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A meta-analysis of the diagnostic accuracy of dengue virus-specific IgA antibody-based tests for detection of dengue infection

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SUMMARY

Immunoglobulin A (IgA)-based tests have been evaluated in different studies for their utility in diagnosing dengue infections. In most of the studies, the results were inconclusive because of a small sample size. Hence, a meta-analysis involving nine studies with 2096 samples was performed to assess the diagnostic accuracy of IgA-based tests in diagnosing dengue infections. The analysis was conducted using Meta-Disc software. The results revealed that IgA-based tests had an overall sensitivity, specificity, diagnostic odds ratio, and positive and negative likelihood ratios of 73·9%, 95·2%, 66·7, 22·0 and 0·25, respectively. Significant heterogeneity was observed between the studies. The type of test, infection status and day of sample collection influenced the diagnostic accuracy. The IgA-based diagnostic tests showed a greater accuracy when the samples were collected 4 days after onset of symptoms and for secondary infections. The results suggested that IgA-based tests had a moderate level of accuracy and are diagnostic of the disease. However, negative results cannot be used alone for dengue diagnosis. More prospective studies comparing the diagnostic accuracy of combinations of antigen-based tests with either IgA or IgM are needed and might be useful for suggesting the best strategy for dengue diagnosis.

Key words: Dengue fever, dengue haemorrhagic fever, immunology, meta-analysis, IgA.

INTRODUCTION

Dengue, caused by four serotypes of dengue virus (DENV), poses a major threat to public health systems in tropical and subtropical countries and is an emerging threat to countries where it has not yet been reported. Disease burden studies from South East Asian countries have estimated 209 million dengue episodes and 5906 deaths during 2001–2010 [1].

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Dengue is a complex disease with diverse range of clinical manifestations ranging from mild forms (undifferentiated fever and dengue fever) to severe forms of the disease (dengue haemorrhagic fever, dengue shock syndrome and expanded dengue syndrome/isolated organopathy/unusual manifestations) [2]. During the febrile phase, the symptoms mimic those caused by a wide spectrum of viral, bacterial and protozoal infections, making diagnosis difficult.

Rapid and accurate diagnosis is essential for effective clinical management of dengue cases. Nucleic acid-based tests offer the best opportunity for early diagnosis of dengue but are expensive and not available in resource-limited settings. Non-structural

protein 1 (NS1) antigen-based enzyme linked immunosorbent assays (ELISAs) and rapid immunochromatographic tests (ICTs) offer a better alternative for nucleic acid-based tests in early diagnosis of dengue. These tests are less expensive and are used even in primary health centres. However, recent studies have indicated that the sensitivity of NS1 antigen-based tests are lower in secondary infections and in DENV-4 serotype infections [3–5].

The anti-DENV IgM capture ELISA (MAC-ELISA) test is widely used for probable diagnosis of dengue in resource-limited settings. IgM antibodies against DENV appear 3–5 days after the onset of symptoms and can persist for 2–3 months. The sensitivity for MAC-ELISA is above 90% and the specificity is 98% for samples collected after 5 days of onset of symptoms [6]. However, IgM levels are lower in secondary infections and about 28% of secondary infections were not detected by MAC-ELISA [7]. Anti-DENV IgG-based ELISAs have been utilized for discriminating between primary and secondary infections as well as to rule out past infections [6].

Based on the observation that the anti-DENV IgA antibodies appear at the same time as IgM antibodies and persist for shorter periods compared to IgM, Talarmin *et al.* tested an IgA capture ELISA (AAC-ELISA) for its utility in diagnosing dengue infections [8]. An antigen capture anti-DENV IgA ELISA (ACA-ELISA) for diagnosis of dengue has been reported with higher sensitivity and specificity [9]. An ICT, which detects anti-DENV IgA antibodies was evaluated for its utility in diagnosing dengue in various countries [10]. Recently, a commercial dengue IgA capture ELISA kit was made available and tested for its utility in dengue diagnosis [11]. Most of these studies have been conducted with small sample sizes and a meta-analysis may provide more reliable estimates about the diagnostic accuracy of IgA-based tests. In the present study, we undertook a systematic review and meta-analysis of the diagnostic accuracy of IgA-based tests for diagnosis of dengue.

MATERIALS AND METHODS

Search criteria and study selection

The articles used for the meta-analysis were searched in NCBI Pubmed using the following search criteria: (immunoglobulin A OR IgA) AND (dengue OR dengue fever OR dengue hemorrhagic fever OR dengue shock syndrome) AND diagnosis AND sensitivity. Articles published up to March 2015 were included. Additional studies were included by searching the references of the articles derived from Pubmed and also by searching citations of articles in Google Scholar. Inclusion criteria were: (1) studies that evaluated the sensitivity and specificity of any IgA-based assay against the gold standard assay for diagnosis using serum samples, and (2) studies in English language. The following types of studies were excluded: (1) studies with incomplete data for calculating true positives and true negatives, (2) conference abstracts, commentaries, review articles and editorials, and (3) studies which used samples other than serum (capillary blood, saliva, urine). The criteria suggested by World Health Organization for diagnosis of confirmed dengue were considered as the gold standard [12]. The samples met any one of the following criteria:

(1) Samples which were positive either by virus isolation or by reverse transcriptase–polymerase chain reaction (RT–PCR)/real-time RT–PCR.
(2) Samples which showed IgM seroconversion in paired sera.
(3) Samples which showed IgG seroconversion or increase in IgG titre in paired sera.

The above criteria were considered as confirmation for dengue samples.

The literature search was performed independently by two reviewers.

Data extraction

The following data were extracted from the articles selected for meta-analysis: authors, year of publication, index test used, reference standards used, sample size, number of dengue cases, number of non-dengue cases, day of sample collection, whether primary or secondary infection, serotypes, and the number of true positives, true negatives, false positives and false negatives. If a study contained multiple sample sets, only the sample set which was tested by gold standard reference assays were included. The sample sets with a single sample tested by IgM or IgG or NS1 Ag ELISAs were excluded from the analysis. The following criteria were used for subgroup analysis: type of index test used, primary/secondary infection, and day of sample collection. The data extraction was performed independently by two reviewers and disagreements were resolved by discussion.

Quality assessment

The quality assessment of the selected studies was performed by two reviewers independently using the

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QUADAS 2 tool [13] and discrepancies were resolved by discussion. The risk of bias was assessed in four key domains covering patient selection, index test, reference standard, and flow of patients through the study. The risk of bias in each domain was judged in terms of 'low', 'high' and 'unclear'. The first three domains were also assessed in terms of concerns regarding applicability. Risk of bias and the applicability-concerns graph was generated using Review Manager 5.3 software [14].

Statistical analysis

A meta-analysis was performed using Meta-Disc software version 1.4 [15]. The sensitivity, specificity, diagnostic odds ratio (dOR), positive likelihood ratio (LR+) and negative likelihood ratio (LR–) were calculated for each study and the pooled estimates of these parameters were obtained using a random-effects model (DerSimonian–Laird method). The Cochran Q test was used to test the homogeneity of likelihood ratios and diagnostic odds ratios across the studies. The effect of heterogeneity for each parameter was provided by the I^2 statistic, which described the percentage of total variation across the studies due to heterogeneity. The probable reasons for heterogeneity were investigated by stratifying the data on the basis of type of index test, primary/secondary infection and the day of sample collection. A meta-regression analysis was performed to discover whether the year of publication and sample size affected the diagnostic accuracy of IgA-based tests. Fagan plot analysis was performed in order to estimate how much the result of a diagnostic test changes the probability that a patient has the disease. This analysis was based on the positive and negative likelihood ratios estimated by the meta-analysis and was performed for three pre-test probabilities of 25%, 50% and 75% [16]. Publication bias was assessed by Deeks' funnel plot asymmetry test [17]. Both Fagan plot analysis and Deeks' funnel plot asymmetry test were performed using Stata version 13 (StataCorp., USA).

Ethical approval

This work does not contain any studies with human participants or animals performed by any of the authors.

RESULTS

Search results

Using the search criteria in Pubmed/Medline, 14 studies were obtained. Eleven articles which evaluated the diagnostic accuracy of IgA-based test for diagnosis of dengue were shortlisted. The reference lists and citations of the shortlisted articles were also searched for additional articles leading to the addition of a further four articles. After applying the inclusion and exclusion criteria, nine articles were selected for the final meta-analysis (Table 1). The remaining six articles were excluded for reasons mentioned in Table 2. Of these, one article was excluded because dengue-negative samples were not included in the study while another article was excluded because the data was based on capillary blood samples [18, 20]. Four other articles were excluded due to the use of inappropriate gold standard assays [19, 25, 27, 28].

Characteristics of the studies used for analysis

In total, nine articles were used for the meta-analysis. Three studies used AAC-ELISA, while one study used ACA-ELISA [8, 9, 21, 22]. One study used a commercially available ELISA (Platelia Dengue IgA Capture, Bio-Rad, USA) [11]. An immunofluorescence assay was used in one study [23]. A commercially available ICT (ASSURE® Dengue IgA Rapid test, MP Biomedicals, USA) was used by three studies [10, 24, 26]. In total, 2096 samples were used for the meta-analysis. Only a subset of samples which were tested against the gold standard reference assay was included from four studies [8, 10, 21, 24] (Table 1).

The quality of the studies in terms of the risk of bias was assessed in four key domains covering patient selection, index test, reference standard, and flow of patients through the study and the timing of the index test and reference standards. The summary of the results of quality assessment using the QUADAS 2 tool is given in Figure 1. Three (33·3%) studies had a high risk of bias with regard to selection of samples. All three studies had included healthy controls as dengue-negative subjects [21, 23, 26]. Six (66·7%) studies had an unclear risk of bias with regard to the performance of index test. In all of these six studies, it was unclear whether the index test was performed without the knowledge of results of the reference test [8, 10, 21–23, 26]. One (11·1%) study had a high risk of bias with regard to the performance of the index test [9]. In this study, the reference test results were not blinded to the personnel who performed the index test. All studies had a lower risk of bias with regard to the conduct of the reference test and the flow and timing of samples. There was no or low risk of concern regarding the applicability in terms of patient selection, index test and reference test.

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Table 1. *Characteristics of the studies used for meta-analysis of diagnostic accuracy of IgA in detection of dengue*

Author, year [ref.]	Sample size	Index test	Reference test	Sample timing	Infection status
Talarmin *et al.* 1998 [8]*	280	IgA capture ELISA	Seroconversion, fourfold rise in hemagglutination inhibition titre, virus isolation and RT–PCR	1–29 days	Not known for many samples
Groen *et al.* 1999 [23]	120	IFA	Seroconversion in paired sera	5–8 days	Primary/secondary
Balmaseda *et al.* 2003 [21]*	201	IgA capture ELISA	IgM/RT–PCR/inhibition ELISA in single samples, seroconversion or inhibition ELISA titre rise in paired sera	0–7 days	Not known
Balmaseda *et al.* 2008 [22]	356	IgA capture ELISA	Seroconversion, fourfold rise in inhibition ELISA titre, RT–PCR and virus isolation	0–4 days	Primary/secondary data given (exact number not known)
Ahmed *et al.* 2010 [24]*	204	ICT	Reference ELISAs – IgM, IgG and NS1 Ag in paired sera	Acute phase sera (days not mentioned)	Primary/secondary
Tan *et al.* 2011 [10]*	382	ICT	Reference ELISAs – IgM, IgG and NS1 Ag, RT–PCR, seroconversion in paired sera using RT–PCR and IgM ELISA	Acute phase sera (days not mentioned)	Primary/secondary
Yap *et al.* 2011 [9]	144	Antigen capture anti DENV IgA ELISA	Seroconversion in paired sera using RT–PCR and IgM ELISA	1–8 days	Primary/secondary
de la cruz Hernández *et al.* 2012 [26]	225	ICT	NS1 Ag ELISA and real-time RT–PCR	0–5 days	Primay/secondary
De Decker *et al.* 2015 [11]	184	Platelia Dengue IgA Capture, Bio-Rad	Virus isolation, RT–PCR	3–15 days	Primary/secondary

IFA, Immunoflourescence assay; ICT, immunochromatographic test.
* Only a subset of samples were used.

Table 2. *Studies that were excluded from meta-analysis and reasons for exclusion*

Author, year [ref.]	Reason for exclusion
Nawa *et al.* 2005 [19]	Inappropriate gold standard
Nawa *et al.* 2006 [18]	Dengue negative samples were not studied
Sharmin *et al.* 2012 [25]	Inappropriate gold standard
Hasan *et al.* 2013 [27]	Inappropriate gold standard
Naz *et al.* 2014 [28]	Inappropriate gold standard
Matheus *et al.* 2014 [20]	Data was based on capillary blood samples

Overall diagnostic accuracy of IgA-based test for diagnosis of dengue infection

When all the nine studies were analysed in meta-analysis, the sensitivity values (proportion of test-positives in subjects with disease) ranged from 47·6% to 93·0% and the pooled estimate was 73·9% [95% confidence interval (CI) 71·6–76·0] (Fig. 2*a*). The specificity values (proportion of test-negatives in subjects without disease) ranged from 86·0% to 100% and the pooled estimate was 95·2% (95% CI 93·0–96·9) (Fig. 2*b*).

The LR+ (number of times a positive index test result more likely in the diseased group compared to the non-diseased group) ranged from 6·66 to 107·65 with a pooled estimate of 22·0 (95% CI 8·4–37·3) (Fig. 3*a*). The LR– (number of times a negative

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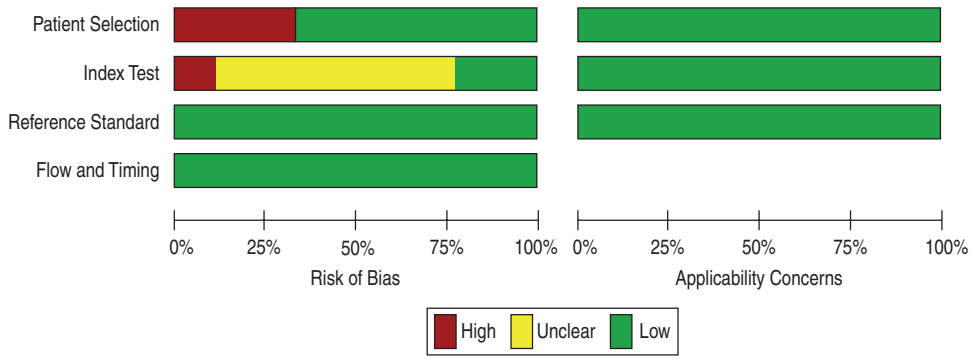

Fig. 1. Risk of bias and applicability-concerns graph: a review of authors' judgements about each domain presented as percentages across the included studies.

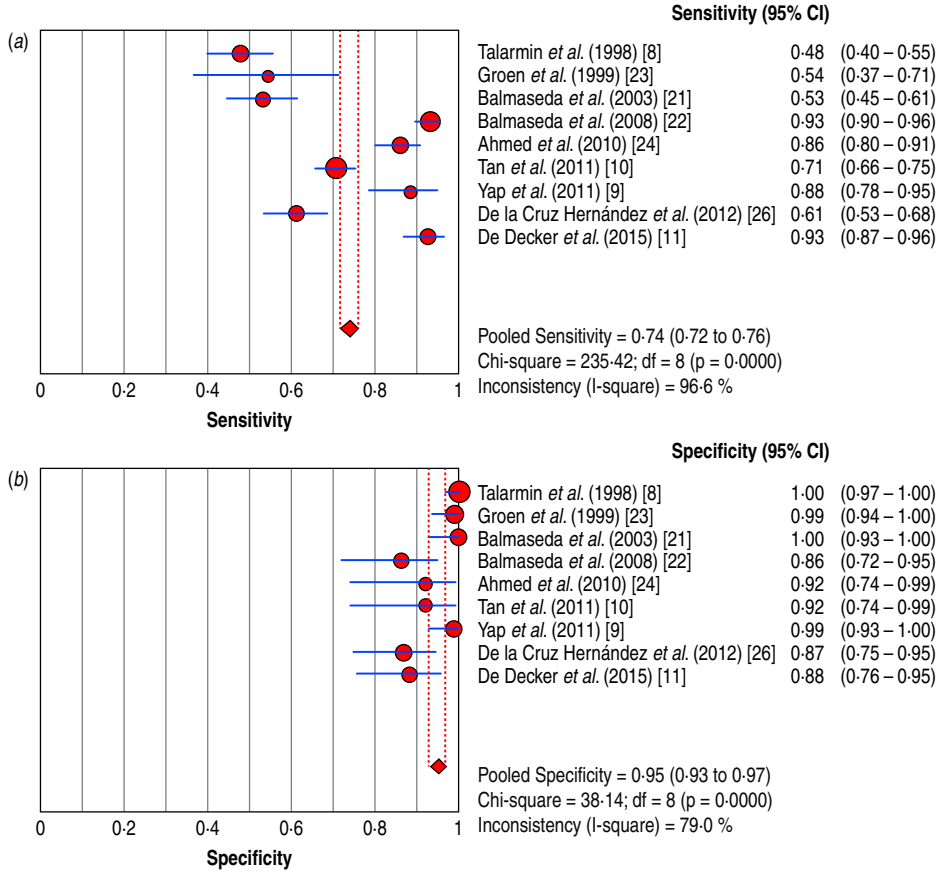

Fig. 2. Forest plots for (*a*) sensitivity and (*b*) specificity of IgA-based tests. Forest plot for sensitivity or specificity of each individual study as well as the pooled estimate are represented by solid circles and the horizontal lines represent 95% confidence intervals (CI).

index test result less likely in the diseased group compared to the non-diseased group) ranged from 0·08 to 0·53 with a pooled estimate of 0·25 (95% CI 0·17–0·36) (Fig. 3*b*).

The dOR (odds of obtaining a positive test result in a diseased individual compared to a non-diseased individual) ranged from 10·30 to 564·25 with a pooled estimate of 66·77 (95% CI 28·70–155·33) (Fig. 4).

The heterogeneity presented as I^2 values (i.e. quantifies the variation in excess of that due to chance across studies) were significantly higher than 50 for all the parameters studied (Figs 2–4).

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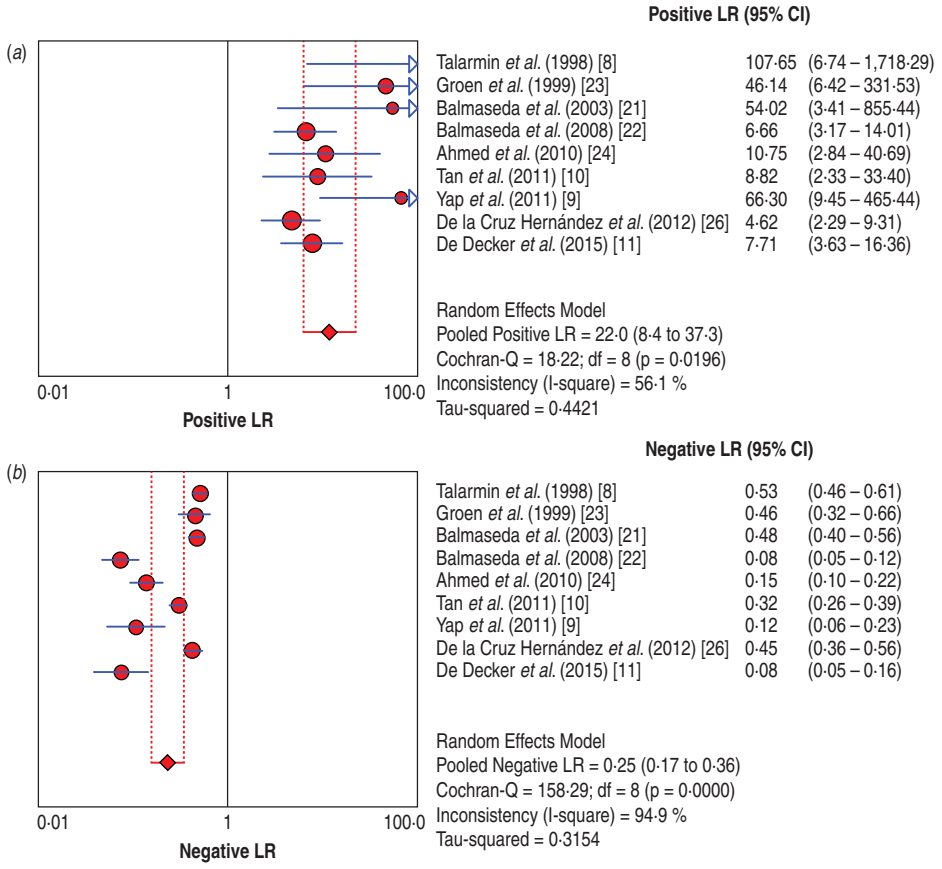

Fig. 3. Forest plots for (*a*) positive likelihood ratio (LR) and (*b*) negative LR of IgA-based tests. LRs of each individual study and the pooled estimate are represented by solid circles and the horizontal lines represent 95% confidence intervals (CI).

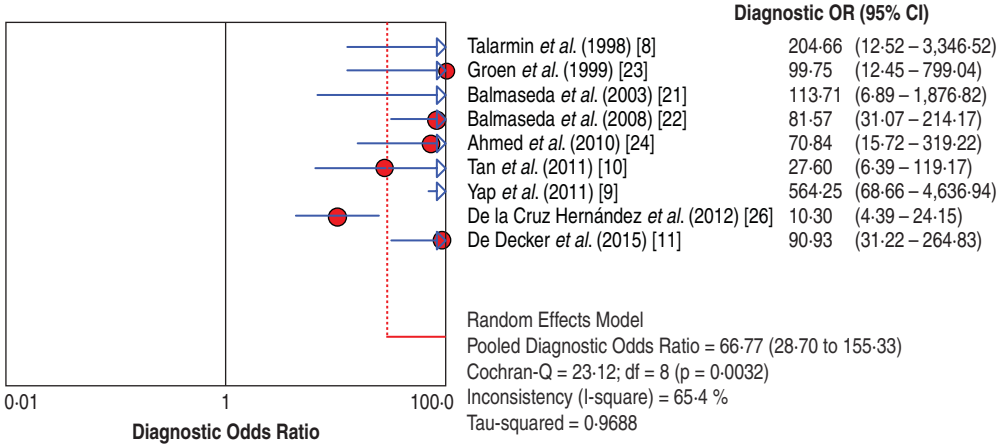

Fig. 4. Forest plot of diagnostic odds ratio of IgA-based tests. The diagnostic odds ratios of each individual study and the pooled estimate are represented by solid circles and the horizontal lines represent 95% confidence intervals (CI).

Influence of the type of test employed, day of sample collection and infection status on the diagnostic accuracy of IgA-based tests in diagnosing dengue

Since the heterogeneity between the studies was significant, a subgroup analysis was performed based on the type of test employed, day of sample collection and infection status to identify the source of heterogeneity.

Five studies had used an ELISA-based test while three studies had used an ICT-based test. The subgroup analysis, based on the type of the test employed,

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revealed that the ELISA-based test had sensitivity and specificity of 76% (95% CI 73–79) and 96% (95% CI 94–98), respectively. The dOR was 108·42 (95% CI 57·07–205·95) while LR+ was 17·11 (95% CI 5·87–49·86) and LR– was 0·19 (95% CI 0·09–0·40). Heterogeneity was not observed between the studies for dOR ($I^2 = 0$). For ICT-based test, the sensitivity, specificity, dOR, LR+ and LR– were 72% (95% CI 69–75), 89% (95% CI 82–95), 23·82 (95% CI 7·44–76·22), 6·03 (95% CI 3·43–10·57), and 0·29 (95% CI 0·17–0·47), respectively. Heterogeneity was not observed between the studies for specificity and LR+ ($I^2 = 0$). A moderate heterogeneity was observed for dOR ($I^2 = 61·3\%$) (Table 3).

Complete data for the day of sample collection after the onset of symptoms was available for four studies. Based on the available data, samples were categorized into two groups, samples collected up to 4 days before and samples collected 4 days after symptom onset. For samples which were collected up to 4 days before, sensitivity, specificity, dOR, LR+ and LR– were 78% (95% CI 74–81), 92% (95% CI 88–96), 35·91 (95% CI 8·78–146·91), 6·99 (95% CI 3·39–14·43), and 0·28 (95% CI 0·14–0·56), respectively. The heterogeneity was significant between the studies for all parameters except for LR+ ($I^2 = 45·4\%$). For samples collected 4 days after onset of symptoms, the sensitivity, specificity, dOR, LR+ and LR– were 86% (95% CI 78–92), 93% (95% CI 88–97), 85·68 (95% CI 15·51–473·25), 13·56 (95% CI 3·01–61·01), and 0·153 (95% CI 0·1–024), respectively. Heterogeneity between the studies was not observed for sensitivity and LR– ($I^2 = 0$) and a moderate heterogeneity was observed for dOR ($I^2 = 65·8\%$) (Table 3).

Six studies had complete data on the infection status and samples were categorized into those from primary or secondary infections. For samples from primary dengue infection, the sensitivity, specificity, dOR, LR+ and LR– were 68% (95% CI 63–73), 96% (95% CI 94–98), 56·44 (95% CI 14·07–226·31), 14·26 (95% CI 5·38–37·83), and 0·32 (95% CI 0·18–0·57), respectively. In samples from secondary infections, the sensitivity, specificity, dOR, LR+ and LR– were 92% (95% CI 89–94), 96% (95% CI 94–98), 321·65 (95% CI 59·84–1728·9), 20·09 (95% CI 7·13–56·64), and 0·09 (95% CI 0·03–0·26), respectively. Heterogeneity was significant between the studies in both the groups ($I^2 > 75\%$) (Table 3).

Influence of the year of publication and sample size on the diagnostic accuracy of IgA-based tests for detecting dengue

Further, to discover whether the year of publication and sample size affected the diagnostic accuracy of IgA-based tests, a meta-regression analysis was performed. For the year of publication, the studies were categorized into those published on or before 2007 and after 2007 (the cut-off was chosen on the basis that the first study on use of IgA for dengue diagnosis was published in 1998 and hence the median year between 1998 and 2015 was considered). To study the influence of sample size, mean study sample size was calculated to be 234 and the studies were categorized into those with sample size $\leqslant 234$ and those with sample size > 234. A meta-regression analysis revealed that the year of publication ($P = 0·74$) and the sample size ($P = 0·28$) had no influence on the diagnostic accuracy of IgA-based tests for detecting dengue.

Fagan plot analysis to estimate the post-test probability based on simulation of an environment with different pre-test probability

To estimate the post-test probability associated with IgA-based diagnostic tests in an environment with different prevalence rates of dengue disease, Fagan plot analysis was performed for three pre-test probabilities: 25%, 50% and 75%. The analysis revealed that diagnosis using an IgA-based test would be incorrect in 8%, 20% and 43% of dengue cases when the prevalence rates were 0·25, 0·5 and 0·75, respectively. It further indicated that diagnosis would be correct in 88%, 96% and 99% of dengue cases when the prevalence rates were 0·25, 0·5 and 0·75, respectively (Fig. 5).

Assessment of publication bias

Publication bias was assessed using Deeks' funnel plot asymmetry test. The results revealed that the funnel plot was symmetrical suggesting that there was no publication bias ($P = 0·44$) (Fig. 6).

DISCUSSION

The results of the meta-analysis showed that using IgA-based assays to diagnose dengue had an overall sensitivity of 74% and a specificity of 95%. The overall dOR, LR+ and LR– were 66·8, 22·0 and 0·25, respectively. The results suggest that patients with dengue had a 22-fold higher chance of being positive for IgA, while if the patient was negative for IgA, the probability of the patient having dengue was 25%. Thus, the overall dOR, LR+ and LR– results indicate that IgA-based tests have a moderate level of accuracy and that they are diagnostic of only disease. The negative results cannot be used alone for diagnosis of dengue.

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Table 3. *Subgroup analysis of diagnostic accuracy of IgA for detection of dengue based on the type of index test, sample timing and infection status*

Subgroups and heterogeneity between studies for each subgroup	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic odds ratio (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
Type of index test used					
ELISA-based IgA tests ($n = 1165$)	0·76 (0·73–0·79)	0·96 (0·94–0·98)	108·42 (57·07–205·95)	17·11 (5·87–49·86)	0·19 (0·09–0·40)
I^2	98·0	85·5%	0%*	66·3%	97·2%
ICT-based IgA tests ($n = 811$)	0·72 (0·69–0·75)	0·89 (0·82–0·95)	23·82 (7·44–76·22)	6·03 (3·43–10·57)	0·29 (0·17–0·47)
I^2	93·3%	0%*	61·3%*	0%*	91·6%
Sample timing					
$\leqslant$4 days ($n = 764$)	0·78 (0·74–0·81)	0·92 (0·88–0·96)	35·91 (8·78–146·91)	6·99 (3·39–14·43)*	0·26 (0·14–0·56)
I^2	97·0%	81·1%	77·9%	45·4%	95·0%
>4 days ($n = 1324$)	0·86 (0·78–0·92)	0·93 (0·88–0·97)	85·68 (15·51–473·25)	13·56 (3·01–61·01)	0·153 (0·1–024)
I^2	0%*	74·9%	65·8%*	75·9%	0%*
Infection status					
Primary ($n = 865$)	0·68 (0·63–0·73)	0·96 (0·94–0·98)	56·44 (14·07–226·31)	14·26 (5·38–37·83)	0·32 (0·18–0·57)
I^2	84·5%	81·5%	79·0%	73·8%	90·6%
Secondary ($n = 896$)	0·92 (0·89–0·94)	0·96 (0·94–0·98)	321·65 (59·84–1728·9)	20·09 (7·13–56·64)	0·09 (0·03–0·26)
I^2	88·9%	81·5%	78·1%	79·9%	88·4%

CI, Confidence interval.

Number of studies for ELISA-based tests ($n = 5$), and for ICT-based tests ($n = 3$); four studies for sample timing and six studies for infection status.

Unless otherwise stated the P value for heterogeneity between studies is $< 0·05$.

* Heterogeneity between studies was not significant ($P > 0·05$).

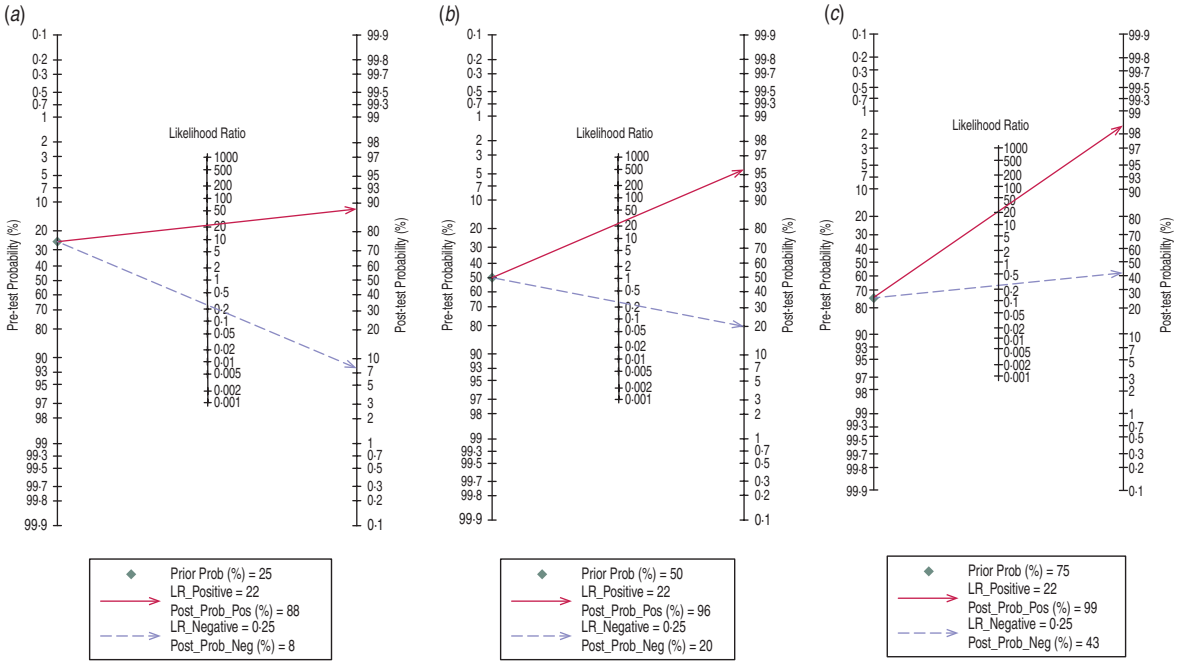

Fig. 5. Fagan's nomogram showing the post-test probabilities associated with IgA-based tests under different pre-test probabilities: (*a*) 25%, (*b*) 50%, (*c*) 75%. Fagan's nomogram consists of three vertical axes, the first axis represents pre-test probability, the middle axis represents the positive and negative likelihood ratios and the last axis represents post-test probability.

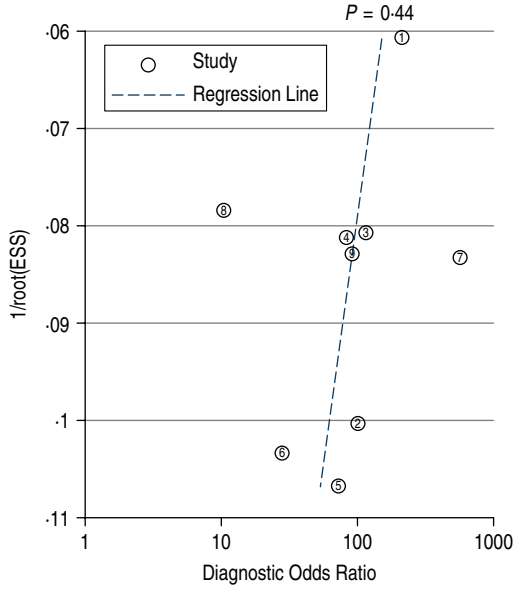

Fig. 6. Deeks' funnel plot for publication bias. Each circle represents an individual study and the dashed line represents the regression line. *P* value = 0·44, suggesting no publication bias.

Significant heterogeneity was observed between the studies. Hence, subgroup analysis was performed on the basis of type of test, day of sample collection and immune status to identify the source of heterogeneity. A meta-regression analysis based on the year of publication and the sample size was also performed. The analysis revealed that type of the test and the day of sample collection could be possible sources of heterogeneity. The DENV serotype responsible for the infection, disease severity, ethnicity and geographical location of the study might also add to the heterogeneity observed in the study. Only three studies had data on serotypes while disease severity data was not available in all the studies except one [10, 11, 26].

Based on the summary statistics, ELISA-based tests were more accurate in diagnosing dengue compared to ICT-based tests. If a patient had a negative test result based on ICT, the probability that the patient had dengue was 29%, while for ELISA-based results it was 19%. All the ELISA-based studies were based on IgA capture ELISA except the study by Yap *et al.* which utilized an antigen capture IgA ELISA [8, 9, 16, 17]. The dOR for ACA ELISA was 564 and thus ACA-ELISA represents a promising test for diagnosis of dengue but needs evaluation in multiple prospective cohorts. Only three studies were available for meta-analysis of ICT-based tests. Although based on the summary statistics, the results of the ICT-based test were promising initially in a

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study with a dOR of 71 and LR– of 0·15 [19], results from subsequent studies with smaller sample sizes were not encouraging and further evaluation in larger prospective cohorts is required [20–23]. ICT-based tests had the advantage of providing point of care diagnosis within minutes without the requirement of costly equipment compared to the ELISA-based tests. However, since the reading is based on visual inspection, the ICT format has the weakness of subjective reading by the operator [29].

Irrespective of the type of test, IgA-based tests were more accurate in detecting dengue in the samples collected 4 days after symptom onset compared to the samples that were collected 4 days before. IgA-based tests had a greater accuracy in diagnosing secondary infections compared to primary infections. An earlier study reported that positivity for dengue-specific IgA was detected at about day 5 of onset of symptoms in primary infection while in secondary infections, positivity for IgA was detected around 4 days after symptom onset [7]. The results suggest that IgA-based tests could be of utility in settings where dengue is endemic and more secondary infections were occurring. It also indicated that the IgA-based tests would be more efficient in diagnosing dengue for the samples taken 4 days after symptom onset.

Prevalence rates of dengue vary according to geographical region. The Fagan plot analysis suggested that with an increase in the prevalence rate of dengue disease, post-test probability of positive IgA test results increased to more than 95% in dengue cases. However, the post-test probability of negative test results in dengue-positive cases also increased. This suggests that the proportion of false-negative IgA test results would be higher under the conditions of higher prevalence rates. In India, where dengue is endemic in most states, positivity rates for dengue in suspected cases ranged from 30% to 60% during different time periods [30]. The Fagan plot analysis revealed that even under a prevalence rate of 0·5, 20% of false-negative results were expected in dengue cases. These results corroborated the finding that negative IgA test results should be confirmed with other dengue diagnostic tests.

Recent studies have reported that the combination of NS1 Ag and IgM antibody-based tests improve the sensitivity of serological tests [29]. Since IgA also appears in the body fluids during the same time period as IgM, the combination of NS1 Ag and IgA antibody-based tests can serve as an alternative. Although IgM- and IgA-based tests are equivalent in terms of sensitivity in detecting dengue [11, 22], IgA-based tests might have an advantage over IgM-based tests in secondary infections. NS1 Ag-based tests also had lower sensitivity during secondary infections [3–5]. Hence, the combination of NS1 Ag and IgA antibody-based tests might help in improving the diagnosis of dengue disease in countries where higher numbers of secondary infections occur. Prospective studies in different settings are needed to evaluate the diagnostic accuracy of combinations of IgM- and IgA-based tests with the NS1 Ag test.

In the present study, no publication bias was observed. However, the existence of publication bias cannot be excluded since the Deeks' funnel plot asymmetry test had low power when the included studies had significant heterogeneity [17]. Quality analysis of the studies included in the meta-analysis revealed that 33·3% of the studies used samples from healthy controls. Using the samples from healthy controls for evaluation of diagnostics might exaggerate the diagnostic accuracy of the study. In 66·7% of the studies, it was unclear whether the reference test results were blinded to the people who performed the index tests. Another limitation of performing and interpreting meta-analysis of the diagnostic accuracy of tests for detecting dengue was the use of multiple reference tests by the study groups. In most of the excluded studies, testing of single serum samples by reference ELISAs (NS1 antigen ELISA, IgM capture ELISA, IgG capture ELISA) was used to define dengue cases. Details about disease severity was lacking in all the studies. Earlier studies have reported higher levels of dengue-specific IgA in severe dengue cases [31, 32]. This calls for more prospective studies with data on disease severity, infection status, day of sampling and uniform criteria/guidelines for the reference test to perform comparative evaluation of IgA-based and other antibody/antigen-based tests to diagnose dengue.

To conclude, the results of the present meta-analysis suggest that IgA-based assays have a moderate accuracy to detect dengue. The type of test, immune status and the day of sampling influenced the diagnostic accuracy of IgA-based tests. IgA-based tests can supplement other diagnostic tests and might be of use in settings of dengue endemicity with more secondary infections.

DECLARATION OF INTEREST

None.

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