

**DENGUE FEVER INFECTION EVALUATION OF ANTI-DENGUE IGM OR IGG USING
NUMEROUS RAPID DIAGNOSTIC TESTS (RDTS)**Won-Shik Kim¹, Yong-Gyoo Lee², Man Kyu Huh³ and Yong Lim^{4*}¹Department of Clinical Laboratory Science, Daejeon Health Institute of Technology, Daejeon, 34504, Republic of Korea.²Department of Social Welfare, Woosong Colledge, Daejeon, 34518, Republic of Korea.³Food Science and Technology Major, Dong-eui University, Busan 47340, Republic of Korea.⁴Department of Clinical Laboratory Science, Dong-eui University, Busan 47340, Republic of Korea.***Corresponding Author: Yong Lim**

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Article Received on 06/10/2018

Article Revised on 27/10/2018

Article Accepted on 17/11/2018

ABSTRACT

Dengue fever (DV) is a mosquito-borne tropical disease caused by one of four dengue viruses. It is necessary to test the commercial rapid diagnostic kits for DV infection. In this study, we evaluated the accuracy and precision of DV relative to the manufacturer's specifications at General Hospital Kulala Lumpur, KL, Malaysia. Three commercially available dengue rapid diagnostic tests (RDT) kits were evaluated. We used Asan Easy Test, BIOLINE Dengue Duo, and PanBio as the RDT in the study. Specificity of Asan Easy Test was 100.0% (75/75) for IgM and its sensitivity was 64.6% (73/113). The detection of IgG was same trend. Specificity of Asan EasyTest was 100.0% (75/75) for IgG and its sensitivity was 31.6% (18/57). Specificity of SD BioLine was 100.0% (75/75) for IgM and its sensitivity was 53.7% (50/113). Specificity of SD BioLine was 100.0% (75/75) for IgG and its sensitivity was 21.0% (12/57). Specificity of PanBio was 100.0% (75/75) for IgM and its sensitivity was 41.5% (47/113). Specificity of PanBio was 100.0% (75/75) for IgG and its sensitivity was 22.9% (13/57). Asan EasyTest had greater overall sensitivity than SD BioLine and PanBio.

KEYWORDS: Asan EasyTest, Dengue fever (DV), dengue rapid diagnostic tests (RDT), IgG, IgM.**INTRODUCTION**

Dengue fever (DV) is a mosquito-borne tropical disease caused by one of four dengue viruses (DENV). DV poses a significant worldwide public health threat with approximately 2.5 to 3 billion people residing in DV endemic areas, among whom 100 to 200 million individuals will be infected and approximately 30,000 patients will succumb to the disease, annually.^[1] Symptoms typically begin three to fourteen days after infection.^[2] This may include a high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash. A vaccine for dengue fever has been approved and is commercially available in a number of countries.

It is caused by four different viruses and spread by *Aedes aegypti* mosquito. Dengue outbreaks have also been attributed to *A. albopictus*, *A. polynesiensis* and several species of the *A. scutellaris* complex. Like other flaviviruses, its genome comprises a single strand of positive-sense RNA encoding 3 structural and 7 nonstructural (NS) proteins.^[3]

Timeline of dengue biomarker appearance in patients experiencing primary and secondary infection. In primary infection (top panel), both nonstructural protein

1 (NS1) and virus can be detected from the onset of disease, with immunoglobulin M (IgM) appearing around day 3 of illness and immunoglobulin G (IgG) appearing toward the end of the acute period.^[4] Secondary infections are characterized by the presence of IgG early in the acute phase of disease and a shorter duration of NS1 and virus detection.

Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5-7 days.^[5] The highest levels (titres) of IgM are detected following a primary infection, but IgM is also produced in reinfection. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months.^[5,6] IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2-3 months. After a primary infection, IgG reaches peak levels in the blood after 14-21 days. In subsequent re-infections, levels peak earlier and the titres are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus.^[7,8] In testing for IgG and IgM antibodies there may be cross-reactivity

with other flaviviruses which may result in a false positive after recent infections or vaccinations with yellow fever virus or Japanese encephalitis.^[7] The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic.^[9]

Detection of the DV nonstructural protein 1 (NS1) has emerged as an alternative biomarker to both serologic and molecular based techniques for diagnosis of acute DV infection. NS1 antigenemia is detectable within 24 hours and up to 9 days following symptoms onset. This overlaps with the DV viremic phase and NS1 is often detectable prior to IgM seroconversion. Concurrent evaluation for the NS1 antigen alongside testing for IgM- and IgG-class antibodies to DV (DENGEM) provides optimal diagnostic potential for both early and late dengue disease.

The diagnosis of dengue fever may be confirmed by microbiological laboratory testing.^[10] Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost.^[11] In this study, we evaluated the accuracy and precision of DV relative to the manufacturer's specifications at General Hospital Kulala Lumpur, KL, Malaysia.

MATERIALS AND METHODS

Clinical specimens and Clinical evaluation of RDT

Serological diagnosis depends on the presence of IgM antibody or a rise in IgG antibody titre in paired acute and convalescent phase sera. IgM antibody becomes detectable during the acute phase of the illness and 90% of patients are IgM positive by the sixth day after onset of symptoms. Currently, the most widely used IgM assay is a capture ELISA (enzyme-linked immunosorbent assay).^[12]

Three commercially available dengue RDT kits were evaluated. The comparative analyzers were performed by General Hospital Kulala Lumpur, KL, Malaysia during October 2011 - January, 2012. A total of 170 virus isolation was attempted for all acute samples, and DENV was identified using serotype-specific IFAs. We used Asan EasyTest as the RDT in the study. The Standard Diagnostics (Korea) BIOLINE Dengue Duo Kit.^[13] as the rapid diagnostic tests (RDTs) was used for reference serology. The Panbio Japanese Encephalitis Dengue IgM Combo ELISA was also used for reference serology (Panbio, Australia; Cat. # E-JED01C; Lot # 110061).^[14]

Assays were performed according to the manufacturers' instructions. In brief, 100µL of whole blood or serum sample was transferred by pipette into the sample well of the freshly unpackaged test device. The appearance of the test and control lines after a specified migration time (15-30 minutes) indicated a positive result.^[15] All RDTs

had a control line and a test line. For each RDT involving the interpretation of the presence of a line, two people read the results independently and concurred on a given call. The technicians carrying out the evaluation of the test articles were blind to the DENV-infection status of the panel of serum samples and interpreted the colour line (red) on the immunochromatographic strip. Whole blood and serum intended for use with the DENV RDT was either used immediately following sampling, or refrigerated for a maximum of 48 hours at 5°C before being equilibrated to room temperature (approximately 25°C) prior to testing.^[16]

Prepare a clean plastic test tube and add 75µl of the diluted solution. Add 1 µl of serum into the solution and mix well. Test strip containing diluted solution and serum add the mixed tube. Place the sample line immersed in the solution in a test tube and let stand for 5 minutes. The diluted solution is completely absorbed in the test strip and allowed to stand for 10 to 15 minutes until it is dry, and the result is read.

Statistical analysis

The sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for the assays were calculated based on "true positive dengue samples" (virus isolation/PCR positives, sero-negative acute sera, acute primary, acute secondary) using the following formula:

$$\% \text{ Sensitivity} = a/(a+c) \times 100\%$$

$$\% \text{ Specificity} = d/(b+d) \times 100\%$$

$$\text{Efficiency} = (a+d)/(a+b+c+d) \times 100\%$$

$$\% \text{ PPV} = a/(a+b) \times 100\%$$

$$\% \text{ NPV} = d/(c+d) \times 100\%$$

Where, a = the number of true positives, b = the number of false positives, c = the number of false negatives, and d = the number of true negatives.

Statistical analysis was performed with Statistica version 18 (StatSoft, Inc., Tulsa, OK). Significance was assigned at $p < 0.05$ for all parameters and were two-sided unless otherwise indicated. Uncertainty was expressed by 95% confidence intervals. Categorical variables between groups were compared by Fisher's exact test. The t-test was used for continuous variables.

RESULTS

The characteristics of the study population (n = 320 cases) that contributed acute plasma to the test panel is shown in Table 1. The panel of dengue cases (n = 170) were consecutively enrolled. A total of 170 prospective serum samples submitted for dengue virus (DV) IgM and IgG testing by the Focus Diagnostics DV IgM and IgG EIAs were also tested by the InBios IgM and IgG DV assays (Figures 1 and 2). The appearance of the control line alone indicated a negative result. The results were compared and the data summarized in Table 1. Specificity of Asan Easy Test was 100.0% (75/75) for IgM and its sensitivity was 64.6% (73/113). The

detection of IgG was same trend. Specificity of Asan EasyTest was 100.0% (75/75) for IgG and its sensitivity was 31.6% (18/57). Specificity of SD BioLine was 100.0% (75/75) for IgM and its sensitivity was 53.7% (50/113). Specificity of SD BioLine was 100.0% (75/75) for IgG and its sensitivity was 21.0% (12/57). Specificity of PanBio was 100.0% (75/75) for IgM and its sensitivity was 41.5% (47/113). Specificity of PanBio was 100.0% (75/75) for IgG and its sensitivity was 22.9% (13/57). The specificity of IgM and IgG tests alone was not significantly different among test samples. However, the sensitivity of their tests alone was significantly different among test samples. Asan EasyTest had greater overall sensitivity than SD BioLine and PanBio.



Figure 1: Validation of the dengue IgG/IgM rapid diagnostic test (RDT) by a comparative evaluation.



Figure 2: The Asan EasyTest showed dengue IgG/IgM rapid strip in a plastic cassette. Serial numbers of clinical specimens are shown on each device.

Table 1: Clinical evaluation of specificity and sensitivity using the three dengue IgG/IgM rapid tests developed in this study

RDTs	Specificity (%)		Sensitivity (%)	
	IgM (n= 75)	IgG (n= 75)	IgM (n= 113)	IgG (n= 57)
Asan Easy Test	100	100	64.6 (73/113)	31.6 (18/57)
SD Bio Line	100	100	53.7 (50/113)	21.0 (12/57)
Pan Bio	100	100	41.5 (47/113)	22.9 (13/57)

DISCUSSION

The utility of the detection of anti-Dengue IgA as a recent infection indicator has already been demonstrated by some researchers.^[17] Talarmin et al.^[18] have

determined the presence of anti-Dengue IgM and IgA antibodies in the sera of 178 patients with classic dengue disease. Groen et al.^[19] also have suggested the diagnostic value of anti-dengue IgA detection in the serum using immunofluorescence assays, even though the highest percentage of IgA detection was observed in acute phase serum samples of secondary infections.

The rapid test used a novel format in order to simultaneously detect both anti-dengue IgG and anti-dengue IgM in infected blood with high sensitivity and specificity.^[20] For example, Carter et al.^[16] prospectively assessed the Standard Diagnostics (Korea) BIOLINE Dengue Duo DENV rapid diagnostic test to NS1 antigen and anti-DENV IgM (NS1 and IgM) in children in Cambodia, with the aim of improving the diagnosis of DENV infection. DENV RDT NS1 antigen alone had a sensitivity of 60.8% in comparison to reference NS1 assay, and RDT anti-DENV IgM had a sensitivity of 32.7% in comparison to reference anti-DENV IgM assay.^[16] For other example, the sensitivity and specificity in diagnosing acute dengue infection in the SD Duo NS1/IgM were 88.65% and 98.75%, respectively.^[2] They reported that of the 320 sera for dengue, 168 (52.5%) tested positive for SD Duo NS1 Ag, and 220 (68.75%) tested positive for SD Duo IgM. A diagnostic strategy combining SD Duo NS1 or IgM (NS1/IgM) gave a 289 (90.31%) positive detection. The assay is sensitive and highly specific. Detection of both NS1 and IgM by SD Duo gave comparable detection rate by either serology or RT-PCR.^[21] Basically, a test configuration is similar to what is used in other test kits.

Test results should be used in conjunction with clinical evaluation, including exposure history and clinical presentation. False-positive results, particularly with the dengue virus IgG enzyme-linked immunosorbent assay (ELISA), may occur in persons infected with other flaviviruses, including West Nile virus and St. Louis encephalitis virus. Obtaining a detailed exposure history and further laboratory testing may be necessary to determine the infecting virus. Moreover, the ELISA cross-reacts with other flaviviruses. Samples positive for IgM antibody alone are thus not confirmatory for current infection, and are reported only as “probable” dengue. For a diagnosis of “confirmed” dengue, dengue virus should be identified by isolation, immunohistochemistry in necropsy tissue, or there should be a four-fold rise in antibody titre using a type-specific plaque reduction neutralisation test.^[22,23] In case of conjunction with other parameters such as ELISA or RT-PCR, the performance data of the Asan Easy Test can help determine which dengue diagnostics should be used during the first few days of illness, when the patients are most likely to present to a clinic seeking care.

CONCLUSION

Three commercially available dengue rapid diagnostic tests (RDT) kits were evaluated. We used Asan Easy Test, BIOLINE Dengue Duo, and PanBio as the RDT in

the study. Asan EasyTest had greater overall sensitivity than SD BioLine and PanBio.

REFERENCES

1. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature*, 2013; 496: 504-7.
2. Kularatne SA. Dengue fever. *BMJ*, 2015; 351: h4661.
3. Perera R, Kuhn RJ. Structural proteomics of dengue virus. *Curr Opin Microbiol*, 2008; 11: 369-77.
4. Muller DA, Alexandra CI, Depelsenaire ACI, Young PR. Clinical and Laboratory Diagnosis of Dengue Virus Infection. *The Journal of Infectious Disease*, 2017; 215: S89-S95.
5. WHO. Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control. 2nd ed., Geneva: World Health Organization, 1997.
6. Innis B, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg*, 1989; 40: 418-27.
7. Simmons CP, Farrar JJ, Nguyen VV, Wills B. Dengue. *The New England Journal of Medicine*, 2012; 366: 1423-32.
8. Varatharaj A. Encephalitis in the clinical spectrum of dengue infection. *Neurology India*, 2010; 58: 585-91.
9. Gubler DJ. Dengue viruses. In: Mahy BWJ and Van Regenmortel MHV (eds.). *Desk Encyclopedia of Human and Medical Virology*, Boston; Academic Press, 2010; 372-82.
10. Wiwanitkit V. Dengue fever: diagnosis and treatment. *Expert Review of Anti-Infective Therapy*, 2010; 8: 841-5.
11. WHO. Dengue Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva: World Health Organization: 2009.
12. Burke DS, Nisalak A, Ussery MA. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. *J Clin Microbiol*, 1982; 16: 1034-42.
13. SD BIOLINE. Dengue Duo NS1 Ag + Ab Combo. PLOS; 2014; <http://www.alere.com/ww/en/product-details/sd-bioline-dengue-duo-ns1-ag-ab-combo.html>.
14. Panbio. Japanese encephalitis-Dengue IgM Combo. PLOS, 2014; <http://www.alere.com/ww/en/product-details/panbio-japanese-encephalitis-dengue-igm-combo.html>.
15. Pal et al. Evaluation of Dengue NS1 Antigen Rapid Tests and ELISA Kits Using Clinical Samples. PLOS, 2014; doi.org/10.1371/journal.pone.0113411.
16. Carter MJ, et al. Diagnostic accuracy and incorporation into diagnostic algorithms. PLOS, 2015; DOI:10.1371/journal.pntd.0003424.
17. Dutra NR, et al. The Laboratorial Diagnosis of Dengue: Applications and Implications. *J Glob Infect Dis.*, 2009; 1: 38-44.
18. Talarmin A, Labeau B, Lelarge J, Sarthou JL. Immunoglobulin capture enzyme-linked immunosorbent assay for the diagnosis of dengue fever. *J Clin Microbiol*, 1998; 36: 1189-92.
19. Groen J, Velzing J, Copra C, et al. Diagnostic value of dengue virus specific IgA and IgM serum antibody detection. *Microbes Infect*, 1999; 1: 1085-90.
20. Lee J, et al. Enhanced performance of an innovative dengue IgG/IgM rapid diagnostic test using an anti-dengue EDI monoclonal antibody and dengue virus antigen. *Scientific Reports*, 2015; PMC4676027. doi: 10.1038/srep18077.
21. Wang SM and Sekaran SD. Early diagnosis of Dengue infection using a commercial Dengue Duo rapid test kit for the detection of NS1, IGM, and IGG. *Am J Trop Med Hyg*, 2010; 83: 690-5.
22. Russell PK, Nisalak A, Sukhavachana P, Vivona S. A plaque reduction test for dengue virus neutralizing antibodies. *J Immunol*, 1967; 99: 291-96.
23. Vorndam V, Kuno G. Diagnosis of dengue virus infections. In: Gubler DJ and Kuno G (eds.). *Dengue and Dengue Hemorrhagic Fever*, Wallingford, UK; CAB International, 1997; 313-33.