Contents lists available at ScienceDirect

International Journal of Infectious Diseases

Performance of rapid tests in the management of dengue fever imported cases in Lazio, Italy 2014-2019

Giulia Matusali*, Francesca Colavita*, Fabrizio Carletti, Eleonora Lalle, Licia Bordi, Francesco Vairo, Giuseppe Ippolito, Maria R. Capobianchi, Concetta Castilletti

National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS, Via Portuense 292, 00149 Rome, Italy

A B S T R A C T

Background: In Italy, dengue virus is the most frequent agent of imported viral infections. The use of rapid diagnostic tests (RDTs) may be of help as a preliminary user-friendly quick assay to facilitate dengue diagnosis, as ordinary laboratory diagnosis of dengue fever may require special efforts in terms of tools availability, interpretation of results, and skilled personnel. The performance of RDTs, however, may vary according to different epidemiological and laboratory background.
Methods: We reviewed five years of laboratory records of two dengue RDT results (Colorimetric SD-Bioline Dengue-Duo-RDT and Fluorimetric SD-Biosensor-STANDARD-F-Dengue-RDT), able to detect viral NS1 antigen and specific IgM and IgG. Diagnostic parameters were calculated using as reference the results of molecular (RT-PCR) and serological (immunofluorescence, IFA) tests. Overall performance, calculated considering the final case definition, was included in the accuracy assessment of RDTs.
Results: The combined use of NS1 and IgM/IgG RDT for the detection of acute dengue cases resulted in an overall sensitivity and specificity of 87.2% and 97.9% for Colorimetric RDT, 96.2% and 96.2% for Fluorimetric RDT. NS1 was the most reliable marker of acute infection, while IgM resulted falsely positive in nine samples, including sera derived from 2 Zika and 4 non-arbovirus infected patients.
Conclusions: The inclusion of RDT in the diagnostic algorithm is of undeniable help in the prompt management and surveillance of dengue infection in non-endemic areas. Confirmatory tests are, however, necessary to rule in or rule out dengue fever diagnosis.

INTRODUCTION

Dengue is a mosquito-borne infection caused by four distinct viruses (DENV 1-4) which belong to the flavivirus genus and may lead to diverse outcomes: can be asymptomatic (75% of cases), cause Dengue fever (DF), or result in severe dengue (Bhatt et al., 2013).

The main vector for DENV transmission is *Aedes (Ae.) aegypti*, but other species, such as *Ae. albopictus*, may be involved in viral spreading, while only sporadic cases of human-to-human direct transmission (e.g. mother-to-child; blood-borne; and sexual transmission) have been reported (Levi, 2016; Lee and Lee, 2019; Paixão et al., 2016; Wiwanitkit, 2010).

DENV is endemic in tropical and subtropical areas and geographical expansion of the dengue epidemic has been occurring during the last decades due to urbanization and climate change (Messina et al., 2014). International travel contributes in spreading dengue infections and determines imported cases in non-endemic areas. DENV is the most common cause of febrile illness among people seeking for medical care after travel to Latin America or Asia (Wilson, 2017); and in Europe, is second only to malaria as the febrile illness causing most hospitalizations after return from abroad (World health organization WHO, 2012).

From august 2010, local European cases in areas where *Ae. albopictus* is active have been reported in Croatia (3 cases), Southern France (25 cases), and Spain (7 cases) (ECDC, 2018; ECDC, 2019). Moreover, in 2012 about 2100 cases have been registered in the Atlantic island of Madeira (Portugal) where *Ae. aegypti* established in 2004 (Lourenço and Recker, 2014; Almeida et al., 2007).

* Corresponding authors at: Via Portuense 292, 00149, Rome, Italy.
   E-mail addresses: giulia.matusali@inmi.it (G. Matusali),
francesca.colavita@inmi.it (F. Colavita).

Clinical diagnosis may be challenging due to the wide spectrum of manifestations, which can overlap with those of other pathogens co-circulating in the endemic geographic areas.

The laboratory diagnosis, essential to recognize dengue cases, is based on viral isolation, serological tests, and detection of viral RNA. Isolation is cumbersome, requires high biocontainment and often takes too long for clinical management. IgM or IgG detection must be performed on more than a single blood sample and needs to be confirmed by neutralization assays due to the high cross reactivity with other flaviviruses. RT-PCR and sequencing of DENV RNA are highly specific, but may be expensive or not available in routine laboratory settings. In addition, low levels and short-lived DENV viremia (up to 6 days from symptom onset) narrows the window of molecular detection (Muller et al., 2017).

For the above-explained reasons, rapid dengue tests may be particularly useful in dengue endemic regions where laboratory capabilities and specimen storage conditions may be limited. In non-endemic countries, these user-friendly and quick tools may be used as first-line tests, to facilitate initial dengue diagnosis, timely medical treatment decisions, and to inform surveillance activities.

In Italy, the surveillance of human cases of Dengue is active throughout the year. However, during the period of competent vector activity (June - October) the monitoring system must guarantee the best timeliness and sensitivity, to allow immediate identification of imported (and/or autochthonous) cases and the prompt adoption of control measures. It is mostly during this period that the use of RDTs may improve disease control actions.

Here, we report results obtained after a four-year experience in the use of a colorimetric rapid diagnostic test (col-RDT) and one year of a fluorimetric RDT (fluor-RDT). Both tests contains two devices, one to detect NS1 DENV protein, one for DENV-specific IgM and IgG. We discuss assays performances and benefits in the use of RDTs as a first-line tool in diagnostic algorithms.

METHODS

Patients' data source

We retrospectively considered patients with dengue-like symptoms who spontaneously sought treatment in healthcare units of Lazio Region (Central Italy) from January 2014 through July 2019. Samples from all suspect patients were delivered to the Regional Reference Laboratory for arboviral infections, National Institute for Infectious Diseases "Lazzaro Spallanzani" (INMI), Rome, for virological diagnosis and case confirmation.

Case definition was performed according to the EU protocols and Italian Ministry of Health guidelines (Ministero della Salute, 2018; THE EUROPEAN COMMISSION, 2018):

Laboratory tests

A colorimetric test detecting the NS1 viral antigen and anti-DENV IgM and IgG (SD Bioline Dengue Duo, Abbott Diagnostics) was used as preliminary assay between January 2014 and December 2017. From August 2018, the colorimetric test was replaced by fluorimetric RDTs (STANDARD F Dengue NS1 Ag FIA + STANDARD F Dengue IgM/IgG FIA, SD Biosensor, Gyeonggi-do, Republic of Korea). The overlap period in the use of these RDTs (January-July 2018) has been excluded from the analysis. The complete laboratory diagnostic algorithm included, as confirmatory tests, two molecular assays to detect DENV RNA: a real time RT-PCR (DENV-1-4 Real-Time RT-PCR Multiplex Assay, developed by CDC (Centers for Disease Control and Prevention (CDC), 2019)) and a Pan-flavivirus specific nested PCR targeting NS5 region (modified from (Moureau et al., 2007)), followed by sequencing. In addition, multiple indirect immunofluorescence

assays (IFA, Flavivirus Mosaic 2 or Arboviral Fever Mosaic-2, IgM and IgG, Euroimmun, Hamburg, Germany) were performed for specific IgM and IgG titres measurement and differential diagnosis.

Statistical analysis

Test results were retrieved from laboratory records. Diagnostic performance, concordance and k coefficient of agreement were established. Mann-Whitney and Fisher tests were carried out to compare age of DENV cases, or gender and serotype distribution (GraphPad Software, La Jolla California USA).

Ethic statement

The data used in this study are the results of diagnostic tests performed at INMI in the context of diagnostic and surveillance activities, the collection of additional biological samples from patients was not required. The information template accompanying each sample submitted to the Regional Reference Laboratory was the source of essential epidemiological and clinical data. All data are aggregated and non-identifiable.

RESULTS

Patients undergoing RDT

From January 2014 through July 2019, DF diagnosis was confirmed at the Regional Reference Laboratory in 102 out of 804 (12.7%) febrile patients tested. RDTs have been performed on samples derived from 651 individuals, representing the 80.9% of possible cases tested at our Institute (Supplementary Fig. 1).

In this report, we present results obtained from two different RDTs (detecting NS1 viral antigen and anti-DENV IgM and IgG): a colorimetric test (col-RDT) used between January 2014 and December 2017, and a fluorimetric test (fluor-RDT) in exclusive use since august 2018.

The 65 DENV confirmed cases (35 male and 30 females, median age: 37 years old, min 14, max 79) presented with fever (97%), arthralgia (63%), rash (60%), asthenia (78%), headache (75%), myalgia (59%), and retro-orbital pain (44%), while no signs of meningo-encephalitis were ever observed (Table 1). Most patients reported travel history from Asia (44 patients), followed by Latin America (16 patients). Only one case was imported from Oceania and two from Africa (Table 1).

NS5 sequence-based DENV serotype was established for 54 out of 65 patients, resulting in 23 DENV-1, 12 DENV-2, 16 DENV-3 and 3 DENV-4 infections (Table 1).

Performance of the NS1 RDTs in comparison to real-time RT-PCR

The results of DENV-specific real time RT-PCR were used as reference information to assess NS1 RDTs sensitivity and specificity for the diagnosis of acute Dengue.

The two RDTs showed high diagnostic sensitivity (95.8 and 84.6% for col- and fluor-RDT, respectively); and specificity (97.9 and 100% for col- and fluor-RDT, respectively) (Table 2).

For the col-RDT (n = 212), the analysis revealed 5 discordant samples: one false negative and 4 NS1 positive but RT-PCR negative results. However, one discordant sample derived from a patient resulted DENV-4 positive by flavivirus-specific RT-PCR and NS5 sequencing.

Two false negative results were observed using the fluor-RDT (n = 65).

Table 1
Characteristics of DF cases confirmed after RDT results (2014-2019)

Case #	Sex	Age	Fever	Arthralgia	Rash	Asthenia	Headache	Myalgia	Retro-orbital pain	Travel History	DENV serotype
1	M	28	yes	yes	no	yes	yes	no	no	Oceania	DENV-1
2	M	31	yes	yes	no	yes	yes	yes	no	Thailand	unknown
3	F	29	yes	yes	yes	yes	yes	no	no	Santo Domingo	DENV-2
4	M	26	unknown	unknown	unknown	unknown	unknown	unknown	unknown	Thailand	DENV-1
5	F	43	yes	yes	no	yes	yes	no	no	Indonesia	unknown
6	M	40	yes	yes	yes	yes	yes	yes	yes	Colombia, Panama	DENV-3
7	M	51	yes	yes	no	yes	no	yes	no	Maldives	DENV-1
8	M	40	yes	yes	yes	yes	unknown	unknown	unknown	Maldives	DENV-1
9	F	24	yes	no	yes	yes	yes	no	yes	Haiti	DENV-1
10	M	79	yes	no	no	no	yes	no	no	Brazil	DENV-1
11	M	56	yes	yes	no	no	yes	yes	no	Myanmar	DENV-1
12	F	51	yes	yes	no	yes	yes	yes	yes	Philippines	DENV-2
13	F	20	yes	yes	yes	yes	yes	yes	yes	Mexico	DENV-1
14	M	24	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	DENV-2
15	M	52	yes	yes	yes	yes	yes	yes	no	Maldives	DENV-1
16	M	22	yes	yes	yes	yes	yes	yes	yes	India	DENV-2
17	F	54	yes	yes	no	no	no	yes	no	Brazil	DENV-1
18	M	57	yes	yes	yes	no	no	yes	no	India	DENV-4
19	F	64	yes	yes	yes	yes	yes	yes	yes	Cuba	DENV-2
20	F	41	yes	no	yes	yes	yes	no	no	Brazil	DENV-1
21	M	65	yes	no	no	yes	yes	no	yes	Cuba	DENV-3
22	M	49	yes	no	yes	yes	unknown	no	yes	Indonesia	DENV-2
23	M	38	yes	no	yes	no	yes	no	yes	Dominican Republic	DENV-1
24	F	59	unknown	unknown	unknown	unknown	yes	unknown	unknown	Dominican Republic	DENV-1
25	F	32	yes	yes	yes	yes	yes	no	no	Indonesia	DENV-3
26	M	55	yes	yes	yes	yes	no	yes	yes	Malaysia	DENV-3
27	F	26	yes	no	yes	yes	no	no	no	Thailand	DENV-4
28	M	67	no	no	no	yes	no	yes	no	India	DENV-3
29	M	24	yes	no	no	yes	yes	no	yes	Thailand	unknown
30	F	14	yes	no	yes	no	yes	no	no	Philippines	DENV-1
31	F	32	yes	yes	yes	yes	yes	yes	yes	Vietnam	DENV-1
32	F	27	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	DENV-2
33	M	37	yes	yes	yes	no	no	no	no	Thailand, Cambodia, Indonesia	DENV-2
34	M	26	yes	no	yes	yes	yes	yes	yes	Maldives, Thailand	unknown
35	M	25	yes	no	yes	no	yes	yes	yes	Sri Lanka	DENV-2
36	M	51	yes	unknown	no	yes	no	yes	no	Nigeria	DENV-3
37	M	58	yes	no	yes	no	yes	no	no	Philippine	DENV-3
38	M	32	yes	no	yes	no	yes	yes	yes	India	DENV-3
39	F	35	yes	yes	no	no	yes	no	no	Sri Lanka	DENV-2
40	F	26	yes	no	yes	yes	yes	no	yes	Thailand	DENV-1
41	M	28	yes	yes	yes	yes	yes	yes	no	Maldives	DENV-3
42	F	31	yes	yes	yes	yes	no	yes	no	India, Maldives	DENV-3
43	F	31	yes	yes	no	yes	yes	yes	no	Thailand	DENV-1
44	F	71	yes	yes	no	no	yes	yes	no	Indonesia	unknown
45	F	69	yes	yes	no	yes	yes	no	no	Indonesia	DENV-3
46	M	45	yes	yes	yes	yes	yes	yes	no	Colombia	DENV-1
47	F	27	yes	yes	yes	yes	yes	yes	no	Brasil, Jamaica	DENV-3
48	M	36	yes	no	yes	yes	yes	yes	yes	Thailand	DENV-1
49	M	39	yes	no	no	yes	yes	yes	no	Bangladesh	unknown
50	M	34	yes	yes	yes	yes	no	yes	no	Mexico	unknown
51	M	59	yes	yes	no	yes	yes	yes	yes	Indonesia	DENV-2
52	M	59	yes	yes	yes	yes	no	no	no	Thailand, Vietnam, Cambodia	unknown
53	F	34	yes	yes	yes	yes	yes	no	no	Thailand	DENV-4
54	M	50	yes	no	no	yes	yes	no	no	Maldives	DENV-3
55	F	16	yes	unknown	unknown	unknown	unknown	unknown	unknown	Thailand	DENV-1
56	F	29	yes	yes	yes	yes	yes	yes	yes	India, Thailand	unknown
57	F	59	yes	yes	yes	yes	yes	yes	yes	Philippines, Singapore	DENV-3
58	M	37	yes	no	yes	yes	yes	yes	yes	Cuba	unknown
59	F	26	yes	yes	no	yes	yes	yes	yes	Bangladesh	DENV-3
60	M	51	yes	no	no	yes	yes	no	yes	Maldives	DENV-1
61	F	16	yes	no	yes	yes	no	no	yes	DRC	DENV-1
62	F	36	yes	yes	no	yes	no	yes	no	India	DENV-1
63	M	30	yes	yes	no	yes	no	no	no	Bangladesh	DENV-3
64	M	67	no	no	no	no	no	no	no	Brasil	DENV-2
65	F	47	yes	yes	yes	yes	yes	yes	yes	Thailand	unknown

IgM/IgG RDTs comparison to Immunofluorescence assays (IFA)

The comparison of the col-RDT with reference IFA showed a good agreement for IgM detection (94.07% concordance, k coefficient = 0.704; n = 253), and low concordance for IgG results (69 %, k coefficient = 0.065; n = 253), with much higher rate of positivity by IFA (n = 82) than by RDT (n = 4). Noteworthy, samples resulting DENV IgG positive by IFA were, in most cases, cross-reactive to other flaviviruses (e.g. ZIKV, YFV).

Table 2
Diagnostic performance of NS1 RDT as compared to DENV-1-4 RT-PCR

Col-RDT	%	95% CI
Diagnostic sensitivity	95.8	78.9-99.9
Diagnostic specificity	97.9	94.6-99.4
PPV	85.2	68.5-93.8
NPV	99.5	96.4-99.9
Diagnostic accuracy	97.6	94.6-99.2

Fluor-RDT	%	95% CI
Diagnostic sensitivity	84.6	54.5-98.1
Diagnostic specificity	100	93.2-100
PPV	100	-
NPV	96.3	87.9-98.4
Diagnostic accuracy	96.9	87.9-99.6

PPV = positive predictive value; NPV = negative predictive value

Table 3
Combined use of NS1 and IgM/IgG RDTs

Col-RDT	%	95% CI
Diagnostic sensitivity	87.2	72.6-95.7
Diagnostic specificity	97.9	95.8-99.2
PPV	82.9	69.8-91.1
NPV	98.5	96.7-99.3
Diagnostic accuracy	96.8	94.5-98.3

Fluor RDT	%	95% CI
Diagnostic sensitivity	96.2	80.4-99.9
Diagnostic specificity	96.2	89.3-99.2
PPV	89.3	73.3-96.2
NPV	98.7	91.8-99.8
Diagnostic accuracy	96.2	90.5-99.0

PPV = positive predictive value; NPV = negative predictive value.
RDT positive results do not include the sample showing only IgG detection since not indicative of acute infection. RDT negative results include triple negative samples.

When we established the concordance of IFA and fluor-RDT, a good agreement of results was observed for both IgM (98.11%; k = 0.933; n = 106) and IgG (84.91%; k = 0.623; n = 106).

These data indicate higher concordance of the fluorimetric than the colorimetric RDT (p = 0.002 in chi square test) with IFA IgG results.

Overall reliability of RDTs

To analyse the performance of the NS1 and IgM/IgG RDTs in detecting acute dengue infections, we analysed their results in a population of febrile patients with a final case definition of confirmed dengue or non-dengue fever (Ministero della Salute, 2018; THE EUROPEAN COMMISSION, 2018). In our population, IgG only positive tests were never indicative of acute infection.

Accordingly, the sole presence of IgG (in the absence of IgM, viral RNA or antigen detection) is considered as indicative of previous dengue infection or cross reactivity (e.g. previous flavivirus infections or YFV vaccination).

NS1 antigen was the most reliable marker of dengue acute infection. Indeed, col-NS1 and fluor-NS1 RDT detected 82% (32/39) and 88% (23/26) of dengue cases, respectively, while the IgM/IgG RDT only revealed 56% (22/39 col-RDT) and 73% (19/26 fluor-RDT) of cases.

NS1 RDTs also showed higher specificity (99% col- and 100% fluor-RDT) compared to IgM/IgG (98% col- and 89% fluor-RDT) tests.

To evaluate the benefit in the combined use of NS1 RDT and IgM/IgG RDT, we then considered as positive result any RDT reactivity with the exception of IgG only reacting tests.

In a population of 373 febrile patients, the col-RDT showed 7 false positive (6 IgM+, 1 NS1+) and 5 false negative results (Table 3). Noteworthy, 5 IgM false positive sera derived from patients with Plasmodium spp (n = 2), measles (n = 1), and Zika (n = 2) infection. No other cross-reactivities were observed when testing patients infected by non-dengue arboviruses (14 Zika, 1 yellow fever, and 10 chikungunya cases).

Considering the fluor-RDT, we observed 3 false positive tests (IgM+, one from a Plasmodium spp infected patient) and one false negative result (n = 105).

Hence, IgM detection by the two RDTs in 9 samples from non-cases suggests a careful interpretation of an NS1- /IgM+/IgG- result (Table 3).

RDTs reactivity at different time post symptom onset

As reported by the CDC and WHO, in primary infection 80% of all dengue cases have detectable IgM antibody by day 5 of illness, while anti-dengue IgG levels start to raise at the end of the first week from symptom onset (FSO). The NS1 antigen is the earliest marker of infection, whose level can be detectable even before the onset of symptoms, till about the end of the first week of illness (Centers for Disease Control and Prevention (CDC), 2020).

To determine the reactivity of the RDTs at different time point of infection, 57 specimens from DENV cases with known date of symptom onset were considered.

The RDTs failed to detect the NS1 antigen in 8 cases during the first week of illness (Fig. 1A). DENV IgM were detected starting at day 4 (Fig. 1B), and IgG were sporadically observed at day 5, 6, 7 and 13 of illness (Fig. 1C).

DISCUSSION

Fast recognition of dengue viremic patients is highly informative for addressing public health control measures in countries where imported cases may ignite autochtonous spread of the infection, such as Italy, where suitable mosquito vectors are
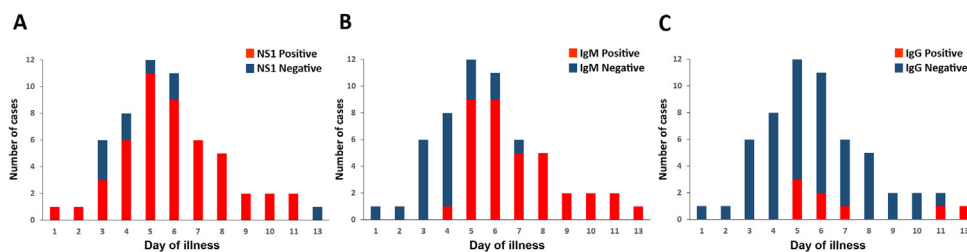

Fig. 1. NS1 (A), IgM (b), and IgG (C) detection by RDTs at different day from symptom onset. Red bars indicate positive and blue bars negative results, respectively.

present. Based on currently available laboratory diagnostic tools, nucleic acid detection assays may identify dengue RNA within 24–48 hours from sample collection, viral isolation may take weeks, and serology requires paired sera collected at (at least) one week of distance. Moreover, all the traditional tests require highly experienced technicians. On the contrary, the use of RDTs significantly shortens to less than 30 minutes the laboratory turnaround time for diagnosis of acute dengue infection and are currently used for preliminary diagnosis.

One limitation of DENV RDTs can be the diagnostic accuracy; indeed, previous studies report good specificity, usually around 90%, but lower sensitivity, which can range from 10 to 99% (Kikuti et al., 2019; Lim et al., 2017). Variation in sensitivity may be linked to demographic and epidemiological differences (endemic vs imported cases, primary vs secondary infection, DENV serotypes, time from symptom onset etc.), laboratory settings and resources (e.g. storage of samples), time of sampling, and type of test used (e.g. NS1 vs IgM/IgG detection) (Kikuti et al., 2019; Blacksell et al., 2006; Blacksell, 2012).

In our study population, the performance of the NS1 RDTs resulted in high sensitivity and specificity while IgM/IgG RDTs showed low sensitivity for acute dengue detection, with time of sampling certainly affecting the results.

Nevertheless, the concomitant use of NS1 and IgM/IgG RDT cartridges may increase diagnostic sensitivity, while only slightly affecting test specificity.

One important observation is that dengue diagnosis was not confirmed in the majority of samples (78%) in which the RDTs were positive only for the IgM. Which pathogen or patient-derived factor(s) can determine non-specific IgM result needs further investigation.

Moreover, IgG only positive tests (n = 13, none from confirmed cases) were indicative of previous dengue infection or cross-reactivity. Importantly, we never detected secondary infections, which are frequent in endemic countries, and may be revealed by high titres of IgG even in the presence of IgM or NS1 detection (Casenghi et al., 2018)

Taking into account that details on the evaluation (i.e. study population characteristics) are not fully provided by the RDTs companies, thus not allowing a proper comparison, we observed a lower diagnostic sensitivity (col-RDT: 92.4% vs 82% for NS1 Ag, 94.2% vs 56% for specific IgG/IgM; fluo-RDT: 100% vs 88% for NS1 Ag, 98% vs 73% for specific IgG/IgM). On the contrary, similar, even higher, values of specificity were obtained for both RDTs (col-RDT: 98.4% vs 99% for NS1 Ag, 96.4% vs 98% for specific IgG/IgM; fluo-RDT: 100% vs 100% for NS1 Ag, 99% vs 89% for specific IgG/IgM). Discrepancies may be due to type of samples (i.e plasma vs serum vs whole blood) different reference (RT-PCR assay, IFA, ELISA, or case definition), and population tested (DENV serotypes' distribution, time from symptom onset etc.).

Further studies in larger cohorts of patients are necessary to analyse the effect of disease phase, DENV serotype, or viral load on RDT performances.

There are some limitations in our study. It is a retrospective survey, the follow-up as well as complete epidemic-clinical information of some patients are missing. Moreover, we could not perform a direct comparison between the two RDTs as the evaluation was based on different periods, leading to a smaller number of individuals evaluated by the fluor-RDT. Nonetheless, the two groups of patients evaluated with either RDT were comparable in terms of gender, age, and DENV serotype distribution. Overall, the results indicate no remarkable differences in diagnostic accuracy of the two RDTs. Two advantages of the fluorescence-based test are the automatic output -that avoid interpretation bias-and the possibility to record the results.

Although confirmatory tests are still necessary, our experience strongly supports the use of combined NS1 and IgM/IgG rapid dengue tests as first line tools for prompt case identification. The use of these tests aids the clinical management, surveillance activities, and vector control strategies. The set-up of control measures being crucial to arrest infection spread and establishment in non-endemic countries where competent vectors are present.

Conflict of Interest

The Authors declare no conflict of interest

Ethical approval

The data used in this study are the results of diagnostic tests performed at INMI in the context of diagnostic and surveillance activities, the collection of additional biological samples from patients was not required. All data are aggregated and non-identifiable.

Author contributions

GM performed diagnostic assays, analysed data, and wrote the paper; FCo analysed data and performed serological assays; CC, FCa, EL and LB performed diagnostic assays; FV recorded epidemiological data; MRC and CC reviewed the manuscript; GI, MRC, and CC acquired funding.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.07.008.

References

Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.

Levi JE. Dengue Virus and Blood Transfusion. J Infect Dis. 2016;213(5):689–90.

Lee C, Lee H. Probable female to male sexual transmission of dengue virus infection. Infect Dis (Auckl) 2019;51(2)150–2 Available from: https://www.tandfonline.com/doi/full/10.1080/23744235.2018.1521004.

Paixão ES, Teixeira MG, Costa M da CN, Rodrigues LC. Dengue during pregnancy and adverse fetal outcomes: a systematic review and meta-analysis. Lancet Infect Dis 2016;16(7)857–65 Available from: https://linkinghub.elsevier.com/retrieve/pii/S1473309916000888.

Wiwanitkit V. Non vector-borne transmission modes of dengue. J Infect Dev Ctries. 2010;.

Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, et al. Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol 2014;22(3) 138–46. . Available from: https://linkinghub.elsevier.com/retrieve/pii/S0966842X13002734.

Wilson ME. Fever in Returned Travelers. Centers for Disease Control and Prevention (CDC), editor. Travelers' Health. Chapter 5 Post-Travel Evaluation. Available

from: https://wwwnc.cdc.gov/travel/yellowbook/2018/post-travel-evaluation/fever-in-returned-travelers.

World health organization WHO. Infectious diseasess in a changing climate Information for public health officials in the WHO European Region. Available from:. 2012. http://www.euro.who.int/__data/assets/pdf_file/0009/234198/Dengue-in-the-WHO-European-Region.pdf.

ECDC. Autochthonous transmission of dengue in EU/EEA in Europe, 2010-2018 [Internet]. Available from:. 2018. https://www.ecdc.europa.eu/en/all-topics-z/dengue/surveillance-and-disease-data/autochthonous-transmission-dengue-eueea-europe.

ECDC. Autochthonous cases of dengue in Spain and France 1 October 2019 [Internet]. Available from:. 2019. https://www.ecdc.europa.eu/sites/default/files/documents/RRA-dengue-in-Spain-France_1Oct2019.pdf.

Lourenço J, Recker M. The 2012 Madeira Dengue Outbreak: Epidemiological Determinants and Future Epidemic Potential. PLoS Negl Trop Dis 2014;8(8).

Almeida AP, Gonçalves YM, Novo MT, Sousa CA, Melim M, Gracio AJ. Vector monitoring of Aedes aegypti in the Autonomous Region of Madeira, Portugal. Wkly releases 2007;12(46). . Available from: http://www.eurosurveillance.org/content/10.2807/esw.12.46.03311-en.

Muller DA, Depelsenaire ACI, Young PR. Clinical and Laboratory Diagnosis of Dengue Virus Infection. J Infect Dis 2017;215(suppl_2)89–95 Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/jiw649.

Ministero della Salute. Piano nazionale di sorveglianza e risposta alle arbovirosi trasmesse da zanzare invasive (Aedes sp.) con particolare riferimento ai virus chikungunya, dengue e zika - 2018 [Internet]. Available from:. 2018. http://www.salute.gov.it/portale/news/p3_2_1_1_1.jsp?menu=notizie&id=3374.

THE EUROPEAN COMMISSION. COMMISSION IMPLEMENTING DECISION (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Off J Eur Union. Available from:. 2018. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0945.

Centers for Disease Control and Prevention (CDC). CDC DENV-1-4 Real-Time RT-PCR Multiplex Assay [Internet]. 2019 Available from: https://www.cdc.gov/dengue/healthcare-providers/testing/molecular-tests/realtime.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fdengue%2Fclinicallab%2Frealtime.html.

Moureau G, Temmam S, Gonzalez JP, Charrel RN, Grard G, de Lamballerie X. A Real-Time RT-PCR Method for the Universal Detection and Identification of Flaviviruses. Vector-Borne Zoonotic Dis 2007;7(4)467–78 Available from: https://www.liebertpub.com/doi/10.1089/vbz.2007.0206.

Centers for Disease Control and Prevention (CDC). Testing for Dengue Virus [Internet]. Available from:. 2020. https://www.cdc.gov/dengue/clinicallab/laboratory.html.

Kikuti M, Cruz JS, Rodrigues MS, Tavares AS, Paploski IAD, Silva MMO, et al. Accuracy of the SD BIOLINE Dengue Duo for rapid point-of-care diagnosis of dengue. PLoS One. 2019;14(3).

Lim JK, Alexander N, Di Tanna GL. A systematic review of the economic impact of rapid diagnostic tests for dengue. BMC Health Serv Res. 2017;17(1).

Blacksell SD, Newton PN, Bell D, Kelley J, Mammen MP, Vaughn DW, et al. Accuracy of Rapid Serological Tests for Dengue • CID [Internet]. Available from:. 2006. https://academic.oup.com/cid/article-abstract/42/8/1127/282608.

Blacksell SD. Commercial Dengue Rapid Diagnostic Tests for Point-of-Care Application: Recent Evaluations and Future Needs?. J Biomed Biotechnol. 2012;2012:1–12.

Casenghi M, Kosack C, Li R, Bastard M, Ford N. NS1 antigen detecting assays for diagnosing acute dengue infection in people living in or returning from endemic countries. Cochrane Database Syst Rev 2018; Available from: http://doi.wiley.com/10.1002/14651858.CD011155.pub2.