The use of rapid dengue diagnostic tests in a routine clinical setting in a dengue-endemic area of Colombia

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There is insufficient evidence of the usefulness of dengue diagnostic tests under routine conditions. We sought to analyse how physicians are using dengue diagnostics to inform research and development. Subjects attending 14 health institutions in an endemic area of Colombia with either a clinical diagnosis of dengue or for whom a dengue test was ordered were included in the study. Patterns of test-use are described herein. Factors associated with the ordering of dengue diagnostic tests were identified using contingency tables, nonparametric tests and logistic regression. A total of 778 subjects were diagnosed with dengue by the treating physician, of whom 386 (49.5%) were tested for dengue. Another 491 dengue tests were ordered in subjects whose primary diagnosis was not dengue. Severe dengue classification [odds ratio (OR) 2.2; 95% confidence interval (CI) 1.1-4.5], emergency consultation (OR 1.9; 95% CI 1.4-2.5) and month of the year (OR 3.1; 95% CI 1.7-5.5) were independently associated with ordering of dengue tests. Dengue tests were used both to rule in and rule out diagnosis. The latter use is not justified by the sensitivity of current rapid dengue diagnostic tests. Ordering of dengue tests appear to depend on a combination of factors, including physician and institutional preferences, as well as other patient and epidemiological factors.

Key words: dengue - diagnosis - health services - decision making

Dengue is an international public health emergency due to its rapid case number increase and fatality rate (Guzman et al. 2010). It is caused by one of four serotypes within the genus Flavivirus and is transmitted to humans by *Aedes* mosquitoes. Although it is present in most tropical and subtropical regions, the highest risk areas are in the Americas and Asia (Bhatt et al. 2013). The clinical presentation of dengue varies with age and immunological status and ranges from asymptomatic to severe and fatal infections. However, the factors associated with disease severity are not yet clearly understood. Abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, liver enlargement > 2 cm and an increase in haematocrit concurrent with a rapid decrease in platelet count have been proposed as warning signs of disease progression to help improve case management (Alexander et al. 2011). Disease is considered severe in the presence of severe plasma leakage with shock and/or fluid accumulation with respiratory distress, severe bleeding or severe organ impairment (Alexander et al. 2011). It is expected that, based on these definitions, clinicians will be able to classify subjects as having dengue, with or without warning signs of severe dengue and treat them according to international guidelines (WHO/TDR 2012). There is not a specific antiviral treatment for dengue and hence, case management comprises adequate fluid support, rest, paracetamol and close monitoring until recovery (WHO/TDR 2012).

Dengue cases are confirmed by virus isolation, antigen or RNA detection, seroconversion or a fourfold increase in specific IgM or IgG titres (Kao et al. 2005). Several dengue diagnostic assays are available, but they are used mainly for research or surveillance due to the infrastructure they require, including a prolonged testing period, relatively high cost and the need for patient follow-up (Kao et al. 2005). There are commercially available rapid dengue diagnostic tests that are more suitable for routine use in health care settings (Blacksell 2012). However, laboratory diagnosis of dengue is not necessary for clinical management except in atypical cases or when ruling out differential diagnoses (WHO/TDR 2012). In Colombia, the national guidelines stipulate the use of dengue diagnostic tests for surveillance purposes only (MPS/INS 2010). Despite this, rapid dengue diagnostic tests are frequently used within the country, perhaps due to the difficulty of diagnosis. Dengue diagnosis, under routine clinical care, is challenging because the typical clinical and laboratory characteristics of dengue in its febrile phase (temperature ≥ 38.5ºC plus headache, vomiting, myalgia, joint pain and

doi: 10.1590/0074-02760140359
Financial support: UniValle, COMFANDI
This study was partially funded by Standard Diagnostics Inc/Alere. However, the diagnostic test manufacturers do not have a role, either directly or through a third party, in the gathering or analysis of data, in the preparation or decision to submit the results for publication.
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Received 30 September 2014
Accepted 1 April 2015

sometimes macular rash, haemorrhagic manifestations, thrombocytopaenia, leukopaenia and elevation of hepatic aminotransferase levels) or critical phase (increasing haemoconcentration, hypoproteinaemia, haemorrhagic manifestations, pleural effusion, ascites, narrowing of the pulse pressure, liver failure, myocarditis, encephalopathy, thrombocytopaenia, increase in the activated partial-thromboplastin time and decrease in fibrinogen levels) overlap with other diseases prevalent in the same endemic regions (Simmons et al. 2012). The importance of considering clinicians in the development and implementation of diagnostic tests has been highlighted, as they are the most knowledgeable concerning the many contributions of new technologies to health care (Feinstein 2002). Here, we sought to analyse how dengue rapid diagnostic tests (RDTs) are been routinely used in health care settings in endemic areas to inform research and development and health services.

SUBJECTS, MATERIALS AND METHODS

Study design and population - A prospective study was conducted from March-December 2012 at 14 health care institutions in Cali, Colombia. This city is one of the three largest in Colombia with a total population of 2,294,653 inhabitants. Dengue is considered hyperendemic due to the circulation of all four dengue serotypes. In 2010, one of the largest dengue epidemics hit the area with 11,047 cases, 5.7% severe dengue and 16 attributable deaths. The 14 health care institutions included in the study were selected as they represent different levels of care (primary, secondary and tertiary) and have permanent access to rapid dengue diagnostic tests. Personnel at the participating institutions had been trained in dengue diagnosis and treatment in 2010 and 2011, therefore, the level of knowledge of dengue among physicians was considered high. Exhaustive sampling in the participating institutions was performed by including all subjects (regardless of age, sex or signs and symptoms) attending any of the 14 health care centres who were seen by a physician and were clinically diagnosed with dengue or for whom a dengue test was ordered during the study period. The study was approved by the Ethical Review Board of University of Valle and Family Compensation Fund of Valle del Cauca.

Rapid dengue diagnostic tests - Rapid dengue diagnostics were routinely available to all 14 institutions at a central lab. In August 2012, the largest institution (named here as A) was moved to a new building with its own lab and could perform dengue tests locally. At all 14 institutions, dengue diagnostics could be ordered at the physicians discretion at any time and results were made available through the computerised central clinical record system. During the study, the rapid dengue diagnostic test for the simultaneous detection of dengue-specific IgM and IgG (Standard Diagnostics Inc) was available at the laboratory facilities. The SD BIOLINE Dengue Duo kits (Standard Diagnostics Inc) that simultaneously detect IgM, IgG and NS1 were only occasionally available. Samples were processed by experienced laboratory technicians following the manufacturer's instructions. The study personnel did not have any direct or indirect contact with physicians to avoid any potential bias.

Data collection and quality control - Dengue cases were identified from clinical records with a diagnosis of dengue according to the codes of the International Classification of Diseases version 2010 (ICD-10) and from the forms used to notify the national surveillance system, which is compulsory (WHO 2010). Information on patient identification and demographics, health care institution, date and ward in which the subject was seen by the physician was provided by the centralised statistics department of the health care institutions. Data on all subjects for whom a dengue diagnostic test was ordered and the results of those tests were provided by the computerised laboratory information system of the central laboratory. Quality control of the data was performed by verifying respective patient identification numbers present in both databases. Indirect quality control of the technician in charge of dengue at the central lab was performed at the beginning of the study with three blind samples prepared by the research team. The results of these three samples remained consistent and further training was not considered necessary.

Statistical analysis - Clinical, laboratory and surveillance databases were merged and exported to Stata 10 (Stata Corp LP, USA) for data analysis. A descriptive analysis was conducted to identify the relative frequencies of the following: severity of dengue, cases in which a diagnostic test was ordered and IgM positivity index. The latter was defined as the percentage of IgM positive results out of the total IgM performed. Dengue cases were considered to be clinically diagnosed if one of ICD-10 codes for dengue, A90 or A91, were reported in the clinical record. The dates when subjects were clinically diagnosed and tested for dengue were compared to ensure the clinical diagnosis preceded the lab test. When these dates were the same, the first reported diagnosis on the date was considered. Factors associated with ordering a dengue test in subjects with a clinical diagnosis of dengue were identified using contingency tables with the corresponding odds ratio (OR) and 95% confidence interval (CI) and chi-squared test or the Fisher exact test when necessary. For quantitative variables, nonparametric tests were used. Multivariate logistic regression models were fitted to estimated adjusted OR with their corresponding 95% CIs. A p-value < 0.05 was considered statistically significant. Repeated dengue tests and those performed in subjects without an ICD-10 for dengue in their clinical records were analysed separately. It was assumed that the subjects clinical diagnosis was other than dengue when there were not matches between the lab database and both the dengue clinical and surveillance databases. For subjects for whom a dengue test was ordered, but whose clinical diagnosis and tests results were other than dengue, a descriptive analysis was performed.

RESULTS

A total of 778 subjects had a clinical diagnosis of dengue, of whom 39 (5%) were classified as having severe dengue; 465 (59.8%) were men and 275 (35.4%) were zero-14 years old. The majority of cases (51.9%) were reported by two (A and M) of the 14 institutions. Institution A (26.6% of cases), a referral institution, was classified as offering

TABLE I
Characteristics of subjects with clinical diagnosis of dengue

Characteristic	Total (n = 778) n (%)	Dengue cases: Nonsevere (n = 739; 95%) n (%)	Dengue cases: Severe (n = 39; 5%) n (%)	OR (95% CI)	p
Sex					
Male	465 (59.8)	445 (60.2)	20 (51.3)	-	0.2
Female	313 (40.2)	294 (39.8)	19 (48.7)	1.4 (0.7-2.7)	
Years of age					
Median (range in years)	20.8 (0.6-91.8)	20.9 (0.6-91.8)	19.4 (0.6-60.3)	-	0.9
0-4.9	72 (9.3)	68 (9.2)	4 (10.3)	1	
5-14.9	203 (26.1)	193 (26.1)	10 (25.6)	0.8 (0.2-3)	0.8
15-44.9	411 (52.8)	392 (53.1)	19 (48.7)	0.8 (0.2-2.5)	0.7
45-64.9	74 (9.5)	68 (9.2)	6 (15.4)	1.5 (0.4-5.6)	0.5
≥ 65	18 (2.3)	18 (2.4)	0 (0)	0	0.3
Institution					
A	207 (26.6)	199 (27)	8 (20.5)	1	0.6
B	6 (0.7)	6 (0.8)	0 (0)	0	
C	73 (9.4)	70 (9.5)	3 (7.7)	1 (0.2-4.1)	
D	40 (5.1)	38 (5.1)	2 (5.1)	1.3 (0.2-6.4)	
E	26 (3.3)	25 (3.4)	1 (2.5)	1 (0.1-8.3)	
F	23 (3)	23 (3.1)	0 (0)	0	
G	81 (10.5)	79 (10.7)	2 (5.1)	0.6 (0.1-3)	
H	40 (5.1)	35 (4.7)	5 (12.8)	3.5 (1-11.6)	
I	23 (3)	22 (39)	1 (2.5)	1.1 (0.1-9.5)	
J	11 (1.4)	10 (1.3)	1 (2.5)	2.4 (0.2-22)	
K	36 (4.6)	34 (4.6)	2 (5.1)	1.4 (0.3-7.2)	
L	1 (0.1)	1 (0.1)	0 (0)	0	
M	197 (25.3)	183 (24.7)	14 (36)	2 (0.7-4.6)	
N	14 (1.8)	14 (1.9)	0 (0)	0	
Ward					
Outpatient	408 (52.4)	389 (52.6)	19 (48.3)	1	
Emergency	370 (47.6)	350 (47.4)	20 (51.3)	1.1 (0.6-2.2)	0.6
Month					
March	127 (16.3)	120 (16.2)	7 (18)	1	0.1
April	84 (10.8)	79 (10.7)	5 (12.8)	1.1 (0.3-3.5)	
May	57 (7.3)	55 (7.4)	2 (5.1)	0.6 (0.1-3.1)	
June	57 (7.3)	55 (7.4)	2 (5.1)	0.6 (0.1-3.1)	
July	56 (7.2)	53 (7.1)	3 (7.7)	0.9 (0.2-4)	
August	61 (7.8)	61 (7.8)	0 (0)	0	
September	72 (9.2)	72 (9.2)	9 (23.1)	2.4 (0.8-7)	
October	80 (10.3)	80 (10.3)	5 (12.8)	1.1 (0.3-3.7)	
November	61 (7.8)	61 (7.8)	2 (5.1)	0.6 (0.1-3)	
December	123 (15.8)	123 (15.8)	4 (10.3)	0.6 (0.1-2)	
Total dengue tests					
0	393 (50.5)	380 (51.4)	13 (33.3)	1	
1	320 (41.1)	297 (40.2)	23 (59)	2.2 (1.1 -4.5)	0.01
2	53 (6.8)	50 (6.8)	3 (7.7)	1.7 (0.5-6.3)	0.4
3	11 (1.4)	11 (1.5)	0 (0)	0	0.5
5	1 (0.2)	1 (0.1)	0 (0)	0	0.8

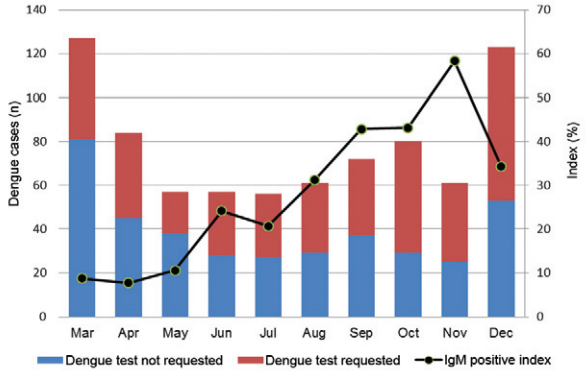
CI: confidence interval; OR: odds ratio.

a tertiary level of care, while the institution M (25.3% of cases) was classified as offering secondary level of care. Subjects with severe cases were more frequently female, between 45-64.9 years old and seen at the emergency wards with the highest frequency in September. However, there were no statistically significant differences between severe and nonsevere cases regarding sex, age, institution, month or emergency consultation (Table I).

At least one dengue diagnostic test was ordered in 386 (49.5%) subjects with slight monthly variations (Figure). Age and sex of subjects were not associated with the ordering of a dengue test, but with severity of disease, emergency consultation, institution and month. All of these factors remained independently associated in the multivariate model; however, institution was withdrawn from the model as a correlation with emergency consultation existed (Table II).

The IgM positivity index was 30% (114/386), but it varied monthly reaching up to 58% in November (Figure). The IgM positivity index was twice as high in severe dengue cases (46.1%-12/26) as in nonsevere dengue cases (28.3%-102/360) cases (p = 0.05). During the study period, 491 dengue tests were ordered for subjects who did not have an explicit clinical diagnosis of dengue in their clinical record, but another diagnosis (such as upper and lower respiratory tract infections, unspecified viral disease, meningitis, unspecified thrombocytopenia, human immunodeficiency virus, diarrhoea, malaria, leptospirosis, among others). In this group, the IgM positivity index was 3.6% (18/491).

More than one dengue test was ordered in 65 subjects: two tests in 53, three in 11 and five in one person. There was no difference in the frequency of repeated dengue tests between severe (3-7.7%) and nonsevere dengue cases (62-8.4%). Of the 58 subjects with negative IgM or IgG results in the first test, IgM seroconversion was observed in 15 subjects in the second test and three subjects in the third test. Similar results were found with IgG, with seroconversion observed in 15 subjects in the second test and three subjects in the third test. No seroconversion was observed in subsequent tests. Only two subjects were tested twice with NS1 and negative results occurred in both cases. Changes from positive to negative IgM or IgG results were observed in two subjects.



Monthly trends in number of dengue rapid diagnostic tests requested and IgM positive results.

DISCUSSION

In the present study, we analyse the use of rapid dengue diagnostic tests under routine conditions in health care settings in an endemic area of Colombia. An emerging pattern in this study was the use of RDTs to either rule in or rule out dengue as a differential diagnosis. Half of the subjects with a clinical diagnosis of dengue had a dengue test ordered (i.e., a test used to rule in dengue) but subjects with other clinical diagnosis were also tested for dengue (i.e., a test use to rule out dengue). The use of dengue tests to confirm dengue diagnosis is supported by the relatively high specificity of the currently available tests, which is required to rule in a diagnosis. The reported specificity of the two tests used in the study sites, namely SD BIOLINE Dengue IgM/IgG and SD BIOLINE Dengue Duo NS1, IgM/IgG RDT, ranges from 86.8-92.3% and from 83.9-100%, respectively (WHO/TDR 2009, Tricou et al. 2010, Blacksell et al. 2011, Gan et al. 2014). However, to date, there is no evidence that the dengue test results influence the physicians behaviour or impact the prognosis of a subject for whom a clinical diagnosis of dengue was already made (Andries et al. 2012). Hence, it is necessary to further assess the impact and cost-effectiveness of implementing rapid dengue diagnostics tests in real-life settings, accounting for evidence-based decisions. Contrastingly, high sensitivity of the test is required to rule out a diagnosis. Rapid dengue diagnostic tests have shown to have a large variation in sensitivities ranging from 47-79.2% for IgM/IgG and from 78.4-93.9% for NS1/IgM/IgG (WHO/TDR 2009, Osorio et al. 2010, Gan et al. 2014). Consequently, they are not suitable for ruling out dengue or for screening purposes. Understanding why physicians decide to order dengue tests is therefore worth exploring, while using their input to improve current rapid diagnostic methods.

The results show that severe dengue presentation, emergency consultation and month were all independently associated with the ordering of dengue diagnostic tests. Several explanations could be considered for these findings. First, due to the potential fatal consequences of misdiagnosis in a severe case, clinicians may consider confirmation of these cases to be more relevant than in the nonsevere cases or seek assurances for themselves and the patient/patient's family in the validity of a positive result when ordering a dengue diagnostic test. The need for assurance can be interpreted as defensive in case of possible litigation or ones own expectations of self-efficacy (i.e., performing as expected), while the need to reassure a worried patient/patient's family can be either real or perceived due to social pressure (van der Weijden et al. 2002). Secondly, RDTs are expected to be used at bedside; hence in the context of emergency care, rapid dengue diagnostic tests could be thought of as an accessible tool to speed up the differential diagnosis process and corresponding management (Peeling et al. 2010). Alternatively, this factor may be explained by the inclusion of emergency departments located in the secondary and tertiary levels of care where it is known that diagnostic tests are more commonly used to rule in a disease. In contrast, diagnostics are used more often to rule out a condition or to determine the need for referral in primary care centres

TABLE II

Factors associated with requesting a dengue rapid diagnostic test in subjects with clinical diagnosis of dengue

Characteristic	Dengue test requested: Yes (n = 386) n (%)	Dengue test requested: No (n =392) n (%)	OR (95% CI)	p	Adjusted OR (95% CI)	p
Sex						
Male	236 (61.1)	229 (58.4)	1		-	
Female	150 (38.9)	163 (41.6)	0.8 (0.7-1.2)	0.4	-	
Years of age						
Median (range in years)	20.8 (0.6-79)	20.9 (0.3-91.8)	-	0.3	-	
0-4	31 (8)	41 (10.4)	1		-	
5-14	104 (27)	100 (25.3)	1.4 (0.8-2.3)	0.2	-	
15-44	206 (53.4)	205 (52.3)	1.3 (0.8-2.2)	0.2	-	
45-64	39 (10.1)	35 (9)	1.5 (0.7-2.8)	0.2	-	
≥ 65	6 (1.5)	12 (3)	0.6 (0.2-2)	0.4	-	
Dengue classification						
Nonsevere	360 (93.2)	379 (96.7)	1		-	
Severe	26 (6.8)	13 (3.3)	2.1 (1-4.2)	0.03	2.2 (1.1-4.5)	0.02
Institution level of care						
Primary	147 (38.1)	221 (56.4)	1		[a]	
Secondary	93 (24.1)	104 (26.5)	1.3 (0.9-1.9)	0.09	-	
Tertiary	146 (37.8)	67 (17.1)	3.3 (2.2-4.7)	< 0.001	-	
Ward						
Outpatient	171 (44.3)	237 (60.5)	-		-	
Emergency	215 (55.7)	155 (39.5)	2 (1.4-2.5)	< 0.001	1.9 (1.4-2.5)	< 0.001
Month						
March	46 (12)	81 (20.7)	1		1	
April	39 (10.1)	45 (11.5)	1.5 (0.8-2.6)	0.1	1.4 (0.7-2.4)	0.2
May	19 (5)	38 (9.7)	0.8 (0.4-1.7)	0.7	0.7 (0.4-1.5)	0.5
June	29 (7.5)	28 (7.1)	1.8 (0.9-3.4)	0.06	1.7 (0.9-3.3)	0.08
July	29 (7.5)	27 (6.9)	1.9 (1-3.5)	0.05	1.6 (0.8-3.1)	0.1
August	32 (8.3)	29 (7.4)	1.9 (1-3.6)	0.03	1.9 (1-3.6)	0.04
September	35 (9)	37 (9.4)	1.6 (0.9-3)	0.08	1.5 (0.8-2.7)	0.2
October	51 (13.2)	29 (7.4)	3 (1.7-5.5)	< 0.001	3.1 (1.7-5.5)	< 0.001
November	36 (9.3)	25 (6.4)	2.5 (1.3-4.7)	0.004	2.3 (1.2-4.4)	0.008
December	70 (18.1)	53 (13.5)	2.3 (1.4-3.8)	0.001	2 (1.2-3.4)	0.006

a: withdrawn from the model because of colinearity with institution; CI: confidence interval; OR: odds ratio.

(Whiting et al. 2007). Finally, the association between ordering rapid dengue diagnostic tests with the month of the year may suggest that clinicians use diagnostic tests to detect outbreaks of febrile diseases, which is relevant to countries such as Colombia, where there is not a distinctive seasonality of dengue incidence. Of the subjects meeting clinical criteria for dengue, the probability of in fact having dengue is lower in nonepidemic periods than during epidemics. Hence, laboratory diagnosis may help physicians to adjust the predictive values of the clinical definition. This epidemiological reason for ordering dengue tests could be useful in routine care by adjusting of the predictive values of the clinical definitions of disease in subsequent patients or to increase awareness of diseases. Hence, improved laboratory based surveillance that informs clinicians of dengue outbreak at the local level have been proposed to assist them (Lorenzi et al. 2013). This epidemiological use of health technology is rarely recognised and could be added to the five categories of factors (diagnostic, therapeutic and prognostic, patient-related, doctor-related and policy and organisation-related) that influence ordering of diagnostic tests previously proposed (Whiting et al. 2007).

During field work, a dengue epidemic was not declared in the study site, but the IgM positivity steadily increased reaching 58% in November. However, this

increase in IgM positivity preceded an epidemic that was declared early in 2013 in the study site. This finding further supports the monitoring of IgM positivity as a potential tool for early outbreak detection (Hati 2009). A cut-off point above which an IgM positive index suggests a dengue epidemic will need to be validated. NS1 positive index could also be explored. There were too few positive NS1 samples in the present study to assess this marker. Performing more than one RDT in the same subject was useful to identify dengue cases through seroconversion of IgM and IgG specific antibodies, but further studies are required to assess the cost-effectiveness of this practice in the routine care.

There are several limitations to the present study. Reasons for ordering diagnostic tests are multiple and complex. While our approach was eminently quantitative and allowed us to identify factors associated with the use of rapid dengue diagnostic tests, a qualitative approach would be complementary to identify other factors that are not measurable by quantitative methods. Dengue diagnosis and classification was based on the ICD-10 available in the computerised system, which does not include the most recent definitions proposed by World Health Organization (WHO/TDR 2012). Hence, it was not possible to explore the patterns of use of RDTs in cases classified as dengue with warning signs. Time-series analysis was not performed because the number of monthly observations (n = 10) was considered insufficient to yield reliable results. Finally, detailed information of signs, symptoms and onset of fever were not available and could not be explored for their association with ordering diagnostic tests. Particularly, the latter is expected to be critical to the use of dengue RDT as the sensitivity of these tests varies with time. After data analysis, we were able to retrieve information on date of disease onset on 296 subjects from the dengue surveillance database at the local public health office. Dengue RDTs were performed in 109 of these subjects with a median of four (range 0-21) days of symptoms compared to five (range 0-180) days in subjects who were not tested. Surveillance records also contain detailed information of signs and symptoms, but neither this nor dates of onset of disease were used in the analysis because this data was not validated and was unavailable for most study subjects.

In conclusion, rapid dengue diagnostic tests are been used to both rule in and rule out disease. The latter highlights the need for improved sensitivity of currently available rapid dengue diagnostic tests. Further studies, which consider the influence of nonevidence-based reasons for using health technology, such as reassurance for clinicians or patients/families as well as epidemiological reasons, are required to assess the cost-effectiveness of implementing rapid dengue diagnostic tests in routine care in endemic areas.

ACKNOWLEDGEMENTS

To Dr Martha Lucia Ramos, from COMFANDI, for support to perform the study, and to Melissa Pelaez, Liliana Soto and Claudia Vidal, from UniValle, for their assistance.

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