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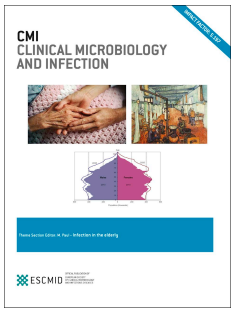

Rapid diagnostic tests for determining dengue serostatus: a systematic review and key informant interviews

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Rapid diagnostic tests for determining dengue serostatus: a systematic review and key informant interviews

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Abstract

Objectives: Vaccination for dengue with the live attenuated tetravalent CYD-TDV vaccine (Dengvaxia®) is only recommended in individuals who have had prior dengue virus (DENV) infection. Rapid diagnostic tests (RDT) for past DENV infection would offer a convenient method for pre-vaccination screening at point-of-care. A systematic review was conducted to evaluate the performance of current dengue rapid diagnostic tests (RDTs) for determining dengue serostatus, using IgG antibodies against DENV as a marker of past infection.

Methods: PubMed and EMBASE databases were searched from 2000 to 2018 to identify studies evaluating dengue RDTs in individuals with known or possible previous DENV infection. Study quality was evaluated using GRADE and QUADAS-2 criteria. Semi-structured interviews were also performed with available dengue RDT manufacturers.

Results: The performance of 4 dengue IgG RDTs was determined in 3137 individuals across 10 studies conducted in 13 countries, with serum used in most of the studies. No studies reported data for determining dengue serostatus, and limited data were available regarding cross-reactivity with other viruses. The majority of studies demonstrated sensitivities and specificities between 80-100% for dengue IgG detection in samples from secondary infection or convalescent timepoints after recent infection.

Conclusions: Although current dengue IgG RDTs have shown reasonable performance compared to laboratory-based tests in secondary infection, additional research is needed to determine how RDTs would perform in relevant populations targeted for vaccination. New RDTs or modifications to current RDTs are feasible and may optimize the performance of these tests for use in a pre-vaccination screening approach.

Introduction

Dengue is a flavivirus infection spread by *Aedes aegypti* and *Aedes albopictus* mosquitoes and is estimated to infect up to 400 million people worldwide each year [1]. Four distinct dengue virus serotypes (DENV-1 through DENV-4) cause dengue. After infection with one serotype, an individual develops lifelong immunity to that serotype, but subsequent infection with another serotype increases the risk of severe dengue due to antibody dependent enhancement of infection [2]. The annual incidence of DENV infections has increased exponentially over the past decades, accompanied by continual geographic expansion to new areas [3,4]. International travelers are also increasingly affected [5-8]. Effective vector control strategies are not sustainable [3], community-based approaches have had mixed results [9,10], and compliance with personal protective measures is difficult [11]. Hence, a dengue vaccine would be an important tool to combat the dengue burden.

Currently, the only commercially available dengue vaccine is a tetravalent live attenuated recombinant vaccine, CYD-TDV (Dengvaxia®), that was developed by Sanofi-Pasteur. Licensed in approximately 20 countries (as of July 2018) for use in individuals between 9 and 45 years of age in most countries, it is given with a 3-dose schedule six months apart [12]. In late 2017, Sanofi-Pasteur released long-term safety data stratified by serostatus [13]. Serostatus refers to whether a person has had a previous DENV infection prior to vaccination: a seropositive person has had at least one past DENV infection, whereas a seronegative person is dengue-naïve [14]. Follow-up data of trial participants who were seronegative prior to administration of the vaccine

showed a 1.75-fold increased risk of hospitalizations due to dengue and severe dengue from year 3 onwards in comparison with unvaccinated seronegative participants. This unanticipated outcome is thought to be mediated by antibody dependent enhancement of infection, where non-neutralizing antibodies can facilitate greater viral entry into monocytes through Fc receptor binding. This can lead to higher viral load, greater immune activation, and increased risk for severe dengue. In seropositive individuals, the vaccine was efficacious and safe, conferring long-term protection [13]. Consequently, in April 2018, WHO`s Strategic Advisory Group of Experts on Immunization (SAGE) revised its recommendations to state a "pre-vaccination screening strategy" would be the preferred option for countries seeking to use Dengvaxia®, a strategy whereby only dengue-seropositive individuals should be vaccinated [15-17].

The choice of tests for dengue diagnosis depends on the timing and purpose. For the diagnosis of acute DENV infection, tests are based on DENV isolation, presence of dengue viral antigens, detection of viral nucleic acid in blood through techniques such as RT-PCR, IgM seroconversion, and/or a four-fold or greater rise in IgG antibody titer in paired blood samples collected at least 14 days apart [18]. Dengue virus and antigen detection are the most accurate diagnostic tools during the first 5 days of illness, as IgG and IgM antibodies are not produced until 5-7 days after the onset of symptoms in primary infections [19,20]. IgM levels can become undetectable after 3-6 months, while IgG levels often persist over an individual's lifetime and can be used to indicate previous DENV infection [19]. Thus, for the detection of past DENV infections, IgG antibodies to DENV serve as a marker of past DENV infection. Enzyme-linked immunosorbent

assays (ELISAs) are the most commonly used laboratory-based serology assays to measure DENV IgG. However, ELISAs are time-consuming and require significant laboratory infrastructure, including instrumentation, trained staff, and refrigeration for reagents. Delays in turnaround time would hamper vaccination campaigns as patients would likely be lost to follow-up if required to return several days later for vaccination. The plaque reduction neutralization test (PRNT), which measures the titer of neutralizing antibodies against DENV infection, has also been used to evaluate dengue serostatus, but is even more laborious and expensive than ELISAs, and hence not routinely used [20]. All serological assays can exhibit some degree of cross-reactivity with other flaviviruses such as Zika, Japanese encephalitis and yellow fever [21].

Rapid diagnostic tests could enable quick, simple screening in dengue endemic areas, which are often resource-limited and do not have the laboratory capacity to perform ELISA or PRNT testing. RDTs would also provide with results at the point-of-care to ensure safe vaccine administration. However, the disadvantage of currently available RDTs is that they have not yet been validated for screening for past DENV infection and may lack sufficient sensitivity and specificity to ensure effective vaccination strategies. Since RDTs have typically only been evaluated in the context of acute DENV infection and not for the detection of past infection, a systematic review was performed to evaluate the sensitivity and specificity of commercially available RDTs used for detecting IgG antibodies against DENV as a marker of previous DENV infection.

Methods

A systematic review was performed according to the Preferring Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [22]. PubMed and EMBASE databases were searched from January 1, 2000, to May 31, 2018 to identify relevant publications in peer-reviewed journals as original scientific research. Search terms were based on a PICO (population, intervention, comparator, and outcome) question format. The population encompassed individuals with known or possible previous DENV infection. The intervention was use of RDTs for detection of DENV IgG antibodies, with the comparator being a validated laboratory-based ELISA or PRNT assay. The primary outcome was previous DENV infection, which was measured in studies by the sensitivity and specificity for IgG detection. The search was performed using the following terms: (dengue OR “dengue virus” OR “dengue fever”) AND (“rapid diagnostic test” OR “rapid test”) AND (IgG OR sensitivity OR specificity OR “commercially available” OR “prior infection” OR “previous infection” OR “convalescent” OR seropositive OR seropositivity).

After all studies were retrieved, two reviewers independently reviewed all potentially relevant studies in full. Disagreements between reviewers were resolved with further discussion between the two primary reviewers. Studies were included if they met the following inclusion criteria: studies evaluating the performance of RDTs that are able to test for DENV IgG, studies comparing RDTs to an established laboratory-based reference standard for determining DENV infection status, and studies involving samples from patients with prior DENV infection. Studies were excluded if they contained one or more of the following exclusion criteria: studies on the use of RDTs for diagnosing acute primary infection only, studies on non-commercially available

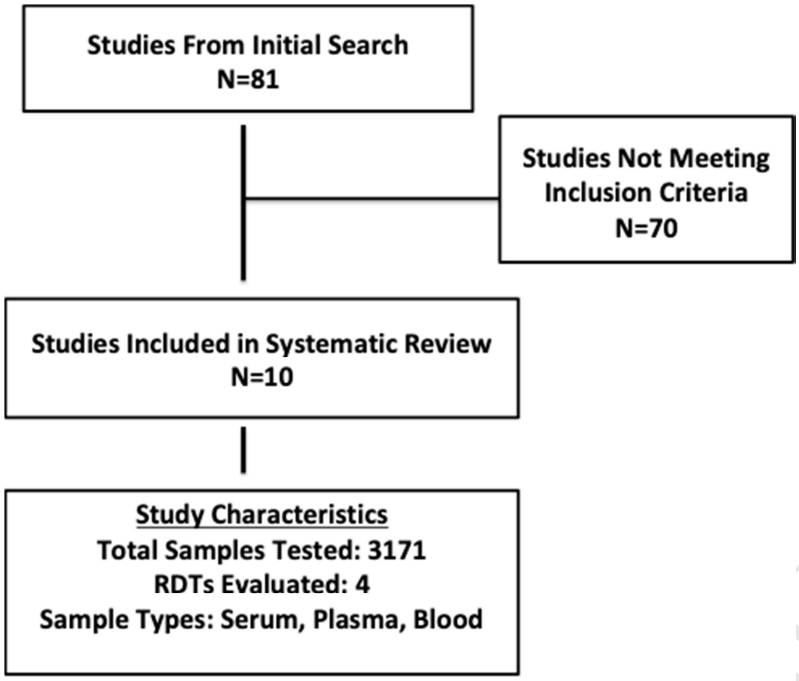
124 assays, studies not including an RDT, studies using a reference standard that has not been
125 independently validated, or studies only having a clinical diagnosis as a reference standard.
126
127 Studies were summarized outlining the principal components of each cohort. The summary
128 included the authors, sample size, study location, test characteristics and reference standard.
129 Study results were extracted and summarized for all included studies. Impact on test
130 performance based on variables such as geographic location (if endemic for dengue and other
131 flaviviruses), travel history, previous vaccination status, DENV serotype, and previous infection
132 with other flaviviruses, were also considered. Data from all studies were aggregated, and
133 frequency statistics were run to describe the population tested across all studies. Forest plots
134 were generated to depict the range of sensitivity and specificity results for the RDTs studied.
135 The quality of each study was assessed following QUADAS-2 guidelines, and the complete body
136 of evidence was evaluated using GRADE guidelines [23,24].
137
138 Semi-structured telephone interviews were conducted by the primary author with dengue rapid
139 test product managers from all available dengue RDT manufacturers with commercially-
140 available tests that had published data. Questions were asked regarding the performance,
141 regulatory status, regional availability, intended use, and scientific principles regarding their
142 dengue RDT technology, the availability of data regarding serostatus determination with RDTs,
143 and the feasibility of updating the RDTs for use in determining dengue serostatus. Information
144 was evaluated qualitatively, and common answers regarding the current capabilities of dengue

RDTs and the potential for detection of dengue serostatus that were mentioned by a majority of manufacturers were identified.

Results

The initial search identified 81 potential published studies for evaluation. Of these, 70 studies did not meet the inclusion and exclusion criteria for the systematic review. Twenty-three studies contained information on the sensitivity and specificity of dengue RDTs compared to conventional laboratory-based ELISA testing. However, 13 of these studies were excluded since they only evaluated RDT performance for acute primary DENV infection and did not provide data on the performance of the IgG component for known or possible previous infection. After filtering studies based on all inclusion and exclusion criteria, 10 studies were included in the final systematic review (Fig 1).

Fig 1. PRISMA Diagram



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162 The 10 studies included prospective and retrospective cohort studies [25-34]. Four dengue RDT
163 brands were represented: SD BIOLINE Dengue Duo (Alere/Abbott), Panbio Dengue Duo
164 (Alere/Abbott), OneStep Dengue Fever IgG/IgM RapiCard InstaTest (Cortez), and the GenBody
165 Dengue IgG/IgM test. Table 1 shows a summary of all studies, including information on the
166 types of samples tested and patient characteristics.

167 **Table 1. Study Summary**

Author, year, country	Test(s) Evaluated	Sample Size	Sample Type	Patient Characteristics	Reference Method	IgG Sensitivity (95% CI)	IgG Specificity (95% CI)
Vickers 2017 Jamaica	OneStep Dengue Fever IgG/IgM RapiCard InstaTest	339	Retrospective Serum	Suspected dengue; all ages	ELISA	All Samples: 44.4% (38.2-50.7%)	All Samples: 95.1% (88.0-98.7%)
Piedrahita 2016 Colombia	SD BIOLINE Dengue Duo	41	Prospective Serum	Suspected dengue; ages <18 years	ELISPOT-MNT	All Samples: 26.9% (7.9-41%)	All Samples: 66.7% (39.5-93.9%)
Vickers 2015 Jamaica	SD BIOLINE Dengue Duo	339	Retrospective Serum	Suspected dengue; Secondary infection:	ELISA	All Samples: 39.1% (33.3-45.2%) Secondary	All Samples: N/A Secondary

				IgM/IgG ratio <1.2; all ages		Infection: 52.1% (42-62%)	Infection: 100% (95.6-100%)
Krishnanant-hasivam 2015 Sri Lanka	SD BIOLINE Dengue Duo	143	Prospective Plasma	Suspected dengue	ELISA	All Samples: 38.8% (30.1-48.1%)	All Samples: 95.5% (77.1-99.2%)
Lee 2015 Malaysia	GenBody Dengue IgG/IgM, SD BIOLINE Dengue Duo, Panbio Dengue Duo	311	Prospective Whole Blood	Known dengue IgG positive and negative samples	ELISA	IgG-positive Samples Genbody: 96.7% SD BIOLINE: 82% Panbio: 75.3%	IgG-negative Samples Genbody: 100% SD BIOLINE: 100% Panbio: 100%
Pal	SD BIOLINE	834	Prospective	Suspected	IgG Capture	Convalescent	Convalescent

2015 Peru, USA, Cambodia, Venezuela	Dengue Duo, Panbio Dengue Duo		and Retrospective Serum, Plasma, and Fingerstick Whole Blood	dengue; Convalescent timepoints: 15+ days after symptom onset; All ages	ELISA	Samples SD BIOLINE: 93.9% (90.2-96.6%) Panbio: 98% (95.5-99.4%)	Samples SD BIOLINE: 87.1% (84.1-89.8%) Panbio: 58.3% (54.2-62.4%)
Sanchez-Vargas 2014 Mexico	SD BIOLINE Dengue Duo	397	Prospective Serum	Secondary infection: IgG positive regardless of NS1 or IgM results; Negative samples from other febrile	IgG Capture ELISA	All Samples: 90.1% (85.3-94.8%) Secondary infection: 83.7% (72.3-95.0%)	All Samples: 92.5% (88.8-96.1%)

				illnesses			
Pan-Ngum 2013 Sri Lanka	Panbio Dengue Duo	549	Prospective Serum	Suspected dengue, ages ≥ 16 years	ELISA	All Samples: 61.9% (50.7-72.3%)	All Samples: 79.6% (75.6-83.1%)
Moorthy 2009 India	Panbio Dengue Duo	86	Retrospective Serum	Dengue-like illness	IgG Capture ELISA	All Samples: 87.5%	All Samples: 66.6%
Groen 2000 Curacao, Indonesia, Netherlands	Panbio Dengue Duo	132	Retrospective Serum	Suspected dengue; other viral infections	Consensus of multiple immunoas-says	All samples: 52%	All samples: 100%

168

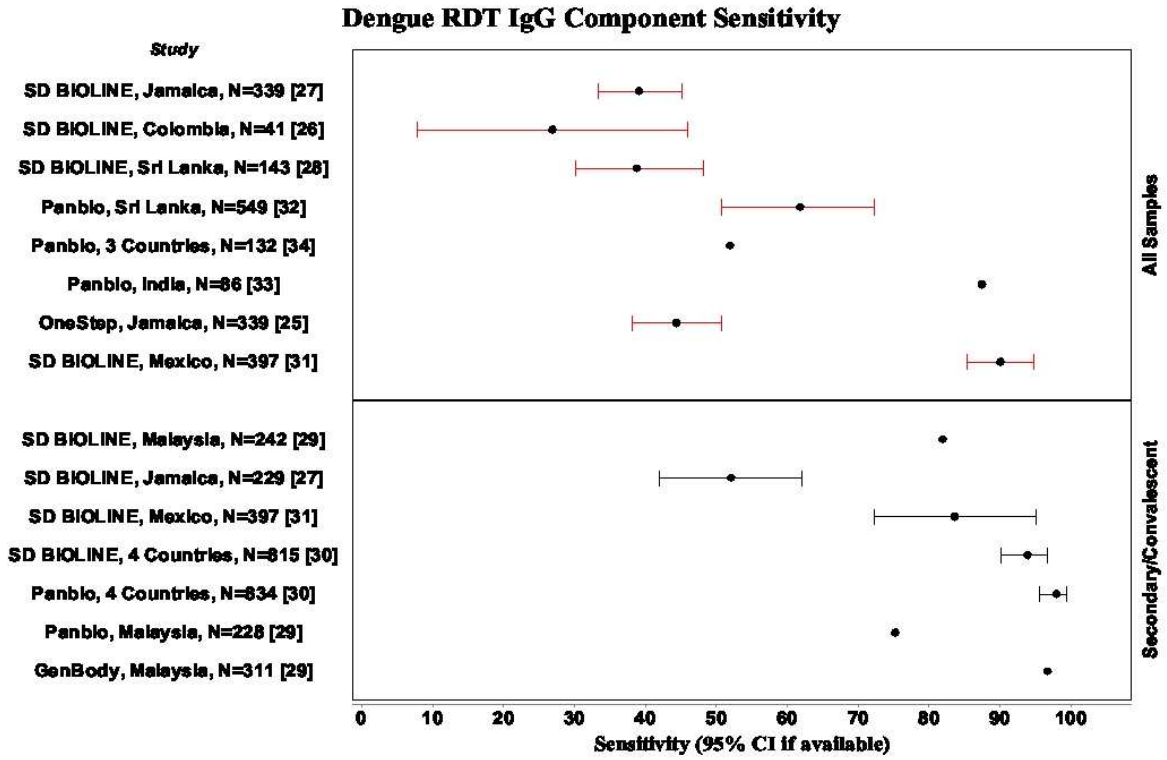
169 Abbreviations: ELISA, enzyme-linked immunoassay. ELISPOT-MNT, enzyme-linked immunospot microneutralization test

In total, 3171 samples were tested with RDTs across all the studies. Sample types included whole blood, plasma, and serum. No studies examined the performance of dengue RDTs to detect remote previous infection. Eight of the 10 studies evaluated the ability of the IgG component of the RDT to detect DENV IgG antibodies present in samples from all suspected or known dengue patients being evaluated for DENV infection, compared to a laboratory-based IgG ELISA test. Additionally, four of the 10 studies included samples from individuals described as having secondary DENV infection (defined in studies as documented previous infection or IgG positivity) or convalescent timepoints after recent infection (i.e., defined in one study as 15 days or more after symptom onset), providing some insight into the performance of the IgG component of the RDTs in individuals who had been infected with DENV previously.

Figure 2 shows the sensitivity of the dengue RDT IgG component from studies evaluating all samples from patients with suspected or known DENV infection as well as studies with separate categories for secondary DENV infection or convalescent timepoints after recent infection.

Fig 2. Dengue RDT IgG Sensitivity Results

Figure 2 shows the sensitivity estimates and 95% confidence intervals (when reported) for detection of dengue IgG for each RDT evaluated. Sensitivity in samples from all suspected and known dengue patients is shown in the top half of the figure, followed by sensitivity in samples from secondary infections or convalescent timepoints after recent infection in the bottom half.

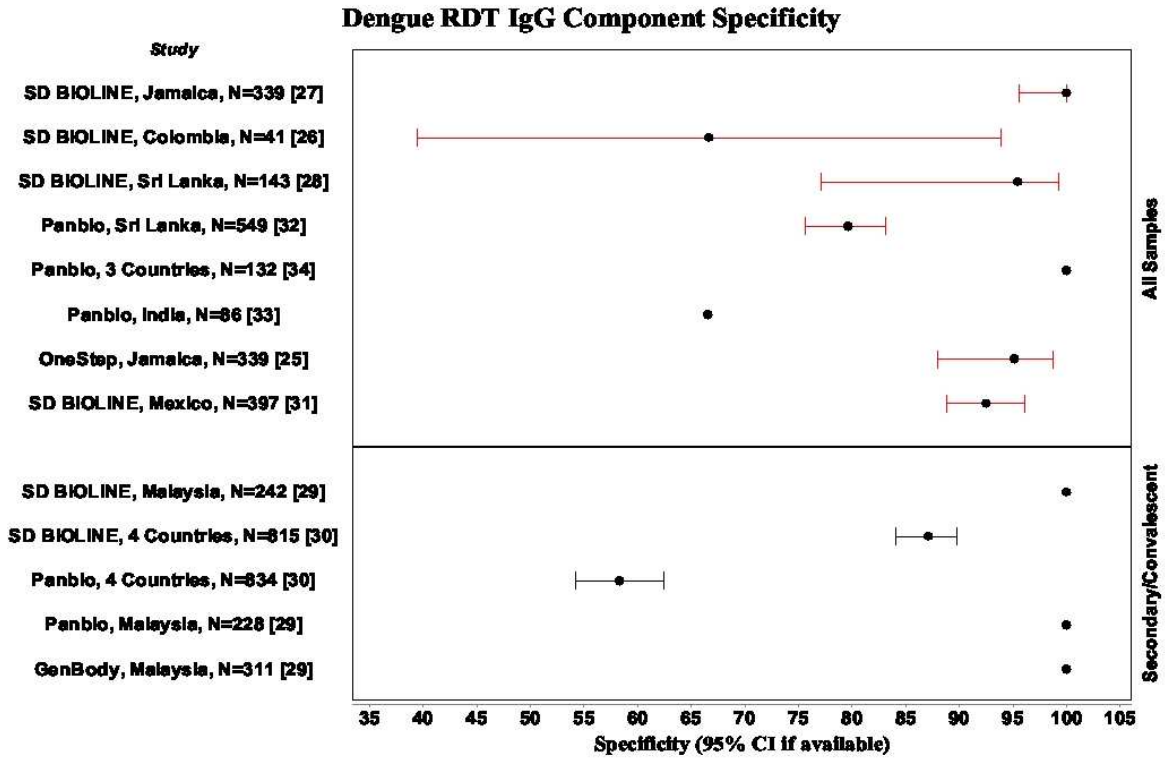


The most commonly studied RDT was the SD BIOLINE Dengue Duo test, followed by the Panbio Dengue Duo test, and all RDTs in this review could detect both IgG and IgM. When used in the context of all samples being tested for DENV infection, the sensitivity of the RDT IgG component typically ranged between 30-60%. However, when evaluated only in secondary infection or convalescent timepoint samples, the sensitivity of the RDT IgG component was significantly higher, typically between 75-98% with wide confidence intervals. This is consistent with the fact that all samples under evaluation for DENV infection included cases of acute primary infection, which would have had much lower levels or no IgG antibodies present, depending on when in the course of infection the samples were drawn.

Figure 3 demonstrates the specificity of the dengue RDT IgG component reported across the studies. In all samples under evaluation for DENV infection, the specificity of the dengue RDTs' IgG component ranged from 65-100%, again with wide confidence intervals. When evaluated only in cases of secondary infection or convalescent timepoints after recent infection, the specificity rose to between 85-100% in most studies.

Fig 3. Dengue RDT IgG Specificity Results

Figure 3 shows the specificity estimates and 95% confidence intervals (when reported) for detection of dengue IgG for each RDT evaluated. Specificity in samples from all suspected and known dengue patients is shown in the top half of the figure, followed by specificity in samples from secondary infections or convalescent timepoints after recent infection in the bottom half.



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217 Overall, there were no studies that directly evaluated the use of RDTs for determination of
218 dengue serostatus, as all studies examined RDT performance in the context of either all samples
219 from patients with possible DENV infection, and/or a subset from samples of secondary
220 infection or convalescent timepoints after recent DENV infection. Although all studies included
221 samples from dengue-endemic areas, none of them provided information on vaccination or
222 infection status of patients for other flaviviruses, all of which may lead to cross-reactivity with
223 dengue serological testing. Lack of cross-reactivity data is a major limitation for pre-vaccination
224 screening, since false-positive results due to cross-reactivity to other co-circulating flaviviruses
225 could lead to inappropriate vaccination of dengue-naïve individuals. Studies using samples that
226 have been well-characterized with either ELISA or PRNT for exposure to other flaviviruses,
227 particularly Zika virus given its genetic similarity to dengue, were absent. Additionally, the

majority of studies tested serum samples, and not whole blood samples, which are more relevant for testing at the point-of-care. There were not enough studies using whole blood to conclude whether or not the sensitivity and specificity of the test differed compared to serum or plasma.

Table 2 summarizes the QUADAS-2 assessment by study, while Table 3 summarizes the GRADE assessment of the complete body of evidence, using criteria from published guidelines [23,24]. In the QUADAS-2 assessment, there were high patient selection applicability concerns for all studies, since none of the RDT tests were exclusively performed on patients with remote previous DENV infection. This also lead to unclear applicability of the index test, since the interpretation of an IgG positive result is complicated by the possible detection of IgG in acute infections and the potential absence of IgG in some cases of previous infection.

Table 2. QUADAS-2 Assessment of Studies

	Risk of Bias				Applicability Concerns		
Study	Patient Selection	Index Test	Reference Standard	Flow & Timing	Patient Selection	Index Test	Reference Standard
Vickers 2017	Low	Low	Low	Low	High	Unclear	Low
Piedrahita 2016	Low	Low	Low	Low	High	Unclear	Low
Vickers 2015	Low	Low	Low	Low	High	Unclear	Low
Krishnananthasivam 2015	Low	Low	Low	Low	High	Unclear	Low
Lee 2015	Unclear	Low	Low	Low	High	Unclear	Low
Pal 2015	Low	Low	Low	Low	High	Unclear	Low

Sanchez-Vargas 2014	Low	Low	Low	Low	High	Unclear	Low
Pan-Ngum 2013	Low	Low	Low	Low	High	Unclear	Low
Moorthy 2009	Low	Low	Low	Low	High	Unclear	Low
Groen 2000	Low	Low	Unclear	Low	High	Unclear	Unclear

In the GRADE assessment, the overall certainty of evidence for using dengue RDTs for determination of dengue serostatus was low. The indirectness of evidence was serious given the fact that no study evaluated the RDTs for the detection of remote previous DENV infection only. Additionally, the inconsistency of the studies was serious, as studies varied in how they defined secondary infection, the population studied, the laboratory reference standard used, the cutoffs used to define a positive and negative IgG result, and how samples were chosen for inclusion in the evaluations. Due to these differences across studies, a meta-analysis of data was not conducted. Further data analysis and subpopulation analyses were not done due to the absence of data relating to vaccination status, age groups, other flaviviruses, and time since infection, as well as the overall heterogeneity of study designs.

Table 3. GRADE Evaluation of Evidence Quality

Number of Studies	Study Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Quality	Importance

10	Cohort Studies	Not Serious	Serious	Serious	Not Serious	Low	Critical

Semi-structured interviews were conducted with dengue rapid test product managers from 4 dengue RDT manufacturers (Abbott/Alere, Bio-Rad, CTK Biotech, and GenBody) who responded to an interview request, along with Sanofi-Pasteur, the manufacturer of Dengvaxia®. Manufacturers noted that dengue RDTs have typically been designed to detect the higher levels of IgG that can be present soon after primary and secondary DENV infections and not low-level IgG, though the specific limit of detection for IgG for each test is not publicly available. Furthermore, current RDTs do not have regulatory approval and were not validated for determination of dengue serostatus. An RDT optimized to detect remote prior infection would benefit from having higher sensitivity for IgG than current RDTs, as IgG levels can wane over the course of time; however, additional testing would be needed to ensure that this does not result in increased cross-reactivity with other flaviviruses. Manufacturers also indicated that this should be technically feasible, given the research already done to develop current RDTs as well as the technical expertise from using and developing laboratory-based ELISA tests, which typically have higher IgG sensitivity. Additional discussions over whether total DENV IgG or IgG specific to particular DENV antigens would be helpful, as well as whether other analytes (e.g., IgM or other analytes found in current tests) are needed would also be helpful to guide the final design of an RDT.

Discussion

In acute secondary DENV infections and convalescent timepoints after recent infection, the sensitivity and specificity of the IgG component of dengue RDTs was typically above 75% and 80%, respectively, when compared to IgG detection by laboratory-based ELISA testing. However, a major limitation is the fact that no study evaluated the performance of RDTs for past DENV infection, as studies only used early convalescent samples or samples from presumed acute primary or secondary infection. Therefore, no data are available on RDTs that have tested IgG for DENV infections in the remote past.

Studies varied depending on the population studied, the types of samples included in each study, how secondary infections were determined, what cutoffs were used to categorized IgG levels as positive or negative, and the reference standard test used. Sensitivity and specificity of the IgG component were lower when the dengue samples tested included acute primary infection samples. This may be due to the lack of IgG or low-levels of IgG present in samples taken soon after infection, where IgM constitutes the primary initial immune response.

This systematic review did not identify any studies that specifically evaluated dengue RDTs for determining dengue serostatus in the context of remote prior infection only. The review also identified four major challenges of use of dengue RDTs for detecting prior infection:

1) **Data challenges**

   The studies included in this review evaluated the performance of the IgG component of dengue RDTs in the following groups: 1) all samples from individuals with suspected

DENV infection and/or 2) samples from individuals described as having secondary DENV infection or convalescent timepoints after recent infection. Although these groups are not equivalent to individuals with more remote previous DENV infection, they do provide some insight into the performance of dengue RDTs for identifying IgG antibodies as compared to a laboratory-based test. However, their performance in these populations should be taken as an overestimate compared to an overall population presenting for vaccination screening, since the groups studied typically have higher IgG levels that are much easier to detect compared to a general population. This will primarily impact the sensitivity of the assays. The extent of specificity will vary depending on the population from which dengue-negative reference samples were drawn (e.g., this ranges from using US adults as dengue-negative controls to samples from dengue-endemic populations).

2) Regulatory challenges

Determination of dengue serostatus is not explicitly included as an approved part of the intended use statements of dengue RDTs. This is not surprising, given the fact that the primary intention for these RDTs has been the diagnosis of acute DENV infection in patients with febrile illness. However, the label and intended use for some of these tests, where it is described as an aid to "diagnosis of DENV infection" may be interpreted to include determination of past infection. As an example, the SD Bioline Dengue Duo intended use is to "aid in the presumptive diagnosis between primary and secondary dengue infection." However, IgG can still be detected during and shortly after acute primary infection, which can complicate the distinction between primary and secondary

infection. Regulatory authorities may interpret use of RDTs for determination of past DENV infection as off-label usage, which may lead to procurement and implementation challenges, depending on local policies. Additional research on the use of RDTs for measuring past infection would benefit from following local and regional regulatory requirements, in order to assist RDTs in obtaining an indication for this use.

3) Technical challenges

Since IgG antibody levels can be higher during or soon after acute infection, RDTs that have been optimized to diagnose acute infection may not be suitable for detection of lower IgG antibody levels in individuals with more remote prior DENV infection. This may help to explain the lower sensitivity of the IgG component seen in some studies, although the limit of detection for IgG for each test is not publicly available information. Additionally, dengue serological tests can cross-react with antibodies to other flaviviruses, such as West Nile virus and Zika virus, and none of the studies in this review characterized the occurrence of other flaviviruses in their sample sets. Lowering the titer of IgG antibodies that RDTs can detect in order to increase sensitivity for detection of past infection may also lead to lower specificity. More specific antigens could also be explored. Furthermore, specificity may vary depending on the prevalence of other flavivirus infections as well as vaccinations used for other flaviviruses. Therefore, the relatively high sensitivity and specificity of RDTs may show that they match up well with commercial ELISAs designed to diagnose acute infection only. Additional research would

be helpful to compare RDTs not only to ELISAs, but also to PRNT assays, which may be a more specific measure of DENV exposure and may be a superior reference standard.

4) Impact of Zika virus

Most of the evaluations were conducted before the emergence of Zika virus, which is highly related to DENV. However, recent research on antibody cross-neutralization suggests that ZIKV lies outside the dengue virus serocomplex [35]. In a study on longitudinal serologic specimens from Latin America and Asia, ZIKV neutralizing antibody titers in patients after ZIKV showed low-level cross-reactivity to DENV that was greater in dengue-immune individuals [35]. These antibodies may be able to distinguish ZIKV from DENV infections, although additional research is needed to determine this. Over time it may become harder to distinguish the two viruses, and more specific antigens or tests may be necessary.

Strengths of this systematic review included over 3000 DENV samples tested, including a smaller subset of secondary infections and convalescent timepoints after recent infection, the geographic diversity of studies, and the inclusion of a number of different commercially-available dengue RDTs and sample types. However, the review was limited by the heterogeneity of data and the inability to evaluate factors such as infection with other flaviviruses and the potential impact of other flavivirus vaccines.

With such a paucity of data on the use of dengue RDTs for determining serostatus, further research is necessary to inform pre-vaccination screening approaches for dengue, as it is currently difficult to draw distinct conclusions regarding the performance of RDTs for this use. Studies could examine the performance of current RDTs for the direct purpose of determining serostatus, investigate the performance of the test in areas with co-circulating flaviviruses and vaccination, and assess the use of other reference standards such as PRNT. Based on the performance of currently available dengue RDTs in secondary infection and convalescent timepoints after recent infection, the IgG component of these RDTs do have reasonable performance for detection of these infections compared to conventional laboratory-based ELISA testing. However, further discussion within the scientific and public health community is needed to determine if this performance is sufficient for pre-vaccination screening or not. The decision to use RDTs will likely also depend on local factors, such as dengue seroprevalence, the availability of alternative tests, and the public health risk and benefit from vaccination.

Development of new dengue RDTs or modification of currently available RDTs may be the most beneficial for vaccination screening. Tests with higher sensitivity and specificity, and even new antigen or antibody targets can be investigated and validated by dengue RDT manufacturers, who have the necessary expertise to provide regulatory approved tests suitable for pre-vaccination screening [35]. Alternatively, in settings with sufficient laboratory capacity, laboratory-based testing may also considered, although slower turnaround time of these tests may lead to high rates of individuals not returning for their test results or vaccination [36]. Vaccination programs should evaluate all currently available testing options to determine how

best to evaluate for dengue serostatus in order to ensure safe and effective vaccination. New tests may be needed with high sensitivity and specificity at the point-of-care to avoid excluding individuals who would benefit from vaccination while at the same time preventing the inclusion of individuals who should not be vaccinated.

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Transparency Declaration

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