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Comparative evaluation of validity and cost-benefit analysis of rapid diagnostic test (RDT) kits in diagnosis of dengue infection using composite reference criteria: A cross-sectional study from south India

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ABSTRACT

Background & objectives: Rapid diagnostic test (RDT) kits are widely used in India for the diagnosis of dengue infection. It is important to evaluate the validity and reliability of these RDTs. The study was aimed to determine the sensitivity, specificity and predictive value of four commercially available RDTs [Panbio Dengue Duo cassette, Standard Diagnostics (SD) Bioline Dengue Duo, J. Mitra Dengue Day-1 test and Reckon Dengue IgG/IgM] against composite reference criteria (CRC), and compare the cost of the tests.

Methods: In this prospective observational study for diagnostic accuracy, we tested stored blood samples from 132 cases of dengue and 149 controls of other infections as classified based on CRC, with all the four RDTs. The CRC was based on the epidemiological considerations, common clinical features and laboratory abnormalities. The non-dengue controls were the cases of proven alternative diagnosis. The diagnostic performances of the tests were compared in terms of sensitivity, specificity and predictive value along with the cost involved per test.

Results: The sensitivity of the Panbio and SD RDT kits was found to be 97.7 and 64.3% respectively, and the specificities were 87.8 and 96.6% respectively. The sensitivity of the NS1 antigen capture by SD Duo, Reckon, J. Mitra RDTs was 20.9, 18.6 and 27.1% respectively. The prevalence of dengue specific IgG antibody with Panbio RDT kits was 49.3%. The cost per test for Panbio, SD, Reckon and J. Mitra is US$ 6.90, 4.27, 3.29 and 3.61 respectively.

Conclusion: It was concluded that in dengue outbreak, Panbio IgM capture RDT alone is reliable and easily available test which can be used in acute phase of dengue infection in any resource limited set up. NS1 capture rates by any of the other three RDTs might not be reliable for the diagnosis of acute dengue infection.

Key words Composite reference criteria; dengue; immunochromatography; rapid diagnostic test (RDT) kits

INTRODUCTION

Dengue is the most common arthropod-borne viral disease which occurs in endemic proportions in South-east Asia and pacific countries[1]. According to the estimates, annual apparent cases of dengue infection range between 50 and 96 million worldwide, and nearly 70% of the disease burden occurs in Asia and is on constant rise[2]. The spectrum of infection varies from asymptomatic infection to serious life-threatening hemorrhagic shock. The estimated overall case fatality rate in India is 3–5%[3–6]. Early and accurate diagnosis is crucial in reducing the mortality. Almost 90% of the patients with dengue infection remain asymptomatic[7]. Recognition of acute dengue infection may be challenging since the symptoms are non-specific and mimic several other causes of acute undifferentiated febrile illness. The severe manifestations of dengue include hemorrhagic manifestations, multiorgan dysfunction and distributive shock. Hence, early diagnosis of dengue infection and initiation of appropriate fluid resuscitation is the key component in the management of severe dengue infection. World Health Organization[3] recommends a syndromic case definition that can help in identifying dengue cases in endemic areas[3]. However, the syndromic approach alone may be of limited utility in the diagnosis or management of severe dengue cases[8]. The lack of cardinal features of dengue infection compels the clinicians to depend on diagnostic tests.

The ideal test for early diagnosis of dengue infection

should be able to distinguish it from other diseases of similar clinical spectrum. The test needs to be highly sensitive, rapid, inexpensive, easily performable and can be done at temperatures >30 °C[1]. WHO recommendations for the diagnosis of dengue include ELISA based detection of dengue-specific IgM antibodies or a ≥4-fold increase in the titer of total antibodies to dengue virus in paired acute and convalescent sera or detection of dengue virus by reverse transcription–polymerase chain reaction. However, these tests are costly, time consuming, laborious, technologically demanding and are not always available in most resource limited set ups and during epidemics[2, 9]. Hence, a large fraction of medical practitioners depend on readily available rapid diagnostic test kits which are cheaper and have less turn-around time for the early diagnosis of dengue infection.

The RDTs are based on immunochromatographic methods to detect IgM and IgG antibodies with or without NS1 antigen in the serum of the patients. Many different RDT kits are available commercially which are widely used for the diagnosis of dengue fever in secondary and tertiary care centers across India as these give results within an hour and technically are less demanding. The issue, however, lies with the reliability and performance of these tests. Therefore, there is an urgent need to evaluate the readily available and cheaper commercial rapid diagnostic test (RDT) kits as an adjunct to the clinical criteria for accurate diagnosis of dengue fever[9–14].

In this prospective observational study, we evaluated the sensitivity, specificity and predictive value of four commonly used rapid test kits in India manufactured by four different manufacturers', *viz.* Panbio, Standard Diagnostics, J. Mitra and Reckon diagnostics in a tertiary care centre in south India during the dengue fever outbreak season in September 2012 to February 2013.

MATERIAL & METHODS

Study design, population and setting

We conducted this prospective cross-sectional observational study at Christian Medical College (CMC), Vellore, India which is a 2500 bedded tertiary care teaching hospital in south India. The study proposal was reviewed and approved by the institutional review board and ethics committee of CMC, Vellore. All adult patients (>18 yr) presenting to either medicine outpatient or emergency medicine department with acute (<14 days) febrile illness (AFI) between September 2012 and February 2013 were included in this study. This season corresponds to the dengue fever outbreak in south India. After obtaining the written informed consent from the patient (or nearest relative in case the patient was unable to give consent), the demographic, clinical and relevant blood investigations results were recorded. For the etiological diagnosis, blood tests were performed for serological confirmation (as mentioned below), blood culture and malarial parasite smear. The blood samples were sent to the laboratory of clinical virology at our hospital for testing with dengue RDTs, where it was stored at –80°C in aliquots until batch testing.

Composite reference criteria for diagnosis of dengue infection and its rationale

In south India, outbreak of dengue infection occurs during the months of October to February[15]. It shares the clinical, epidemiological and certain laboratory features with other common infectious etiologies like scrub typhus, malaria, enteric fever and septicemic illness. We developed a CRC for defining the cases of dengue infection. A team of experts comprising of an infectious diseases expert, a virologist and an epidemiologist at CMC, Vellore derived the CRC, which included the clinical features suggested in the WHO guidelines for dengue[3], the clinical and supportive laboratory features seen in the dengue cases, and exclusion of all the competing etiology causing similar clinical illness[14–15]. The rationale for the need for a composite criteria was that there was a lack of accuracy in the WHO syndromic case definition for dengue related illness and that there is no single cheap and readily available test that can be considered to be the gold standard for a rapid diagnosis of dengue.

Although, the CRC was not validated, but this was improvised on the WHO guidelines and exclusion of major differential diagnosis in the outbreak situation will enable to improve the predictive value of the CRC. Thus, CRC was based on three categories, *viz.* clinicoepidemiological data, supportive laboratory parameters and exclusion of the other common etiologies of acute undifferentiated febrile illness (Table 1). The study was conducted during the outbreak period of dengue fever in Indian set up in order to increase the pre-test likelihood of dengue. Hence, we believe that the CRC will be highly specific for selecting dengue cases.

Rationale for selection of non-dengue controls

The cases with confirmed etiology of cause other than dengue were used as non-dengue controls for the study. Parasitological diagnosis of malaria was established by microscopic examination of the Giemsa stained peripheral blood thick and thin smears. Diagnosis of scrub typhus was confirmed by IgM ELISA positivity and/or presence of a pathognomonic eschar with IgM ELISA was performed on serum samples using the scrub typhus.

Table 1. Composite reference criteria for diagnosis of dengue related illness

Part A: Clinico-epidemiological criteria—All four need to be fulfilled
(1) Epidemiological data (onset of illness during the peak season, *i.e.* September–February).
(2) Illness of community acquired origin.
(3) Acute febrile illness—Duration of illness <14 days.
(4) Patient having one or more of the following symptoms—Headache, myalgia and rash.

Part B: Supportive laboratory parameters—At least one criteria need to be fulfilled.
(1) Thrombocyte count <100,000 cells/mm^3.
(2) Total white blood cell count <10,000 cells/mm^3.

Part C: Exclusion of other etiology of acute febrile illness—All four need to be fulfilled.
(1) Malaria: Negative peripheral blood smear for malarial parasites.
(2) Scrub typhus: Absence of an eschar and a negative scrub typhus ELISA* (PanBio Ltd, Brisbane, Australia).
(3) Leptospirosis: Negative for Leptospira IgM ELISA* (Virion/Serion GmbH, Germany).
(4) Bacterial infection: Sterile blood/body fluid cultures.

*ELISA: Enzyme linked immunosorbent assay.

detect (InBios International, Inc., Seattle, USA) as per the manufacturer's instructions. An optical density (OD) >0.5 was considered positive. Enteric fever and other bacteremic illness were diagnosed based on blood culture. Leptospirosis was confirmed, if IgM ELISA (Virion Serion GmbH, Germany) was positive.

Test methods and interpretation

Four commercially available and most commonly used RDTs were selected for the study, from the following manufacturers: Panbio®(Dengue Duo cassette), Standard Diagnostics Bioline (Dengue Duo), J. Mitra (Dengue Day-1 test), and Reckon Diagnostics (Dengue IgG/IgM). These RDTs are based on immunochromatographic detection of anti-dengue IgG and IgM with or without dengue virus NS1 antigen (except Panbio test kit) in whole blood, serum, or plasma. The well of the test kit contains recombinant dengue virus envelope proteins-colloidal gold conjugates and anti-dengue NS1 Ag-colloid gold conjugate. Upon addition of patient's sample with the buffer, antigen-antibody complex is formed which migrates along the length of the test strip by capillary action where it is captured by the anti-human IgG and/or anti-human IgM (in case of anti-dengue IgG and IgM) and anti-dengue NS1 antigen (in case of NS1 antigen) to yield a chromatographic band visible with the naked eye. Appearance of the chromatographic band at IgM and IgG region is interpreted as primary and secondary infection respectively. Appearance of the coloured band at control region only indicates a negative test.

While all the four RDTs detect anti-dengue IgM and IgG, they differ in the detection of NS1 antigen, volume of patient's sample required and time-to-interpretation. The SD Duo, J. Mitra Dengue Day-1 and Reckon RDT kits detected NS1 antigen and anti-dengue IgM/IgG, requiring 100 and 10 µl of the serum samples respectivelys and 20 min for time-to-interpretation. On the other hand, Panbio RDT detected only anti-dengue IgM/IgG, requiring 10 µl of the serum sample and 15 min for the time-to-interpretation.

The serum samples from the patients were stored at –80°C in aliquots until batch testing. All the assays for the RDT kits in this study were performed as per the manufacturer's instructions. The test results were examined and interpreted according to the manufacturer's instructions by two different readers to avoid bias in the interpretation.

Statistical methods

Clinical and hematological results between the dengue cases and non-dengue controls were assessed for statistical significance (p <0.05), using either Student's t-test or the Wilcoxon signed-rank test, with SPSS (version 21). The true-positive, false-positive, false-negative, and true-negative values were calculated by constructing 2-by-2 table using RDT results in comparison with those of the final case diagnosis based on the reference criteria. The standard diagnostic accuracy indices of sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) were calculated for each RDT.

RESULTS

Clinical characteristics of the study population

In total, 281 consecutive unmatched patients with AFI were enrolled in the study from 1 September 2012 to 28 February 2013. As per the CRC, 132 cases of AFI were classified as dengue and 149 cases as non-dengue etiology. The etiological distribution of the non-dengue cases are shown in Table 2. Majority of the non-dengue cases

Table 2. Distribution of controls (patients with other infections)

Distribution of non-dengue cases	n = 149
Scrub typhus (Eschar present; IgM ELISA positive)	69
Scrub typhus (Eschar absent; IgM ELISA positive)	61
Malaria	4
Leptospirosis	3
Blood culture proven other etiology	12
Salmonella typhi	8
Salmonella paratyphi	3
Escherichia coli	1

comprised of scrub typhus (130 out of 149 cases) which shares the same seasonal pattern and clinical features in southern India. The baseline characteristics of the dengue and non-dengue cases are shown in Table 3. The median duration of fever at presentation to the hospital was five days [Inter quartile range (IQR) = 4–7 days] among dengue group as compared to seven days (IQR = 7–10 days) among the non-dengue group ($p < 0.001$). The dengue group differed significantly from the non-dengue group in being comparatively younger in age, shorter duration of illness prior to presentation, higher hematocrit, lower leukocyte count and elevated serum glutamic oxaloacetic transaminase (SGOT) level. This is consistent with the clinical and laboratory profile frequently encountered in dengue related illness and supports that the CRC rightly classified the enrolled cases.

Comparison of the RDT kits for diagnosis of acute dengue infection

For the comparison of the RDTs, laboratory diagnosis of acute dengue febrile illness was defined if either NS1 antigen was positive ($NS1^+/IgM^-$) or IgM was positive ($NS1^-/IgM^+$) or both were positive ($NS1^+/IgM^+$).

The sensitivity, specificity, PPV and NPV for acute dengue febrile illness for each RDT based on the IgM result of all four RDTs and for NS1 antigen result of three RDTs (SD Bioline, Reckon and J. Mitra) are shown in Tables 4 and 5. Based on IgM capture positivity of the four RDT kits, Panbio was found to have the highest sensitivity and NPV (97.7 and 97.7% respectively) followed by SD duo (64.3 and 75.5%). The specificity and PPV was higher for SD duo (96.6 and 94.3% respectively) as compared to Panbio (87.8 and 87.8% respectively). The Reckon RDT had the highest specificity (99.3%). The overall performance of IgM assay by Reckon and J. Mitra was unsatisfactory in the diagnosis of acute dengue infection.

On comparing the performance of the RDT kits for diagnosis of acute dengue febrile illness based on NS1 antigen capture only, all the three RDTs lacked sensitivity, while SD Duo had a high specificity (97.3%). The maximum sensitivity and NPV was recorded for J. Mitra (27.1 and 59.1% respectively). Thus, the performances of all the RDTs were unsatisfactory and NS1 antigen capture based RDT alone was found to be highly unreliable (Table 4).

It is well known that the duration of NS1 antigenemia in acute dengue febrile illness varies from 5 to 9 days after the onset of illness. However, in this study, case definition included duration of febrile episode up to 14

Table 3. Baseline characteristics of dengue and non-dengue cases (other infections)

Baseline characteristics	Dengue (n=132)	Non-dengue (n=149)	p-value
Female[a]	52 (132; 39.39%)	75 (149; 50.34%)	0.04
Myalgia[a]	122 (130; 93.85%)	120 (134; 89.55%)	0.265
Headache[a]	98 (125; 78.4%)	108 (133; 81.20%)	0.261
Rash[a]	22 (104; 21.15%)	14 (124; 11.29%)	0.07
Age (yr)[c]	27 (21–38.5)	38 (26–50)	0.03
Duration of fever (days)[c]	5 (4–7)	7 (6–10)	< 0.001
Haematocrit (%)[b]	43 (6.1)	38 (5.3)	< 0.001
White blood cell count (cells/mm^3)[c]	4300 (2800–7100)	8500 (6200–11000)	< 0.001
Platelet count (cells/mm^3)[c]	86000 (30250–148250)	119000 (65250–174500)	0.002
SGOT (µ/l)[c]	90 (50–177.2)	73 (41.5–127)	0.032
SGPT (µ/l)[c]	39 (27–109.8)	50 (27.5–86)	0.814
Creatinine (mg%)[c]	10.14 (0.99–1.3)	1.13 (0.95–1.4)	0.852

a—Number of patients (%); b—Mean (Standard deviation); c—Median (Inter quartile range).

Table 4. Comparison of the performance of dengue rapid diagnostic tests (RDTs) for IgM assay

Manufacturer	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV[a](%)	NPV[b](%)
Panbio (n = 280)	97.7 (93.5–99.5)	87.8 (81.5–92.5)	87.8	97.7
SD Bioline (n = 276)	64.3 (55.4–72.6)	96.6 (92.2–98.9)	94.3	75.5
Reckon (n = 276)	13.9 (8.6–21.2)	99.3 (96.2–99.9)	94.7	56.8
J. Mitra (n = 276)	36.4 (28.1–45.4)	68.7 (60.6–76.1)	50.5	55.2

a—Positive predictive value; b—Negative predictive value.

Table 5. Comparison of the performance of dengue rapid diagnostic tests (RDTs) for NS1 assay

Manufacturer	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV[a](%)	NPV[b](%)
SD Bioline	20.9 (14.3–28.9)	97.3 (93.2–99.2)	87.1	58.4
Reckon	18.6 (12.3–26.4)	96.6 (92.2–98.9)	82.8	57.5
J. Mitra	27.1 (19.7–35.7)	92.5 (87.0–96.2)	76.1	59.1

a—Positive predictive value; b—Negative predictive value.

days. Tests for NS1 antigens were performed on all patients who presented with fever duration up to nine days. The sensitivity and specificity of the three RDTs are shown in Table 5. Irrespective of the duration of illness, the sensitivity of all the three RDTs varied from 18 to 28%, while all the kits had fairly good specificity.

In order to explore the change in the performance of the RDTs in the diagnosis of acute dengue febrile illness, we calculated the sensitivity and specificity by combining the RDTs results in series and parallel (data not shown). The sensitivity was >98% for combination of Panbio RDT in parallel with any of the three RDTs. Hence, Panbio alone has nearly equal sensitivity and combination of any other RDT only marginally increases the sensitivity and doubles the cost of such testing. On the other hand, the specificity increased to >99% for combination of Reckon in series with any of the three RDTs.

The prevalence of dengue specific IgG antibody among the patients tested with Panbio, SD duo, Reckon and J. Mitra RDT kits was 49.3, 37.7, 39.9 and 15.9% respectively. It is interesting to note that this finding is contrary to the belief that the seroprevalence of dengue specific IgG antibody will be high in a dengue endemic area. This is comparatively lower as compared to the prevalence of IgG seropositivity reported by Rodríguez *et al*[16].

The cost per test (as per manufacturer's quoted price in India) for Panbio, SD, Reckon and J. Mitra were US$ 6.90, 4.27, 3.29 and 3.61 respectively which is considerably lower than dengue specific ELISA testing.

DISCUSSION

Dengue is a major emerging arthropod-borne viral disease which has reached endemic proportion in India. Since 2004 dengue has been incorporated into the governmental epidemiological surveillance under integrated disease surveillance project (IDSP)[3, 17]. The choice of investigation for confirming dengue infection depends upon the intended use of the result of the test. For early diagnosis and point-of care use, the ideal dengue diagnostic test should be highly sensitive, fairly simple, less time consuming and inexpensive while the test intended for surveillance and outbreak investigations should have high specificity, should be able to detect early stages of infection and identify the serotypes[1–4, 6, 17–18]. The sensitivity and specificity of the WHO[3] case definition for dengue guideline is 76.4 (69.6–82.1) and 42.5% (38.9–46.3) respectively. The sensitivity of the 2011 WHO-SEAR expert group case definition for dengue has increased to 87.9% (82.2–91.9) while its specificity is 20.1 (17.2–23.3). Both these case definition have good sensitivity but lacks specificity.

The recommended confirmatory diagnostic test kits for dengue infection (isolation of the virus in cell culture, the identification of viral nucleic acid or antigens, or the detection of virus-specific antibodies by ELISA) should have high specificity, but their high cost, turn-over time and limited availability restricts their use in clinical practice[6, 18–19]. Rapid diagnostic test kits, based on immunochromatographic method to capture NS1 antigen, anti-dengue specific IgM and IgG antibody are commonly used in clinical practice since these are easy to use, require less turn-over time and are less expensive. However, the performance of these commercially available RDTs varies greatly[5, 9–10, 12, 20–22].

In this study, we used the CRC to compare the performance of the four commercially available and widely used RDTs. Anti-dengue IgM capture by Panbio RDT showed maximum sensitivity [97.7% (95% CI 93.5–99.5)], good specificity [87.8% (95% CI 81.5–92.5)], with minimum volume of serum sample required and faster turn-over time. NS1 antigen capture by any of the other three RDTs showed unacceptably low sensitivity but good specificity, up to nine days of illness which is in contrary to several other published reports[9–10, 20–22]. Although, combining Panbio with Reckon in series increases its specificity than any individual RDT alone, but doubling the cost of such testing may be a limiting factor in clinical management. Hence, for early diagnosis and point-of care clinical use, anti-dengue IgM capture by Panbio RDT proved to be a rapid and accurate test that can be performed in a resource limited set up to rule out dengue infection, while for surveillance a combination of Panbio with either of SD Duo or Reckon seems a rapid and specific alternative.

Another interesting observation of the study was that the seroprevalence of IgG positivity in the population by Panbio RDT was 49.3%. A household based survey in Chennai by Rodríguez *et al*[16] showed a seroprevalence rate of 93% (95% CI 89–95) for dengue.

Limitations

In this study, we compared the sensitivity, specificity and predictive values of four commercially available and widely used RDT kits for dengue infection against CRC. Even in other common conditions like tuberculous meningitis where a gold standard test for confirmation of diagnosis is not widely available, a CRC is quite often used. We could not compare the RDTs with standard ELISA based NS1 or IgM capture assay or establish the cross-reactivity with other flavivirus infections due to constraints in resources.

CONCLUSION

We conclude that out of the four RDT kits tested for the diagnosis of acute dengue infection, only Panbio RDT was found to be a reliable kit for commercial use. Further, studies are needed to compare the Panbio RDT with IgM ELISA or PCR based methods for dengue infection.

Conflict of interest

Two manufacturers (J. Mitra and Reckon) provided 400 test kits each for testing free of cost. The SD Bioline and Panbio tests were part of the routine testing for suspected dengue cases in our hospital. None of the manufacturers had any role in patient enrolment, performing the diagnostic tests, interpretation or analysis or write up. There is no conflict of interest by any of the authors.

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