Archived dengue serum samples produced false-positive results in SARS-CoV-2 lateral flow-based rapid antibody tests

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Abstract

Co-endemicity of SARS-CoV-2 and dengue virus (DV) infection is becoming a matter of serious concern as it has been already reported that antibodies (Ab) elicited by SARS-CoV-2 infection can produce false-positive results in dengue IgG and IgM rapid tests and *vice versa*. Here we communicate that five of thirteen DV antibody-positive serum samples from Kolkata, archived in 2017 (predating the COVID-19 outbreak), produced false-positive results in SARS-CoV-2 IgG/IgM lateral flow-based rapid tests. Our results emphasize the importance of implementing tests with higher specificity to conduct sero-surveillance for accurate estimation of SARS-CoV-2/DV prevalence in regions where both viruses now co-exist.

The world is experiencing the coronavirus disease 2019 (COVID-19) pandemic, with 21 294 845 confirmed cases and 761 779 deaths up to 16 August 2020 [1]. SARS-CoV-2 infection is increasing in India, with ~50–60 000 confirmed cases being reported daily for the last several days [1]. Due to this high daily infection rate, rapid tests for SARS-CoV-2 antibodies (Abs) are being increasingly implemented to detect the onset of community transmission, if any, especially asymptomatic and convalescent cases.

It has been previously reported anecdotally from Singapore that the Abs elicited by SARS-CoV-2 infection can produce false-positive results in dengue IgG and IgM rapid tests [2]. Recently, a report from Israel stated that 55 COVID-19 patients' sera produced 12 false-positive results (21.8%) in dengue lateral flow-based rapid tests [3]. It is also noteworthy that the early symptoms of COVID-19 can be mistaken for those of dengue fever, including thrombocytopenia, in highly dengue-endemic countries such as India and Brazil [4].

By this time, with the onset of monsoon in India, dengue infections have started increasing with the COVID-19 pandemic in the background. Most cases of dengue virus (DV) infection are asymptomatic and self-limiting. One report estimated

390 million (95% CI: 284–528) infections per year globally, of which 96 million (CI: 67–136) manifested clinically. About 4 billion people across 129 countries are currently at risk of DV infection, with 70% of the global burden in Asia, namely the Indian subcontinent and Southeast Asia [5].

In this scenario, the obvious question is whether DV Abs, prevalent in people in highly dengue-endemic regions like Kolkata, will cross-react in SARS-CoV-2 rapid antibody detection tests. If this happens, serology-based diagnosis and sero-surveillance for these immunologically cross-reacting viruses have to be carried out with adequate precautions/background and other supporting information, in regions where both viruses are co-existent. Interpretation of results has to be done with caution to avoid arriving at erroneous estimates.

We performed rapid DV IgG and IgM detection tests (SD Bioline, Abbott) on archived serum samples ($n=33$) from DV-diagnosed patients (NS1 ELISA-positive) from the 2017 dengue cases in Kolkata (pre-dating the COVID-19 pandemic). Initially, only DV seropositive samples were subjected to SARS-CoV-2 Ab detection rapid tests. The primary objective was to investigate DV Ab cross-reactivity

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Abbreviations: ACE2, angiotensin-converting enzyme 2; ELISA, enzyme-linked immunosorbent assay; HCoV, human corona virus; MERS, middle east respiratory syndrome; NAT, nucleic acid test; NS1, non-structural protein 1; qRT-PCR, real time quantitative polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

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Fig. 1. Representative images of COVID-19 and dengue rapid IgG and IgM strip tests. (a) Sample 17-D-15; (b) sample 17-D-37; (c) sample 17-D-15 test using AbCheck kit; (d) sample 17-D-16. (a, c) A serum sample confirmed positive for dengue but false-positive for COVID-19. (b) A serum sample confirmed positive for dengue but negative for COVID-19. (d) A serum sample negative for both dengue and COVID-19 antibodies.

in lateral flow-based immunoassay system for SARS-CoV-2 Ab detection.

High incidence of DV infection has been regularly recorded in Kolkata [6] (from where the archived serum samples were collected). This was especially true for the year 2017 [7] and we therefore, envisaged that a substantial population of Kolkata could be seropositive for dengue. More importantly, we selected the DV serum samples from 2017, archived long before the COVID-19 emergence, in order to rule out the probability of pre-existing SARS-CoV-2 Abs in them, that would otherwise, react in the COVID-19 Ab tests.

Thirteen DV Ab rapid test positive sera (Table 1) were subjected to rapid SARS-CoV-2 IgG and IgM detection lateral flow-based strip test (ImmunoQuick, ImmunoScience India) following manufacturers' instructions (Figure 1). AbCheck COVID-19 test kit (IgG and IgM) (NuLifecare) was also used to confirm the cross-reactivity. Each COVID-19 rapid test strip was coated with SARS-CoV-2 antigen(s) as mentioned in the manufacturers' manuals. Ten out of the remaining twenty DV Ab-negative sera were randomly selected to check serological status against SARS-CoV-2 and to assess background cross-reactivity of the DV Ab-negative sera in the COVID-19 Ab kits, if any.

In brief, 20 µl of each sample was added to a specified area of the test strips, followed by the addition of two drops (~80–100 µl) of kit-specific assay buffer to the designated spot, depending on the test kit. The appearance of a 'test line' for all strip tests was confirmed to ensure the validity of the assay. We also used negative control serum samples (both DV and COVID-19 Ab-negative) as shown in Fig. 1d.

Five of the thirteen DV Ab-positive samples were found to produce false-positive bands in SARS-CoV-2 IgG and IgM detection rapid tests (Table 1). The same DV Ab-positive samples were found to produce a false-positive result in two different COVID-19 test kits (Fig. 1a,c). This confirms that DV Abs can, indeed, cross-react with SARS-CoV-2 antigen(s) and give false-positive results in COVID-19 rapid IgG and IgM tests (Table 1). The ImmunoQuick kit insert mentions that seventy-five COVID-19 negative samples were tested for determining the performance characteristics of the kit. No false-positive results were observed. Cross-reactivity with dengue sera was also tested and the results were found negative. Similarly, the product information of the AbCheck kit mentions that no cross-reactivity was observed when twenty-four SARS-CoV-2 qRT-PCR positive and twelve virus-negative sera were tested.

Table 1. Rapid IgG and IgM test results for COVID-19 and Dengue

Sample name	Age (years)	Sex	Clinical symptom(s)	SD-Bioline		ImmunoQuick		AbCheck	
				DV IgG	DV IgM	COVID-19 IgG	COVID-19 IgM	COVID-19 IgG	COVID-19 IgM
17-D-59	24	M	P, W, BA	f+	+++	-	++	-	++
17-D-68	33	M	P, H, BA	+	-	-	-	-	-
17-D-12	53	F	P, H, RE	++	+	-	+	-	+
17-D-1	46	M	P, BA	+	+	-	-	-	-
17-D-7	20	M	P, BA	++	-	-	+	-	+
17-D-11	51	F	P, BA	+	+	-	-	-	-
17-D-25	43	F	P, BA	++	-	-	+	-	+
17-D-31	35	M	P, BA	+	-	-	-	-	-
17-D-30	34	F	P, H, W	+	-	-	-	-	-
17-D-48	23	M	P, H, R	++	+	-	-	-	-
17-D-37	62	M	P, W, BA, H	++	-	-	-	-	-
17-D-50	25	F	P, H, W, LA	-	++	-	-	-	-
17-D-15	35	F	P, BA, N	+	++	++	-	++	-
DV Ab- negative controls n=10*	31 (Median age)	6F/4M	P, BA	-	-	-	-	-	-

"F" denotes female and "M" denotes male.

"P" denotes pyrexia; "W" denotes weakness; "BA" denotes body-ache; "H" denotes headache; "RE" denotes redness of the eye; "N" denotes nausea; "LA" denotes loss of appetite; "R" denotes rash.

"+" signifies positive result; "++" and "+++" signify relative increase in positive band intensity; "f+" stands for faint-positive band in the strip tests

"-" sign signifies a negative result.

"#": randomly selected serum samples from twenty DV Ab-negative (but NS1 ELISA-positive) serum samples.

Four DV serum samples showed false-positive SARS-CoV-2 IgM bands; of these two were DV IgG and IgM both positive and two were only DV IgG positive. One DV IgM and IgG dual positive sample produced a false-positive SARS-CoV-2 IgG but no IgM band (Table 1).

The aforesaid antibody test results corroborated well with our computational modelling (docking) studies that supported with high confidence that human antibodies to DV envelope can potentially bind to “receptor-binding motif (RBM)” of the SARS-CoV-2 Spike protein, with some of the interactions even intercepting the ACE2 receptor binding to RBM[8]. As COVID-19 rapid Ab test kits mostly use immobilized SARS-CoV-2 surface antigen(s), our prediction is supported by the observed DV false-positivity in COVID-19 Ab rapid tests as well as by the Spike protein antibodies detecting ELISA tests [3].

Our results demonstrate that in dengue-endemic countries, COVID-19 Ab detection-based assays can result in false-positive COVID-19 IgM as well as IgG results in case of DV-infected patients. We were the first to detect this dengue cross-reactivity in COVID-19 antibody tests, globally, and these data were previously deposited in an open-access repository as a preprint in July, 2020 for public awareness at the earliest possible during the pandemic [9]. The reverse scenario had been first reported from Singapore, i.e. originally two COVID-19 patients were misdiagnosed for having dengue as antibodies against SARS-CoV-2 cross-reacted in DV antibody tests [2]. Both the aforesaid observations were subsequently further investigated and validated by a study from Israel, where the authors had more extensively probed and confirmed the cross-reactivity between dengue antibodies and SARS-CoV-2 antigen(s) and *vice versa* via lateral flow-based rapid tests and ELISA tests in a larger number of patient samples [3]. It was reported that 21 out of 95 (22%) dengue serum samples (collected before September, 2019, predating the emergence of SARS-CoV-2), showed equivocal/false-positive results in ELISA, that detects antibodies against the Spike protein of SARS-CoV-2. This was in stark contrast to the background false-positivity rate of 4%, estimated from 102 healthy subjects tested using the aforesaid ELISA [3].

From the above scenarios and our computational modelling studies [3, 8], it appears that both these viruses share antigenic similarities resulting in the observed cross-reactivity and warrants further investigation to elucidate this dengue/COVID-19 conundrum.

Since the onset of the pandemic, several reports suggested potential serological cross-reactivity of SARS-CoV-2 virus with other seasonal HCoVs (NL63, HKU1, OC43 and 229E) and endemic coronaviruses (SARS-CoV-1 and MERS) [10–12]. Nevertheless, extremely low/sporadic incidences of SARS-CoV-1 and almost no incidence of MERS and the other four HCoVs had been observed in the Indian subcontinent, so far [13]. Surveying the epidemiological graph of SARS-CoV-1, it was observed that there were only three reported cases from India during the period of 25th April

to 6th May, 2003 [14]. The MERS epidemiological situation report stated that there were no confirmed cases in India from 2012–2019 [9]. The above evidences suggest that there is much less probability of existing seroprevalence against circulating seasonal HCoVs and endemic coronaviruses in the Indian population. Thus, the serological cross-reactivity between SARS-CoV-2 and other human coronaviruses is less likely in the Indian sub-continent.

In conclusion, sero-surveillance needs to be complemented with NAT and/or virus antigen tests for definitive diagnosis of COVID-19 and dengue in regions where both the viral diseases are co-endemic now. It is also necessary to implement more specific immunoassays for accurate differential diagnosis of these cross-reacting flavivirus (dengue) and coronavirus (SARS-CoV-2). One open question that remains to be solved is whether there is a DV serotype specificity to cross-react with the SARS-CoV-2 Spike antigen(s) as approximately 22–38% and not all dengue serum samples produced false-positive results in COVID-19 antibody tests. This may be the reason why only one of the forty-four dengue serum samples collected from travellers before the COVID-19 emergence gave false-positive results in two different COVID-19 rapid antibody tests in a study from Italy [15]. Another pertinent question is whether these two cross-reacting RNA viruses will confer some degree of cross-protection/immunity against the severity of the diseases caused by each of them [8, 16].

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

Ethical approval for the research was granted by the respective Institutional Ethical Committees of CSIR-IICB and Calcutta National Medical College, Kolkata. All experiments were carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all included patients.

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