ISSN: 0377-4929

Indian Journal of Pathology & Microbiology

Volume 54 • Issue 4 • Oct-Dec 2011

Official Publication of
Indian Association of Pathologists and Microbiologists

Impact Factor® for 2010: 0.57

www.ijpmonline.org

Published by Medknow Publications

Dengue rapid strip test: A Sri Lankan experience

Sir,

Some commercial kits have been evaluated against reference tests, but performance can be affected by local conditions/factors in the country of use. The objective of this study was, to evaluate the Dengue Duo IgM and IgG Rapid Strip test, PanBio, Brisbane, Australia (Catalogue No. R-DEN02D; DDRST) as a screening test for dengue and its ability to differentiate primary and secondary infections under local conditions.

Patients with nonbacterial undifferentiated fever ('viral fever'; n=207; March 1998 to July 1999) and 85 clinically suspected dengue patients were recruited (March 1998 to March 2000) from one paediatric and one adult medicine ward of the North Colombo Teaching Hospital, Ragama, Sri Lanka. Patients with a positive blood picture for malaria or who clinically responded to antimalarials were excluded.

Case definition for viral fever: History of fever or recordable temperature after admission of >37.2°C (>99°F); peripheral white blood cell count that did not show elevated total with elevated neutrophil counts (to exclude possible bacterial infections); no definitive diagnosis at the time of discharge, therefore classed as "viral fever". Case definition for clinically suspected dengue infection: World Health Organisation criteria were used.

Paired sera were collected (viral fever = 93; suspected dengue = 50 patients) soon after admission (mean 6.9 days; median 6 days; range 2-21 days following onset of symptoms) and ten days later. Serum was stored at –20°C until tested.

U.S. Armed Forces Research Institute of Medical Sciences (AFRIMS, Bangkok, Thailand) reference enzyme immunosorbent assay (EIA) was the "gold standard" for dengue and Japanese encephalitis virus (JEV). DDRSTwas performed and interpreted according to the manufacturer's instructions. The intensity of the reaction was graded independently by two people, as doubtful positive (DP; very faint colour), low positive (LP; colour less intense than control line) and strong positive (SP; colour equal to or greater in intensity to control line). Interobserver κ agreement scores were calculated for grading of the colour reaction of IgM and IgG test lines by two observers.

Performance of EIA, interpretation of results and classification of dengue infection status was as described previously,[1,2] with a few subsequent modifications to the protocol by AFRIMS. All samples were tested in parallel for dengue virus (DV) and JE virus (JEV) antibodies. Sensitivity and specificity of detecting dengue was 84.1% (37/44) and 93.9% (93/99) using serum collected soon after admission. Although the sensitivity of detecting acute primary dengue (APD) was 90.9% (10/11), sensitivity of detecting acute secondary dengue (ASD) was only 51.5% (17/33).

Table 1: Diagnostic accuracy of DDRST for diagnosing dengue virus infections and differentiation into primary and secondary infections

	% Overall accuracy (95% CI) n = 143				*% Sensitivity for differentiating primary and secondary*	
	Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*	*Primary n=11*	*Secondary n=33*
Acute serum IgM	81.8 (66.8-91.3)	94.9 (88.06-98.1)	87.8 (72.9-95.4)	92.2 (84.6-96.3)	-	-
Acute serum IgM and IgG	84.1 (69.3-92.8)	93.9 (86.7-97.5)	86.0 (71.4-94.2)	93.0 (85.6-96.9)	90.9	51.5
Paired sera	93.2 (80.3-98.2)	92.9 (85.5-96.9)	85.4 (71.6-93.5)	96.8 (90.4-99.2)	100	60.6

DDRST: Dengue Duo IgM and IgG Rapid Strip test

One patient diagnosed as ASD by DDRST criteria (anti-dengue IgG "DP" reaction in convalescent serum only) was diagnosed with JEV infection by the EIA. This patient had anti-JE IgM levels more than 40 EIA units in both acute (56 EIA units) and convalescent samples (105 EIA units), while anti-dengue IgM of 83 EIA units was found only in the convalescent sample. Based on the standard reference EIA this was a false positive of the DDRST. All other false positive reactions were either LP or DP and occurred both in the IgM and IgG test line (different from findings by Cuzzubbo *et al.*[3] Inter-observer κ agreement score was 0.88 (95% CI 0.81-0.95) indicating very good agreement.

Two studies by Blacksell *et al.*[4] report sensitivities of the DDRST compared to the AFRIMS EIA, for detecting acute serum IgM as 65.3% (total = 491) and 10.5% (total = 174) respectively.[4,5] This is considerably lower than what is seen in our study (81.8; 95% CI 66.8-91.3). However, one of the first studies to evaluate the DDRST reports a sensitivity of 90% (total = 143) in acute serum for both IgM and IgG which is comparable to ours of 84.1%.[3] The specificities reported in all four studies were high and comparable, ranging from 86% to 98%.[3-5]

Our study shows that the DDRST underestimates the number of secondary infections by identifying them as primary [Table1]. This finding differs from claims of the manufacturer and evaluation test results of Cuzzubbo *et al.*,[3] who found that the DDRST detected 91% of APD (total = 20) and 86% (total = 16) of ASD.[3] The other two studies also reported (sensitivity for ASD 56.4% and 42.1% respectively) that the DDRST did not reliably differentiate primary from secondary infection, and that it should not be used for this purpose.[4,5] Except for one study where only 25% of the primary cases were identified,[5] all the other studies including ours, did not have a problem in identifying primary infections.[3,4]

Of the seven false positives, one was due to IgG antibodies to JEV cross reacting with dengue antigens. This has been reported in other studies as well.[3-5] As reported by Cuzzubbo *et al.*[3] and also in our study, cross reaction with JEV occurred only in the IgG test line with none in the IgM test line.[3] In a country like Sri Lanka where both JEV and dengue co-exists, a doubtful positive reaction occurring only in the IgG test line has to be interpreted with caution.

Some investigators have found that the DDRST had a very low specificity (54.3%; 19/35) when used in patients positive for *Plasmodium faclciparum* malaria positive blood samples.[6] Cuzzubbo *et al.*[3] reported a specificity of 87% (13/15) in patients with blood smear positive cases of malaria.[3] In contrast 25 sera each with *Plasmodium falciparum* and *Plasmodium vivax* when tested by Blacksell *et al.*[4] did not give rise to any cross reactions.[4] These differences are hard to explain. Our study excluded patients who were positive for malaria and therefore we cannot comment on the performance of the DDRST in malarial patients.

DDRST is not a complicated test to perform and inter-observer agreement was very good. Therefore, differences in diagnostic accuracy reported previously in other studies cannot be accounted for by variations in how the test was performed. Variations with different batches of the kit may account for some of these differences if strict quality control was not adhered to.

DDRST should not be used for differentiation of primary and secondary dengue infections. This study also highlights the need for testing commercial kits under local conditions as results from preliminary evaluation tests may not always apply at the point of use.

ACKNOWLEDGMENT

I wish to extend my special thanks to Lieutenant Colonel P. Mammen Mammen and Dr. Ananda Nisalak of the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, for giving me the opportunity to work in their laboratory. I appreciate the excellent technical assistance and co-operation extended by Ms. Khun Panor.

Kamani M. Gunasekera, Channa P. Senanayake[1], Lalitha Mendis[2]

Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [1]Department of Microbiology, Faculty of Medicine, Colombo, [2]University of Colombo, President Sri Lanka Medical Council, Srilanka

Address for correspondence:
Dr. H.A.K.M. Gunasekera, Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. E-mail: kamani.lk@gmail.com

REFERENCES

1. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya V, Chongswasdi S, Suntayakorn P, *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.
2. Vaughn DW, Nisalak A, Solomon T, Kalayanarooj S, Nguyen MD, Kneen R, *et al*. Rapid serologic diagnosis of dengue virus infection using a

commercial capture ELISA that distinguishes primary and secondary infections. Am J Trop Med Hyg 1999;60:693-8.
3. Cuzzubbo AJ, Endy TP, Nisalak A, Kalayanarooj S, Vaughn DW, Ogata SA, *et al*. Use of recombinant envelope proteins for serological diagnosis of dengue virus infection in an immunochromatographic assay. Clin Diagn Lab Immunol 2001;8:1150-5.
4. Blacksell SD, Newton PN, Bell D, Kelley J, Mammen MP Jr, Vaughn DW, *et al*. The comparative accuracy of 8 commercial rapid immunochromatographic assays for the diagnosis of acute dengue virus infection. Clin Infect Dis 2006;42:1127-34.
5. Blacksell SD, Bell D, Kelley J, Mammen MP JR, Gibbons RV, Jarman RG, *et al*. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. Clin Vaccine Immunol 2007;14:1458-64.
6. Charrel RN, de Lamballerie X. Low specificity of an immunochromatographic serological assay for diagnosis of dengue fever in travelers returning with malaria. Clin Diagn Lab Immunol 2002;9:1400.

Access this article online	
Quick Response Code:	Website: www.ijpmonline.org
	DOI: 10.4103/0377-4929.91537