Tes McNemar dilakukan dan nilai p kurang dari 0,05 dianggap sebagai perbedaan yang signifikan.

Hasil: Deteksi infeksi dengue dengan menggunakan *SD Dengue NS1 Ag* memiliki kesepakatanyang kuat antara dua pengamat dengan nilai kappa 1, akan tetapi memiliki sensitivitas 50%, spesifisitas 91%, nilai prediksi positif 92% dan nilai prediksi negatif 45% dengan RT-PCR sebagai standar emas. Sementara itu uji imunositokimia (IHC) menunjukkan sensitivitas 88% dan nilai spesifisitas 100% dengan nilai prediksi positif 100% dan nilai prediksi negatif 70%.

Simpulan: Metode ICT (SD Dengue NS1 Ag) untuk mendeteksi NS-1 antigen memiliki sensitivitas dan sensitivitas di bawah uji IHC dengan metode SBPC.

Kata kunci: imunositokimia, immunochromatography, Streptavidin Biotin Complex Peroxidase (SBPC), antigen NS-1, demam berdarah dengue

INTRODUCTION

Dengue hemorrhagic fever has been the major burden for the world with more than 100 million people infected yearly. Incidence of dengue has increased in a drastic pattern in the last decade all around the world. Around 2.5 billion people, which is about two- fifth of the world population are at risk of dengue infection. There is a current estimation of 50 million dengue infection occuring worldwide[1].

In the past, diagnosis of dengue hemorrhagic fever is solely based on the clinical symptoms as stated in the criteria set by WHO without further virology confirmation as they were time consuming, thus leading to late diagnosis[1]. Recently, the presence of rapid test kit using immunochromatography method in detecting dengue NS-1 antigen for early dengue infection is available in the market to allow earlier management for dengue infected patient but it still remain costly to most people. To respond this situation, Dengue Team of Universitas Gadjah Mada has developed monoclonal antibodies to detect the presence of dengue NS-1 antigen in leukocytes of infected patient. SBPC immunocytochemistry method to test NS-1 antigen showed 94% sensitivity and 90% specificity when compared to RT-PCR[2,3]. New diagnostic test with high sensitivity and spesificity will benefit in early management of dengue hemorrhagic fever.

The main objective of the research is to compare the accuracy of diagnostic test of SD Dengue NS-1 Ag (a component of SD Dengue Duo rapid test kit-immunochromatography) with the SBPC Immunocytochemistry method on single sera in detecting NS1 antigen. Other objectives are: (1) to evaluate of the reliability and applicability of both the methods in detecting dengue virus in sub-urban

setting of Yogyakarta, (2) to evaluate the sensitivity of immunochromatography (SD Dengue NS1 Ag) method and SBPC immunocytochemistry method in detecting NS-1 antigen in different days of fever, and (3) to determine the validity of immunochromatography (SD Dengue NS1 Ag) method by determining kappa agreement index between two observers.

MATERIALS AND METHODS

Sample used in this study was collected in the previous study. Thirty five samples from febrile patients regardless of any diseases, suffering from fever for 1 to 7 days who visited Panembahan Senopati District Hospital in Bantul during January to March 2010 were used in this study. Those samples had been tested with SBPC immunocytochemistry and RT-PCR and were stored in deep freezer at Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada.

The Dengue Duo Rapid Test Kit contains Dengue NS-1 Ag and Dengue IgG/IgM Combo Device, 10 ìL of capillary pipette and disposable dropper. In the strip included, Gold Conjugates serve as the main component [composing of mouse monoclonal anti-dengue NS1-Gold Colloid (0.27±0.05 µg)], test line (as main component) contains mouse monoclonal anti- dengue NS1 (0.72±0.14 ìg) and the control line (as main component) contains goat anti-mouse IgG (0.72±0.14 µg).

The test device is removed from the foil pouch and place on a flat, dry surface before 3 drops of blood plasma (about 100µL) is added into the sample well (S). The test begins to work with the purple color moving across the result window in the center of the test device. The test result is interpreted at 15-20 minutes. A positive result will not change once it has been established at 15-20 minutes. However, in order to prevent any incorrect result, the test result should not be interpreted after 20 minutes.

The results of the rapid test kit ware indicated by the presence of color line at the control line and test line. Positive result is indicated by presence of both control line and test line. Negative result is indicated by presence of only control line.

However, result is interpreted as invalid if the control line does not appear as the control line serves as procedural control.

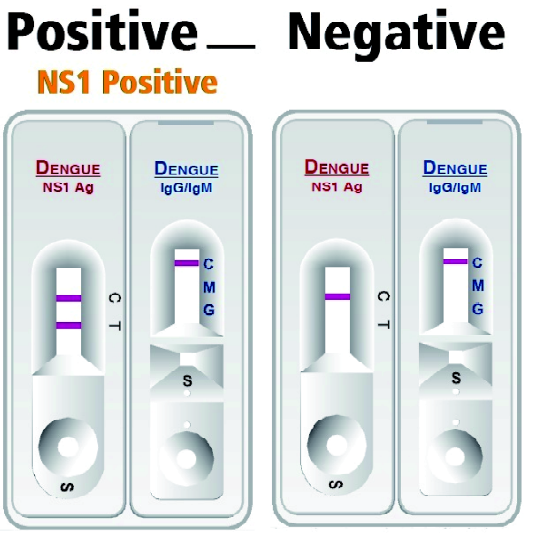

Figure 1. Result Interpretation of SD Dengue NS1 Ag component of SD Dengue Duo rapid test kit (Standard Diagnostics Inc, 2010)[4].

The validity and reliability of the measurement between two observers were evaluated based on kappa values according to Landis and Koch[5].

Sensitivity, specificity, positive predictive value and negative predictive value from the SD Dengue NS1 Ag component of SD Dengue Duo rapid test kit and both the SBPC immunocytochemistry method will be calculated with RT-PCR as the gold standard. The performance were measured based on Hermann formula[6].

RESULTS AND DISCUSSIONS

Thirty five plasma samples were tested using immunochromatography test (SD Dengue Duo NS1)

and SBPC immunocytochemistry method. RT-PCR was used as the gold standard reference. Using Immunochomatography test, thirteen samples showed positive and twenty two were negative results. PCR test confirmed 12 true positive and 1 false positive results out of thirteen plasma that diagnosed as positive result by immunochromatography test and confirmed 12 false negative and 10 true negative results out of 22 patients that tested negative by immunochromatography test (Table 1).

Table 1. Tabulation of immunochromatography (SD Dengue NS1 Ag) result in NS-1 antigen detection against RT-PCR result.

		RT-PCR		
		POSITIVE	NEGATIVE	Total
IMMMUNOCHROMATOGRAPHY (SD Dengue Duo NS1)	POSITIVE	12	1	13
	NEGATIVE	12	10	22
	Total	24	11	35

Sensitivity of Diagnosis	:	12	/ 24	=	0.50
Specificity of Diagnosis	:	10	/ 11	=	0.91
Positive Predictive Value	:	12	/ 13	=	0.92
Negative Predictive Value	:	10	/ 22	=	0.45

Using Immunocytochemistry (SBPC NS1), twenty one samples showed positive results and forteen were negative. PCR test confirmed all 21 samples that diagnosed as positive result by Immunocytochemistry (SBPC NS1) and confirmed 3 false negative and 11 true negative out of 14 patients that tested negative by immunocytochemistry test (Table 2).

Table 2. Performance of immunocytochemistry (streptavidin biotin peroxidase complex) assay in the detection of dengue antigen in the thick blood smear.

		RT-PCR		
		POSITIVE	NEGATIVE	Total
IMMMUNOCYTOCHEMISTRY (SBPC NS1)	POSITIVE	21	0	21
	NEGATIVE	3	11	14
	Total	24	11	35

Sensitivity of Diagnosis	:				
Specificity of Diagnosis	:	11	/ 11	=	1.00
PositivePredictive Value	:	21	/ 21	=	1.00
NegativePredictive Value	:	11	/ 14	=	0.79
Overal accuracy	:	32	/ 35	=	0,91

The samples were used to evaluate the performance of the immunocytochemistry assay in the thick blood smear in terms of sensitivity, specificity, positive predictive value, negative predictive value and the overall accuracy for the detection of dengue antigen in the cytoplasm of

leucocytes, compared to the secondary data that had been obtained by RT-PCR method as a gold standard. The sensitivity and specificity value of the assay were 88% and 100% respectively. The positive and negative predictive values of the assay were 100,0% and 70,0% respectively. The immunocytochemistry assay showed overall accuracy of 91,0%.

Table 3. Comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of immunochromatography (SD Dengue NS1 Ag) and immunocytochemistry (SBPC) method in detecting dengue NS-1 antigen with RT-PCR as gold standard.

	IMMUNOCHROMATOGRAPHY	IMMUNOCYTOCHEMISTRY
SENSITIVITY	0.5	0.88
SPECIFICITY	0.91	1
PPV	0.92	1
NPV	0.45	0.79

Table 3 showed that immunochromatography test (ICT) is less sensitive and specific for detection dengue NS-1 antigen compared to the immunocytochemistry assays. It also showed that the positive and negative predictive values were lower than immunocytochemistry assay. However, no significant statistical differentiation is found based on McNemar test (P=0.77; > 0.05) as shown in Table 4.

Table 4. Tabulation of Immunocytochemistry (SBPC NS1) Result against Immunochromatography (SD Dengue NS1 Ag) Result.

		IMMUNOCYTOCHEMISTRY		
		POSITIVE	NEGATIVE	TOTAL
IMMUNOCHROMATOGRAPHY	POSITIVE	9	4	13
	NEGATIVE	12	10	22
	TOTAL	21	14	35

*McNemar Test: p -value=0.77 (p > 0.05)

Table 5. Comparison of Sensitivity of Immunochromatography (SD Dengue NS1 Ag) and Immunocytochemistry (SBPC NS1) Method in Detecting NS-1 antigen on Different Day of Fever with RT-PCR as reference.

DAY OFFEVER	ICT				IMMUNOCYTOCHEMISTRY				RT=PCR			
	POS	%	NEG	%	POS	%	NEG	%	POS	%	NEG	%
1	0	0	2	18.18	1	4.17	1	9.09	1	4.17	1	9.09
2	0	0	1	9.09	0	0	1	9.09	0	0	1	9.09
3	0	0	2	18.18	1	4.17	1	9.09	1	4.17	1	9.09
4	6	25	11	100	12	50	5	45.45	12	50	5	45.45
5	5	20.83	4	36.36	5	20.83	4	36.36	8	33.33	1	9.09
6	2	8.33	0	0	1	4.17	1	9.09	1	4.17	1	9.09
7	0	0	2	18.18	1	4.17	1	9.09	1	4.17	1	9.09
TOTAL	13	54.17	22	200	21	87.5	14	127.27	24	100	11	100

Note: ICT= immunochromatography test. P os= Positive. Neg= Negative

Table 5 showed that dengue NS1 antigen was detected in the plasma of patient in the 4th day of fever to the 6th day of fever based on imunochromatography test (ICT), but it was detected in the 1st day of fever to the 7th day of fever based on the immunocytochemistry assay in the thick blood smear and RT-PCR in the whole blood of patient.

Table 6. Tabulation of immunochromatography (SD Dengue NS1 Ag) result between 2 different observers

		OBSERVER 2		
		POSITIVE	NEGATIVE	TOTA L
OBSERVER 1	POSITIVE	13	0	13
	NEGATIVE	0	22	22
	TOTAL	13	22	35

OBSERVED AGREEMENT (Po) = (13 + 22)/ 35 = 1.00, CHANCE AGREEMENT (Pe) = [(13/35) * (13/35)] + [(22/35) * (22/35)] = (0.37 * 0.37) + (0.62 *0.62 = 0.1369 + 0.3844 = 0.5213, KAPPA VALUE = (1 – 0.5213) /(1 – 0.5213) = 1

Table 6 showed that there is strong agreement between two observers to detect dengue NS1 antigen in the sera of patient.

Acute dengue virus infection is important to be detected earlier through a laboratory examination to provide appropriate management and early public health control of dengue outbreak. At present, the three basic methods used by most laboratory for diagnosing dengue virus infection are virus isolation and identification, detection of viral genomic sequence by nucleic acid amplification assay (RT-PCR) and detection of dengue virus-specific IgM antibodies by IgM- capture enzyme-linked immunosorbent assay (MAC-ELISA) and/or rapid dengue immunochromatographic test for dengue specific IgM (DIT). Virus isolation and characterization was considered as the gold standard of laboratory diagnosis of acute dengue virus infection, it is however expensive and time consuming in detection as it requires at least 6-10 days for the virus to replicate in tissue culture cells or laboratory mosquitoes (adult or larvae). The current gold standard laboratory diagnosis is by reverse transcriptase-polymerase chain reaction (RT-PCR)[6]. This method is also an expensive method and is not widely available in most hospital diagnostic laboratories. Assay of anti-dengue specific IgM is dependent on the time taken for infected person's immune response to produce IgM antibodies against dengue virus antigens. Hence, both DIT and MAC-ELISA do not provide accurate information on early diagnosis of acute dengue because IgM is commonly firstly detected only on day 4-5 of illness in most cases. Besides, single serological detection of IgM merely indicate recent dengue virus infection and it should not be interpreted as a diagnosis of an acute infection without a paired second serum sample. Hence, a rapid test kit is particularly useful in providing early diagnosis of acute dengue virus infection[7].

The main advantage of using SD dengue duo rapid test kit is the time taken for the procedures to be carried out and the result interpretation is less than half an hour. Besides, it is a combination of both NS-1 antigen detection and differential IgG/IgM antibodies to dengue virus to human blood detection. NS-1 antigen is generally detected during Day 1 and up to Day 9 after onset of fever. Detection of NS-1 is however inhibited if anti-NS1 antibodies

are present. IgM, as mentioned above become detectable by Day 3 to Day 5 after onset of illness in primary dengue and by Day 1 to Day 2 after onset of illness in secondary infections[7].

However, in this study, only SD Dengue NS1 Ag component of the SD Dengue Duo rapid test kit is being evaluated and compared with SBPC immunocytochemistry method in diagnosis of dengue infections. NS-1 antigen detection in SD Dengue NS1 Ag rapid test kit has a sensitivity of 50%, which is significantly lower as compared to recent study[8]. On the other hand, immunocytochemistry (SBPCNS1) has a sensitivity of 88% and specificity of 100%, in which it compares well with recent study.

During testing with McNemar test, the result showed that the probability of change in sensitivity in both tests was not significant. Therefore, there is no significant tendency of change in terms of sensitivity that may occur. The result proves that the sensitivity of both SBPC immunocytochemistry and immunochromatographic methods using SD Dengue NS1 Ag component of SD Dengue Duo rapid test kit has insignificant tendency to changes by chance.

In the comparison of result in terms of day of fever of patient, it was observed that the highest sensitivity of SD Dengue NS1 Ag is at day 5 days, where the result is significantly good as 5 out of 8 positive samples were detected as positive and the accuracy compared to the total positive sample is 20.83%. Sample taken from patient suffer from fever for 4 days has highest percentage of positive detection with accuracy of 25% of the total positive sample. This does not show the sensitivity of test is highest at day 4 due to the total positive sample at day 4 of fever is 12, but the number of sample detected as positive is only 6. Thus, the accuracy at day 4 as compared with RT-PCR is only 50%.

For SBPC immunocytochemistry method, the highest sensitivity and specificity is on day 4 where all positive samples were detected as positive and has accuracy of 50% of total positive samples. All negative samples were also detected as negative with accuracy of 45.45% of total negative sample. On day 5, the sensitivity of SBPC immunocytochemistry is similar to SD Dengue NS1 Ag where 5 out of 8 positive samples were detected as positive and the accuracy is 20.83% when compared to total positive sample.

Day 1, 2, 3, 6 and 7 of fever are however not able to be evaluated well as the samples are less than 3 on those day. Therefore, it does not reflect the actual accuracy on those days because the small number of sample on those day will lead to bias of interpretation.

In a previous review, it was stated where the NS1 antigen detection decreases with increase of days of fever as the time progress, patient will develop antibody against NS-1 antigen. NS-1 antigen detection will also decrease when patient is encountering secondary infection as IgG in patient body will rapidly increase upon second exposure to dengue virus and it will form pre- existing virus-IgG immunocomplexes in the serum of patient. This leads to lower concentration of NS1 antigen present in secondary infection and hence directly compromising sensitivity of test by detection of NS1 antigen[9].

Previous study supported our finding that NS1 antigen detection decreases with increase day of fever. The sample size is also significantly larger than that of this study in evaluating the sensitivity of NS1 antigen detection at different day of fever[10].

From the data that was obtained in this study, it could be seen that the main advantage of SBPC immunocytochemistry is the high sensitivity, specificity, positive predictive value and negative predictive value as compared to SD Dengue NS1 Ag. Despite its high diagnostic significance, SBPC immunocytochemistry is a very complex method in detection of NS1 antigen as the reagents used in this method are not able to be prepared earlier. They can only be prepared during the period of testing[2]. Besides, expertise is required to observe the color change of monocytes and lymphocytes[9].

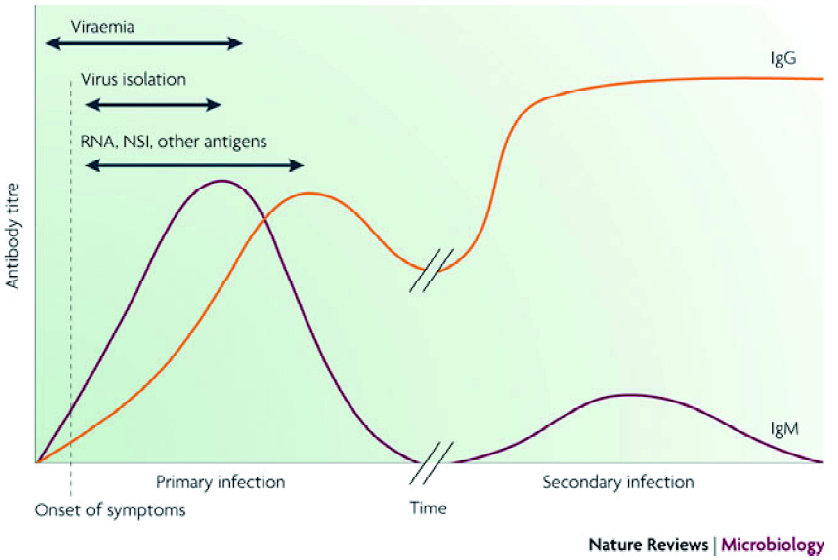

Figure 2. Major Diagnostic Markers for Dengue Infection (Peeling et al., 2010)[9].

Immunochromatography (SD Dengue NS1 Ag), on the other hand, is simple to be conducted as the sample that can be used are serum, plasma or whole blood. No training is required to perform this rapid test because only drops of sample were added into the sample well and interpretation is based on the presence or absence of color line in the kit. The total time needed to perform this test is also very short, approximately only 20 minutes is required to obtain the result of detection[4].

The major limitation in the research conducted is the sample used in the experiment is stored in freezer at the temperature of -80 °C for duration of almost 2 years. There were no previous study regarding the stability of NS-1 antigen in serum at such temperature, thus there is a possibility where the duration of storage may influence the structure of NS1 antigen, leading to low sensitivity. The second limitation is the indication for usage of frozen specimen is not clearly stated. The kit instruction only mentioned that frozen specimen should be brought to room temperature prior to use but there was no clear instruction or indication on the optimal temperature that should be achieved in the specimen before tested. The next is the small sample size that was used in this study. The samples that were tested were only enough to evaluate the sensitivity as compared to RT-PCR and its tendency to changes as compared to SBPC immunocytochemistry. The sensitivity on different days of fever is however not able to be established as the samples on fever day 1, 2, 3, 6 and 7 are subjected to interpretation bias because the samples tested were limited in number. The forth limitation in the data of SBPC immunocytochemistry and RT-PCR are based on previous studies conducted with the same sample used. The procedures of SBPC immunocytochemistry and RT-PCR were not conducted in this research. The last limitation is the study only evaluate the sensitivity and specificity of SD Dengue NS1 Ag and it does not reflect the actual sensitivity and specificity of the SD Dengue Duo rapid test kit as the IgG/IgM component of the SD Duo rapid test kit was not evaluated.

CONCLUSION

This study concludes that immunochromatographic method of NS1 detection using SD Dengue NS1 Ag yields significantly lower sensitivity as compared to SBPC immunocytochemistry method. Evaluation of sensitivity of SD Dengue NS1 Ag and SBPC immunocytochemistry on different day of fever is not able to be performed due to small sample size.

SUGGESTIONS

The combination use of both components in the SD Dengue Duo rapid test kit is needed to diagnose dengue virus infection more accurately as the sensitivity and specificity increases when both IgG/IgM component and NS-1 component were used. Larger sample size is needed in the next study to evaluate the sensitivity of test kit in different day of fever in patient as minimal sample size will not able to yield significant result. Further evaluation of NS1 antigen stability in frozen plasma is needed. Current study does not provide sufficient data to support the assumption of low temperature influence to stability of NS1 antigen. Streptavidin Biotin Peroxidase Complex Immunocytochemistrymethod in diagnosing acute dengue virus infection has huge potential for future use as it has very high diagnostic value significance and the method is more cost-efficient as compared to other commercial diagnostic tools available in the market.

REFERENCES

1. WHO. Dengue Guidelines for Diagnosis, Treatment, Prevention and Control, World Health Organization, 2009.
2. Umniyati SR. Teknik Immunositokimia dengan antibody Monoclonal DSSC7 untuk Kajian Patogenesis Infeksi dan Penularan Transovarial Virus Dengue Serta Survei lansi Virologis Vektor Dengue. [*Disertasi]*, Universitas Gadjah Mada, 2009.
3. Mulyaningrum U. Evaluasi Uji Immunositokimia Untuk Deteksi Infeksi Virus Dengue Pada Sediaan Apus Darah Tipis dan Tebal Penderita Demam. Tesis Untuk Derajad Master dalam Ilmu Kedokteran Tropis, Program Pascasarjana, Universitas Gadjah Mada, 2010.
4. Anonim. SD Dengue Duo rapid test kit, Standard Diagnostics Inc, 2010. Avaliable from: URL: http://www.standardia.com/html_e/mn03/mn03_01_00.
5. Landis JR, and Koch GG. The measurement of observer agreement for categorical data. Biometries, 1977;(33):159-74.
6. Herrmann JE. Immunoassays for The Diagnosis of Infectious Diseases In: P.R. Murray (ed): Manual of Clinical Microbiology. 6th ed. ASM Press. Washington DC, 1995:110-22
7. Shu P, Huang J. Current Advances in Dengue Diagnosis. ClinDiagn Lab Immunol, 2004;11: 642-50.
8. Seok MW, Shamala DS.Early Diagnosis of DengueInfection Using a Commercial Dengue Duo rapid Test Kit for the Detection of NS1, IgM and IgG. Geneva. Am J TropMed Hyg, 2010;83(3):690-5.
9. Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S et al.Evaluation of Diagnostic Tests: Dengue. Nature Reviews Microbiology 8, 2010;S30-7 doi: 10.1038/nrmicro2459
10. Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, et al.: Diagnostic Accuracy of NS1 ELISA and Lateral Flow Rapid Tests for Dengue Sensitivity, Specificity and Relationship to Viraemia and Antibody Responses. PLoS Negl Trop Dis 2009, 3:e360.

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b. ***Abstract*****:** The author should provide two abstract, in Indonesian and English language. All articles should be provided with an sbstract of between 200-300 words in one spacing. The abstract should be written in simple language with structured abstract style. Abstract should describe of the study using below headings: Introduction, Objectives, Methods, Results and Discussion, and Conclusion. Standard nomenclature should be used and abbreviations should be avoided.

c. ***Keywords*****:** A maximum of 5 keywords must be given at the end of the abstract.

d. ***Introduction***: The Introduction should provide the problem statement clearly, the relevant literature on the subject, and the proposed approach or solution.

e. ***Materials and methods*****:** The materials and methods should be clear enough to allow experiments to be reproduced. Previously published research procedure should be cited, and important modifications of it should be mentioned briefly. If the conducted research involved the use of human subjects or animal laboratory, it should be stated that the clearance from the Research Ethics Committee was obtained. The Editor may request a copy of the clearance document or informed consent form for verification.

f. ***Results and Discussion*****:** The Results should be presented with clarity and precision and explained without referring to the literature. The original and important findings should be stated. The Results should be illustrated with figures or tables where necessary but these should be kept to the minimum. The Discussion should interpret the findings in view of the results obtained against the background of existing knowledge. The Discussion should highlight what is new in the paper. Any assumption on which conclusions are made must be stated clearly

g. ***Conclusions:*** State the Conclusions in a few sentences at the end of the paper.

h. ***Acknowledgments***: The Acknowledgments should be presented at the end of the text and before the references. Technical assistance, financial support and advice may be acknowledged.

i. ***Tables:*** The tables should be kept to a minimum and be designed to be as simple as possible. Each table should be numbered consecutively in Arabic numerals and supplied

with a heading and a legend. Tables should be self-explanatory without reference to the text.

j. ***Figure:*** The figures should be numbered consecutively with Arabic numerals. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. The figures should be constructed in such a manner that they can be understood without reading the text. Appropriate symbols should be used on graphs and explained in the legends. Graphs should not duplicate results presented in tables. Title and comments of the figures and photographs should be provied on separate page using MS Word.

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..... it has been reported[1] .....

..... according to Sardjito[2] .....

..... Winstein & Swartz[3] conducted .....

..... by Avon *et al*.[4] .....

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Sample References

Scientific Journal

1. *Standard journal article*

   You CH, Lee KY, Chey RY, Menguy R. Electro-gastro-graphic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980; 79(2):311-14.

   Goate AM, Haynes AR, Owen MJ, Farral M, James LA, Lai LY, et al. Predisposing locus for Alzheimer's disease on chromosome 21. Lancet 1989;1:352-55.

2. *Organization as author*

   The Royal Marsden Hospital Bone-marrow Transplantation. Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977;2:742-44.

3. *No author given*

   Coffee drinking and cancer of the pancreas [editorial]. BMJ 1981;283-628.

4. *Article not in English*

   Massone L, Borghi S, Pestarino A, Piccini R, Gambini C. Localisations palmaires purpuriques de la dermatite herpetiforme. Ann Dermatol Venereol 1987;114:1545-47.

5. *Volume with supplement*

   Magni F, Rossoni G, Berti F, BN-52021 protects guinea-pig from heart anaphylaxis. Pharmacol Res Commun 1988;20 Suppl 5:75-78.

6. *Issue with supplement*

   Gardos G, Cole JO, Haskell D, Marby D, Paine SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

7. *Volume with part*

   Hanly C. Metaphysics and innateness: a psychoanalytic perspective.Int J Psychoanal 1988;69(Pt 3):389-99.

8. *Issue with part*

   Edwards L, Meyskens F, Levine N. Effect of oral isotretinoin on dysplastic nevi. J Am Acad Dermatol 1989;20(2 Pt 1):257-60.

9. *Issue with no volume*
   Baumeister AA. Origins and control of stereotyped movements. Monogr Am Assoc Ment Defic 1978; (3):353-84.
10. *No issue or volume*
    Danoek K. Skiing in and through the history of medicine. Nord Midicinhist Arsb 1982;86-100.
11. *Pagination in roman numerals*
    Ronne Y. Ansvarfall. Bloodtransfusion till fel patients. Vard-facket 1989;13:XXVI-XXVII.
12. *Type of article indicated as needed*
    Spargo PM, Manners JM, DDAVP and open heart surgery [letter]. Anaesthesia 1989;44: 363-64.
    Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondii [abstract]. Clin Res 1987; 35:475A.
13. *Article containing retraction*
    Shishido A. Retraction notice: Effect of platinum compounds on murine lymphocyte mitogenesis [Retraction of Alsabti EA, Ghalib ON, Salem MH. In: Jpn J Med Sci Biol 1979; 32:53-65). Jpn J Med Sci Biol 1980;33:235-37.
14. *Article retracted*
    Alsabti EA, Ghalib ON, Salem Mh. Effect of platinum compounds on murine lymphocyte mitogenesis [Retracted by Shishido A. In: Jpn J Med Sci Biol 1980;33:235-7]. Jpn J Med Sci Biol 1979;32:53-65.
15. *Article containing comment*
    Piccoli A, Bossatti A. Early steroid therapy in IgA neuropathy: still open question [comment]. Nephron 1989;51:289-91.
16. *Article in comment*
    Kobayashi Y, Fujii K, Hiki Y, Tateno S, Kurokawa A, Kamiyama M. Steroid therapy in IgA nephropathy: a retrospective study in heavy proteinuric cases [see comments]. Nephron 1988;48:12-7. Comment in: Nephron 1989;51:289-91.
17. *Article with published erratum*
    Schofield A. The CAGE questionnaire and psychological health [published erratum appears in Br J Addict 1989;84:701]. Br J Addict 1988;83:761-64.

Books and Other Monographs

18. *Personal author(s)*
    Colson JH, Armour WJ. Sports injuries and their treatment. 2nd rev. ed. London: S. Paul, 1986.
19. *Editor(s) as author*
    Diener HC, Wilkinson M, editors. Drug-induced headache. New York: Springer-Verlag, 1988.
20. *Organization(s) as author*
    Virginia Law Foundation. The medical and legal implications of AIDS. Charlottesville: The Foundation, 1987.
21. *Chapter in a book*
    Winstein L, Swartz MN. Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editors. Pathologic Physiology, mechanisms of disease. Philadelphia: Saunders, 1974:457-72.
22. *Conference proceedings*
    Vivian VL, editor. Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference or Child Abuse and Neglect; 1984 Ma 30-31; Chicago. Chicago: American Medical Association, 1985.
23. *Conference paper*
    Harley NH. Comparing radon daughter dosimetric and risk models. In:Gammage RB, Kaye SV, editors. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (MI):Lewis, 1985:69-78
24. *Scientific or technical report*
    Akutsu T. Total heart replacement device. Bethesda (MD): National Institutes of Health. National Heart and Lung Institute; 1974 Apr. Report No.:NIH-NIHI-69-2185-4.
    Disertasi Youssef NM. School adjustment of children with congenital heart disease [dissertation]. Pittsburg (PA): Univ. of Pittsburg, 1988.

25. *Dissertation*
    Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [Dissertation]. St Lucia, Qld: University of Queensland; 2007.
26. *Patent*
    Harred JF, Knight AR, McIntyre JS, inventors. Dow Chemical Company, assignee. Epoxidation process. US patent 3,654,317, 1972 Apr 4.

Other Published Material

27. *Newspaper article*
    Resberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7;Sect. A:2(col. 5).
28. *Audiovisual material*
    AIDS epidemic: the physician's role [videorecording]. Cleveland (OH): Academy of Medicine of Cleveland, 1987.
29. *Computer program*
    Renal system [computer program]. MS-DOS version. Edwardsville (KS): Medi-Sim, 1988.
30. *Legal material*
    Toxic Substances Control Act: Hearing on S. 776 Before the Subcomm. on the Environment of the Senate Comm. on Commerce, 94th Cong., 1st Sess. 343(1975).
31. *Map*
    Scotland [topographic map]. Washington: National Geographic Society (US), 1981.
32. Dictionary or Encyclopaedia
    Ectasia. Dorland's illustrated medical dictionary. 27th ed. Philadelphia: Saunders, 1988: 527.
33. *Classic material*
    The Winter's Tale: act 5, scene I, lines 13-16. The complete works of William Shakespeare. London: Rex, 1973.
34. *In press*
    Lillywhite HB, Donald JA. Pulmonary blood flow regulation in an aquatic snake. Science. In press.

Electronic Material

35. *Journal articel in the internet*
    Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm
36. *Monograph in electronic format*
    CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0 San Diego: CMEA; 1995.
37. Computer program
    Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational System; 1993.

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