



## Research article

## Assessment of SARS-CoV-2 IgG and IgM antibody detection with a lateral flow immunoassay test



Erica Diani <sup>a</sup>, Pier Paolo Piccaluga <sup>b,c,d</sup>, Virginia Lotti <sup>a</sup>, Andrea Di Clemente <sup>a</sup>, Marco Ligozzi <sup>a</sup>, Pasquale De Nardo <sup>e</sup>, Lorenza Lambertenghi <sup>e</sup>, Francesca Pizzolo <sup>f</sup>, Simonetta Friso <sup>f</sup>, Giuliana Lo Cascio <sup>g</sup>, Alice Vianello <sup>h</sup>, Giacomo Marchi <sup>h</sup>, Ercole Concia <sup>i</sup>, Davide Gibellini <sup>a,\*</sup>

<sup>a</sup> Department of Diagnostics and Public Health, Microbiology Section, University of Verona, Italy

<sup>b</sup> Department of Experimental, Diagnostic, and Experimental Medicine, Bologna University School of Medicine, Bologna, Italy

<sup>c</sup> Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy

<sup>d</sup> School of Health, Department of Pathology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

<sup>e</sup> Department of Diagnostics and Public Health, Division of Infectious Diseases, University of Verona, Italy

<sup>f</sup> Department of Medicine, Internal Medicine B, University of Verona, Italy

<sup>g</sup> Azienda Ospedaliera Universitaria Integrata Verona, Verona, Italy

<sup>h</sup> Department of Medicine, Internal Medicine D, Azienda Ospedaliera Universitaria Integrata Verona, Italy

<sup>i</sup> Department of Diagnostics and Public Health, University of Verona, Italy

## ARTICLE INFO

## Keywords:

SARS-CoV-2

COVID-19

LFIA assay

IgM and IgG

## ABSTRACT

The dramatic impact of SARS-CoV-2 infection on the worldwide public health has elicited the rapid assessment of molecular and serological diagnostic methods. Notwithstanding the diagnosis of SARS-CoV-2 infection is based on molecular biology approaches including multiplex or singleplex real time RT-PCR, there is a real need for affordable and rapid serological methods to support diagnostics, and surveillance of infection spreading. In this study, we performed a diagnostic accuracy analysis of COVID-19 IgG/IgM rapid test cassette lateral flow immunoassay test (LFIA) assay. To do so, we analyzed different cohorts of blood samples obtained from 151 SARS-CoV-2 RT-PCR assay positive patients (group 1) and 51 SARS-CoV-2 RT-PCR assay negative patients (group 2) in terms of sensitivity, specificity, PPV, NPV and likelihood ratios. In addition, we challenged LFIA with plasma from 99 patients stored during 2015–2017 period. Our results showed that this LFIA detected SARS-CoV-2 IgM and/or IgG in 103 out of 151 (68.21%) samples of group 1, whereas no IgM and/or IgG detection was displayed both in the group 2 and in pre-pandemic samples. Interestingly, IgM and/or IgG positivity was detected in 86 out of 94 (91.49%) group 1 samples collected after 10 days from symptoms onset whereas only 17 out of 57 of group 1 samples obtained before day 10 were positive to SARS-CoV-2 specific antibodies. We also compared the performance of this LFIA test with respect to other four different LFIA assays in 40 serum samples from multiplex RT-PCR positive individuals. Within the limits of the study size, the results demonstrated that COVID-19 IgG/IgM rapid test cassette LFIA assay displayed valid performance in IgM and IgG detection when compared with the other four LFIA assays.

Hence, this approach might be considered as an alternative point-of-care procedure for SARS-CoV-2 serological investigation.

## 1. Introduction

In December 2019, several cases of fatal pneumonia were observed in Wuhan, China [1]. The description of clinical criteria and the following viral sequencing and isolation has determined the classification of a new disease [2] called coronavirus disease 2019 (COVID-19) and the

identification of a novel coronavirus [3] subsequently designed SARS-Coronavirus 2 (SARS-CoV-2). Although a large array of containment efforts were performed, the global spreading of this infection was dramatically increasing with 116 million of confirmed infections and over 2,500,000 reported deaths thus inducing the world health organization (WHO) to declare the state of pandemic [4, 5]. Although several cases are

\* Corresponding author.

E-mail address: [davide.gibellini@univr.it](mailto:davide.gibellini@univr.it) (D. Gibellini).

<https://doi.org/10.1016/j.heliyon.2021.e08192>

Received 1 July 2020; Received in revised form 19 March 2021; Accepted 13 October 2021

2405-8440/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

asymptomatic, SARS-CoV-2 infection is able to display major symptoms including fever, fatigue and cough, sometimes associated with nasal congestion, diarrhea and neurological symptoms [6, 7]. Severe cases can progress to acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, bleeding, and coagulation impairment often leading to the death of patients [8, 9]. Epidemiological data demonstrate that male patients with age >70 years and associate chronic diseases represent the cohort of individuals with higher incidence of mortality [10].

The diagnosis of SARS-CoV-2 infection is based on RT-PCR assay to detect one or more specific viral sequences either in nasopharyngeal swabs or in lower respiratory districts. This technique is pivotal for SARS-CoV-2 infection diagnosis but the real effectiveness is strictly related to the quality of sample collection, sample type and stage of disease [11, 12]. On the other hand, the development of SARS-CoV-2 specific serological assay could play an important role, especially in the detection of the past infection in asymptomatic individuals and in the epidemiological studies. Comparing to RT-PCR, serological methods exhibit some advantages as high-throughput and faster turn-around time. For example, ELISA and CMIA techniques can detect the antibodies to SARS-CoV-2 antigens with semiautomatic procedure in a large number of samples during the same analytical run [13]. In addition to these classical techniques, rapid lateral test based on immune colloidal gold were set up [14, 15]. These rapid tests detect specific IgM and IgG to SARS-CoV-2 antigens in 10–20 min and can analyze a single specimen of blood, serum or plasma samples as a point-of-care methodology. In this paper, we evaluated the diagnostic accuracy of a rapid lateral flow method (Covid-19 IgG/IgM rapid test cassette LFIA assay), studying its specificity and sensitivity on selected cohorts of patients.

## 2. Materials and methods

### 2.1. Study design and participants

This study was performed on serum samples collected from two groups of patients. Group 1 consisted in 151 SARS-CoV-2 infection positive patients. All these patients were admitted to the hospital with suspected COVID-19. The criteria for the definition of a confirmed case were represented by fever and/or respiratory symptoms, abnormal lung imaging findings and positive detection of SARS-CoV-2 genome sequences with specific RT-PCR. We used the samples whose the materials were available. No further selection was applied.

The diagnosis of SARS-CoV-2 infection was carried out in nasopharyngeal swabs (Copan, Brescia, Italy) with SARS-CoV-2 specific multiplex real-time RT-PCR (multiplex RT-PCR) procedure. Multiplex RT-PCR assay was performed by Allplex nCoV 2019 RT-PCR kit (Seegene, Seoul, Korea) following the manufacturer's procedure. All patients were positive for all three targets (SARS-CoV-2 gene E, N and RdRP). Age and gender information, date of illness onset were achieved from the clinical records. Group 2 consisted of 51 individuals who were not infected by SARS-CoV-2. No amplification was detected in all 51 individuals with SARS-CoV-2 specific multiplex RT-PCR assay. As further negative control group, we analyzed even 99 frozen plasma samples obtained from viral infected patients (i.e. HCV, HBV and HIV) in pre-“SARS-CoV-2 era” during 2015–2017 period. In a next series of experiments, we have collected serial blood samples from a small number ( $n = 12$ ) of patients belonging to Group 1 with the onset of symptoms >10 days in order to determine the LFIA assay impact on the analysis of antibody dynamics. This study was performed at the AOUI Hospital Verona, approved by the local Ethical Committee (2646CESC), and conducted in respect of the Helsinki Declaration. Informed consent was obtained from participants involved in the study.

### 2.2. Detection of antibodies to SARS-CoV-2 with COVID-19 IgG/IgM rapid test cassette LFIA

COVID-19 IgG/IgM rapid test cassette LFIA (Menarini, Florence, Italy) is a colloidal gold LFIA for the detection of IgM and IgG antibodies to SARS-

CoV-2 in blood, plasma and serum samples. The procedure of detection was performed according to the manufacturer's instructions. Briefly, one drop of blood or 5  $\mu$ l of plasma or serum was added on the specimen well followed by two drops of sample buffer (about 80  $\mu$ l) in the sample device. The result is determined by the appearance of colored bands, designed either as positive (control and IgM and/or IgG bands) or negative (control band only). The results should be read in 10 min. The presence of specific bands was observed by eyes and recorded. The other four LFIA assays tested in this study are: i) VivaDiag COVID-19 IgM/IgG rapid test (Viva-Chek Biotech, Hangzhou, China), ii) Prima Professional COVID-19 IgG/IgM Rapid Test, (Prima Lab SA, Balerna, Switzerland), iii) Really Tech 2019-nCoV/COVID-19 IgG/IgM rapid test device, (Really Tech Co Ltd, Hangzhou, China), and iv) Antai Corona Virus (COVID-19) combined rapid test, (Beijing Zhongjian Antai Diagnostics Technology Co. Ltd, Beijing, China). All these LFIA assays were carried out following the manufacturers' indications. Unfortunately, several manufacturers did not indicate the viral antigen(s) used in LFIA assays reporting only undisclosed viral antigens. Notwithstanding, it is displayed in the European web site <https://covid-19-diagnostics.jrc.ec.europa.eu/devices/detail/428> that Prima Professional COVID-19 IgG/IgM Rapid Test and VivaDiag COVID-19 IgM/IgG rapid test exhibited viral nucleoprotein as target.

The other assays for qualitative detection of IgM and IgG antibodies were based on undisclosed SARS-CoV-2 antigen(s) indicated as recombinant antigens. COVID-19 IgG/IgM rapid test cassette LFIA employed S protein as target. In addition, anti-SARS-CoV-2 NCP IgM ELISA kit (Euroimmun, Lubeck, Germany) and anti-SARS-CoV-2 NCP IgG ELISA kit (Euroimmun) were also used to analyze Group 1 patients. The ELISA tests were performed according to the manufacturer's instructions. Borderline results (range between 0.8 and 1.1) were considered as positive detections.

### 2.3. Statistical analysis

The statistical analyses were conducted using GraphPad Prism (version 7). The comparison among the different LFIA devices was performed by Fisher exact test and chi square test. Results were considered significant for p-values < 0.05.

Diagnostic accuracy was evaluated using MedCalc software (Version 20.009; MedCalc software Ltd) and the CatMaker software (Center for Evidence based Medicine, Oxford, UK), in terms of sensitivity, specificity, positive and negative predictive values and likelihood ratios, according to the evidence-based medicine rules and STARD (*Standards for Reporting of Diagnostic Accuracy Studies*) guidelines [16].

## 3. Results

### 3.1. The IgG/IgM rapid test cassette LFIA assay has high specificity and overall good diagnostic accuracy

We first tested the diagnostic accuracy of the index assay against the gold standard (i.e. the RT-PCR assay), according to the STARD guidelines. We selected two groups of patients. The group 1 consisted in 151 patients (88 M and 63 F; median age: 68 years, range 21–98). All patients displayed clinical evidence of suspected COVID-19 and the subsequent analysis of nasopharyngeal samples showed the positive amplification of all three SARS-CoV-2 genes (E, N and RdRP) using specific multiplex real time RT-PCR assay. The second group (group 2) was constituted with 51 individuals (24 M, 27 F; median age: 54 years, range 6–93) who were negative to SARS-CoV-2 multiplex real time RT-PCR assay.

The two groups were tested for IgM and IgG antibodies to SARS-CoV-2 in serum samples using COVID-19 IgG/IgM rapid test cassette LFIA assay kit. The SARS-CoV-2 positive group 1 was positive (Figure 1) for specific IgM, IgG or IgM plus IgG in 103 out of 151 patients (68.21%). In particular, we detected 15 samples positive only for IgM, 18 samples positive only for IgG whereas 70 samples exhibited the double positivity

for IgM and IgG. Specific IgM and IgG response was not detected in 48 samples (Figure 1).

To further assess the specificity of our assay, we tested 51 blood samples obtained from patients who were negative for the detection of viral sequences in nasopharyngeal swabs with multiplex real time RT-PCR. In this context, we have not detected any positive signal for IgM and/or IgG in all 51 patients (Table 1). Accordingly, ST was 68.21%, SP 100%, PPV was 100%, NPV 51.52%, and likelihood ratio – 0.32 (Figure 2A).

The first case of SARS-CoV-2 infection was described in December 2019. To further test the specificity of the analysis, we then challenged an additional set of samples. Specifically, we challenged 99 plasma samples collected between 2015 and 2017 from patients with different viral infection (HIV, HBV and HCV). It is noteworthy that all tested specimens did not show any positive detection for IgM and/or IgG to SARS-CoV-2 (Table 1) confirming the data obtained with the group 2 of patients.

### 3.2. COVID-19 IgG/IgM rapid test cassette LFIA assay showed higher sensitivity after 10 days from symptoms onset

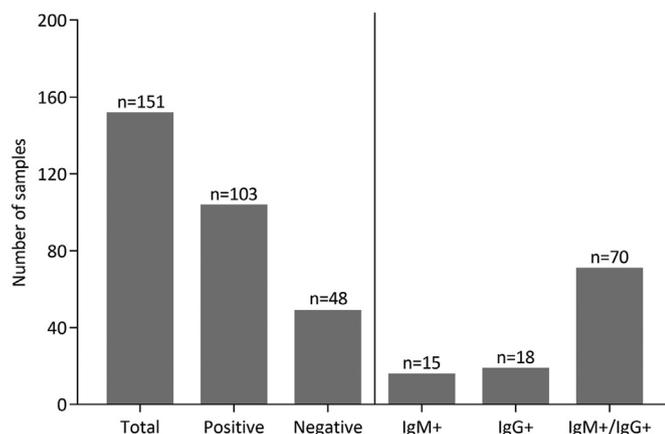
Aiming to improve test sensitivity, we stratified the samples based on different timings of symptoms onset ( $\leq 5$ ; 6–10; 11–15; 16–20;  $> 20$  days). The specimens collected between day 0 and day 5 and between day 6 and day 10 after the onset of symptoms, actually corresponded to lower degrees of test sensitivity (4 out of 26; 15.38% day 0–5 and 13 out of 31; 41.93% day 6–10). The assay showed significantly increased sensitivity when performed after 10 days from symptoms onset. In fact, 37 out of 41 (90.24%), 18 out of 19 (94.73%) and 31 out of 34 (94.17%) samples were positively detected for IgM, IgG or IgM plus IgG when the immunological test was performed at day 11–15, 16–20 and day  $> 20$ , respectively (Figure 3).

The differences were statistically significant when the different settings were compared (0–5 or 6–10 days vs. 11–15 or 16–20 or  $> 20$  days;  $p < 0.0005$  in all analyses by Fisher's exact test).

Overall, the Covid-19 IgG/IgM rapid test cassette LFIA assay showed a remarkably higher sensitivity when performed after at least 10 days from the initial symptoms' onset (86 out of 94; 91.49%) than when performed at earlier time points (17 out of 57; 29.82%;  $p < 0.00001$ ; Fischer's exact test). This corresponded to increased negative predictive values (86.44%) and improved negative likelihood ratio (0.09; Figure 2B).

### 3.3. High diagnostic accuracy of COVID-19 IgG/IgM rapid test cassette LFIA compared to ELISA assays

Based on the large use of ELISA assays in this setting, we sought to compare the diagnostic accuracy of the COVID-19 IgG/IgM rapid test



**Figure 1.** Analysis of Group 1 patients with COVID-19 IgG/IgM rapid test cassette LFIA assay. The number of positive and negative samples is indicated in the left section of figure. In the right section of figure, the number of IgM+, IgG+ and IgM plus IgG positive samples.

**Table 1.** Detection of antibody to SARS-CoV-2.

	Total	Positive samples	Negative samples	Specificity (%)
SARS-CoV-2 RT-PCR negative blood samples (Group 2)	51	0	51	100
Plasma samples 2015–2017	99	0	99	100

cassette LFIA assay with the ELISA Euroimmun kits, specific for the detection of IgM or IgG to SARS-CoV-2 NP protein. Specifically, considering the ELISA as the reference, for the IgM assay, we obtained a ST of 92.42%, SP 74.39%, PPV 74.39%, NPV 92.42%, LLR+ 3.61 and LLR- 0.10 (Supplementary Figure 1). For the IgG assay, we achieved ST 87.91%, SP 91.23%, PPV 94.12%, NPV 82.54%, LLR+ 10.02, and LLR- 0.13 (Supplementary Figure 1).

All in all, we observed a very high degree of concordance (82.43% for IgM and 89.2% for IgG, respectively). It should be noted that the apparently “low” specificity for the IgM assay (recorded since to fulfill the diagnostic accuracy rules ELISA was taken as reference in the analysis) actually hides a higher sensitivity of the index test (LFIA) as indicated by the comparison with PCR based assays (see above).

### 3.4. Analysis of serological dynamics in a small subset of patients by COVID-19 IgG/IgM rapid test cassette LFIA assay

In the next series of experiments, we evaluated COVID-19 IgG/IgM rapid test cassette LFIA assay performance on serial sampling in a small cohort of 12 patients belonging to group 1 (9 M, 3 F; median age 76.5; range 60–90). The first sampling collection was performed between 10 and 34 days from symptoms onset (median 20.5 days).

The analysis of first sample from these patients showed that 7 out of 12 specimens were positive for IgM plus IgG, whereas 3 out of 12 samples were positive only for IgG and 2 out of 12 were negative for all antibodies. The subsequent samplings displayed that two IgM plus IgG positive patients seroconverted to IgG (Table 2). By contrast, no negative case converted to positivity. This indicated, though in a small number of cases, that once the test is performed after 10 days for initial symptoms onset, further delay is probably not associated with sensitivity increases.

### 3.5. Comparison of COVID-19 IgG/IgM rapid test cassette LFIA assay with other LFIA assays

Finally, we performed an analytical comparison among four different LFIA test such as VivaDiag COVID-19 IgM/IgG rapid test (Assay A), Prima Professional COVID-19 IgG/IgM Rapid Test (Assay B), Really Tech 2019-nCoV/COVID-19 IgG/IgM rapid test, (Assay C) and Antai Corona Virus (COVID-19) combined rapid test assay (Assay D) and our index test, (COVID-19 IgG/IgM rapid test cassette LFIA assay; Assay E) on 40 samples (Table 3 and Supplementary Figure 2) selected from group 1 patients (27 M and 13 F, median age 67, range 34–90) among samples obtained from individuals with at least 10 days from symptoms onset.

As indicated in Figure 4A, in our hands, Assay C and E showed SARS-CoV-2 specific immunoglobulin (IgM and/or IgG) in 35 out of 40 samples, Assay D 34 out of 40, Assay B 32 out of 40 and Assay A 31 out of 40. When the analysis was focused on IgG detection, all five LFIAs displayed a number of positive specimens between 30 and 32 samples (Figure 4B). A significant variation was instead detectable when Assay A or B were compared with Assay C, D or E for IgM. Assay E showed a positive result in 32 out of 40 samples, Assay C and D in 31 out of 40 samples, whereas Assay A displayed IgM in 21 out of 40 samples and Assay B in 18 out of 40 samples, respectively. The significant results were observed when the analysis was determined between Assay C vs Assay A ( $p < 0.05$ ); Assay E vs Assay A ( $p < 0.05$ ), Assay C vs Assay B ( $p < 0.01$ ); Assay E vs Assay B ( $p < 0.005$ ), Assay D vs Assay A ( $p < 0.05$ ); Assay D vs Assay B ( $p < 0.01$ ); Fisher's exact test).

Test		Target Disorder		95% Confidence Intervals
		PCR		
		Present	Absent	
COVID-19 IgG/IgM LFIA assay	Positive	103 (a)	0 (b)	
	Negative	48 (c)	51 (d)	
Sensitivity		$a/(a+c)$	68.21%	60.15 to 75.54
Specificity		$d/(b+d)$	100%	93.02 to 100
Pre-test Probability (Prevalence)		$(a+c)/(a+b+c+d)$	74.75%	68.18 to 80.59
Positive Predicted Value		$a/(a+b)$	100%	
Negative Predicted Value		$d/(c+d)$	51.52%	45.69 to 57.30
Likelihood Ratio +		$sens/(1-spec)$		
Likelihood Ratio -		$(1-sens)/spec$	0.32	0.25 to 0.40

Test		Target Disorder		95% Confidence Intervals
		PCR		
		Present	Absent	
COVID-19 IgG/IgM LFIA assay > 10 days	Positive	86 (a)	0 (b)	
	Negative	8 (c)	51 (d)	
Sensitivity		$a/(a+c)$	91.49%	83.92 to 96.25
Specificity		$d/(b+d)$	100%	93.02 to 100
Pre-test Probability (Prevalence)		$(a+c)/(a+b+c+d)$	65%	56.43 to 72.57
Positive Predicted Value		$a/(a+b)$	100%	
Negative Predicted Value		$d/(c+d)$	86.44%	76.67 to 92.52
Likelihood Ratio +		$sens/(1-spec)$		
Likelihood Ratio -		$(1-sens)/spec$	0.09	0.04 to 0.17

**Figure 2.** Diagnostic accuracy evaluation of COVID-19 IgG/IgM rapid test cassette LFIA assay versus multiplex RT-PCR. In A: Analysis of entire series. In B: Analysis of data obtained from patients with symptoms onset >10 days.

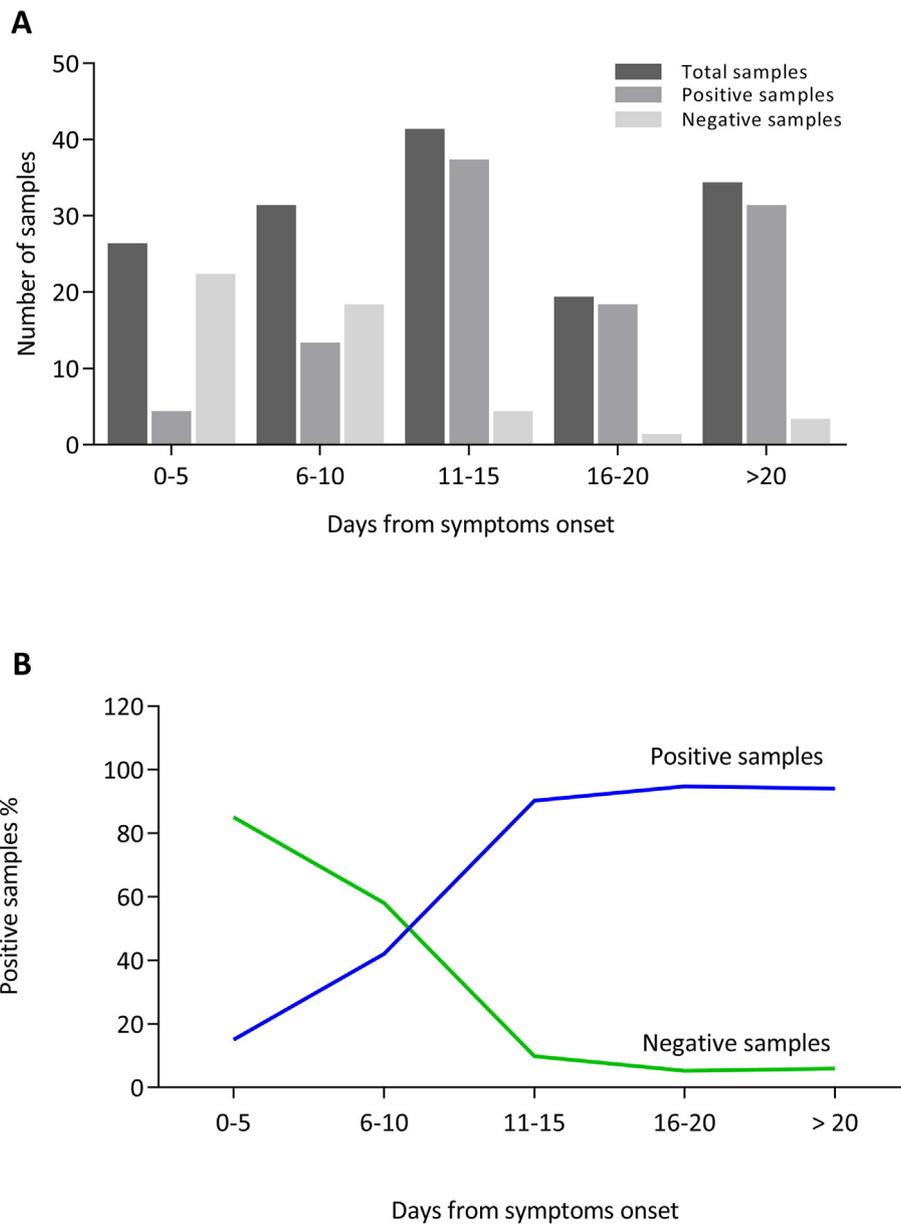
When all LFIA analytical performances were compared with ELISA assays for IgM and IgG, once again a rather good degree of concordance was observed. Specifically, for the IgM detection, the median ST was 86.21% (ranging between 55.17 and 89.66%), while the median SP was 45.45% (range, 45.45–81.82%). For IgG detection, the median ST was 93.75% (range 90.62–96.88%), while the median SP was 87.5% (range 75–87.5%; [Supplementary Figure 3](#)).

#### 4. Discussion

In this study, we evaluated the analytical performance of a novel LFIA assay represented by COVID-19 IgG/IgM rapid test cassette LFIA assay for detecting the IgM and IgG immunological response to SARS-CoV-2. The sensitivity of this LFIA test was investigated using blood samples from SARS-CoV-2 RT-PCR positive patients. We demonstrated that the 68.21% of these specimens were positive for the determination of IgM and/or IgG to SARS-CoV-2. These data confirmed the overall sensitivity noted in other studies with different LFIAs but the detection of antibodies to SARS-CoV-2 is obviously dependent on time course analysis. To investigate this aspect, we stratified the data in five separate groups using the onset of symptoms as reference parameter. In this way, LFIA assay detected antibodies to SARS-CoV-2 in the 29.82% of tested specimens when the samples were collected in the first 10 days after the symptoms onset, whereas a strong sensitivity (91.49%) was demonstrated when the samples were collected after 10 days. These data are in accordance with several studies that reported the seroconversion with a median of 13 days [17]. COVID-19 IgG/IgM rapid test cassette LFIA assay showed a specificity of 100% on different groups of patients. We have also tested 99 plasma samples stored between 2015 and 2017, before SARS-CoV-2 pandemic to determine the specificity of our LFIA assay. The choice of this particular group of samples is clearly due to epidemiological considerations. In fact, these specimens cannot be positive for SARS-CoV-2 infection thus representing a useful negative control. SARS-CoV-2 emerged for the first time in December

2019, probably, through a previous adaption of a coronavirus bat strain in an intermediate host (Malayan pangolin) and then in human beings [18, 19]. No evidence indicates the pre-2019 presence of SARS-CoV-2 as human virus. It should be noted, however, that so high specificity value might reflect a relatively limited sample size. It cannot be ruled out, therefore, that increasing by at least two or three times of examined samples, the specificity value may slightly below 100%.

When compared to ELISA, a type of test commonly used in the clinical community, the concordance of COVID-19 IgG/IgM rapid test cassette LFIA assay was overall very high, ranging from 82.43% for IgM and 89.2% for IgG. Comparable results were also obtained when all the LFIA assays were tested against ELISA, confirming the clinical value of this type of assay. Although the number of tested samples was too small to provide a conclusive interpretation, the comparative analyses between COVID-19 IgG/IgM rapid test cassette LFIA assay and other four LFIA devices, indicated that, in our hands, the analytical performance of our assay for IgG is similar in the total number of positive samples with the other four LFIAs. On the other hand, these LFIA exhibited different number of IgM positive specimens. In fact, two assays (assay A and B) showed a significant lower number of IgM positive samples. Interestingly, the comparative analytical performance of five LFIA assays on this group of samples displayed similar results when Assay C, D and E were employed as well as Assay A showed similar results when compared with Assay B. Unfortunately, the lack of indications on the characteristics of employed viral antigen target (i.e. viral protein, full-length or partial viral protein, viral protein sequence, glycosylation of target) in all LFIAs does not allow an exhaustive evaluation of this phenomenon. At our knowledge, the available information indicated that assay A and B viral targets were represented by N viral nucleoprotein whereas Assay E exhibited S protein target. Liu and coworkers [20] determined that ELISA assays, based on recombinant S protein targeting, were more sensitive than recombinant N protein-based ELISA when specific IgM detection is evaluated. This observation might partially explain the different



**Figure 3.** Stratification of samples classified with days from symptom onset. In A: Percentage of positive samples when five group of patient were constituted (0–5, 6–10, 11–15, 16–20 and >20 days from symptom onset). In B: Analysis of different percentages of positive and negative samples in the five stratified groups.

**Table 2.** Study of antibody dynamics in blood samples from 12 patients using COVID-19 IgG/IgM rapid test cassette LFIA.

Patients	First sample			Second sample			Third sample		
	IgM	IgG	Symptoms onset (days)	IgM	IgG	Symptoms onset (days)	IgM	IgG	Symptoms onset (days)
1	+	+	15	+	+	20	ND	ND	-
2	-	-	10	-	-	15	-	-	20
3	+	+	19	+	+	24	+	+	29
4	-	+	12	-	+	17	-	+	20
5	+	+	34	+	+	39	+	+	43
6	-	+	25	-	+	30	ND	ND	-
7	+	+	17	+	+	22	-	+	30
8	-	+	25	-	+	30	ND	ND	-
9	+	+	22	+	+	27	ND	ND	-

(continued on next page)

Table 2 (continued)

Patients	First sample			Second sample			Third sample		
	IgM	IgG	Symptoms onset (days)	IgM	IgG	Symptoms onset (days)	IgM	IgG	Symptoms onset (days)
1	+	+	15	+	+	20	-	+	25
11	-	-	26	-	-	31	-	-	35
12	+	+	30	+	+	35	ND	ND	-

ND: not done.

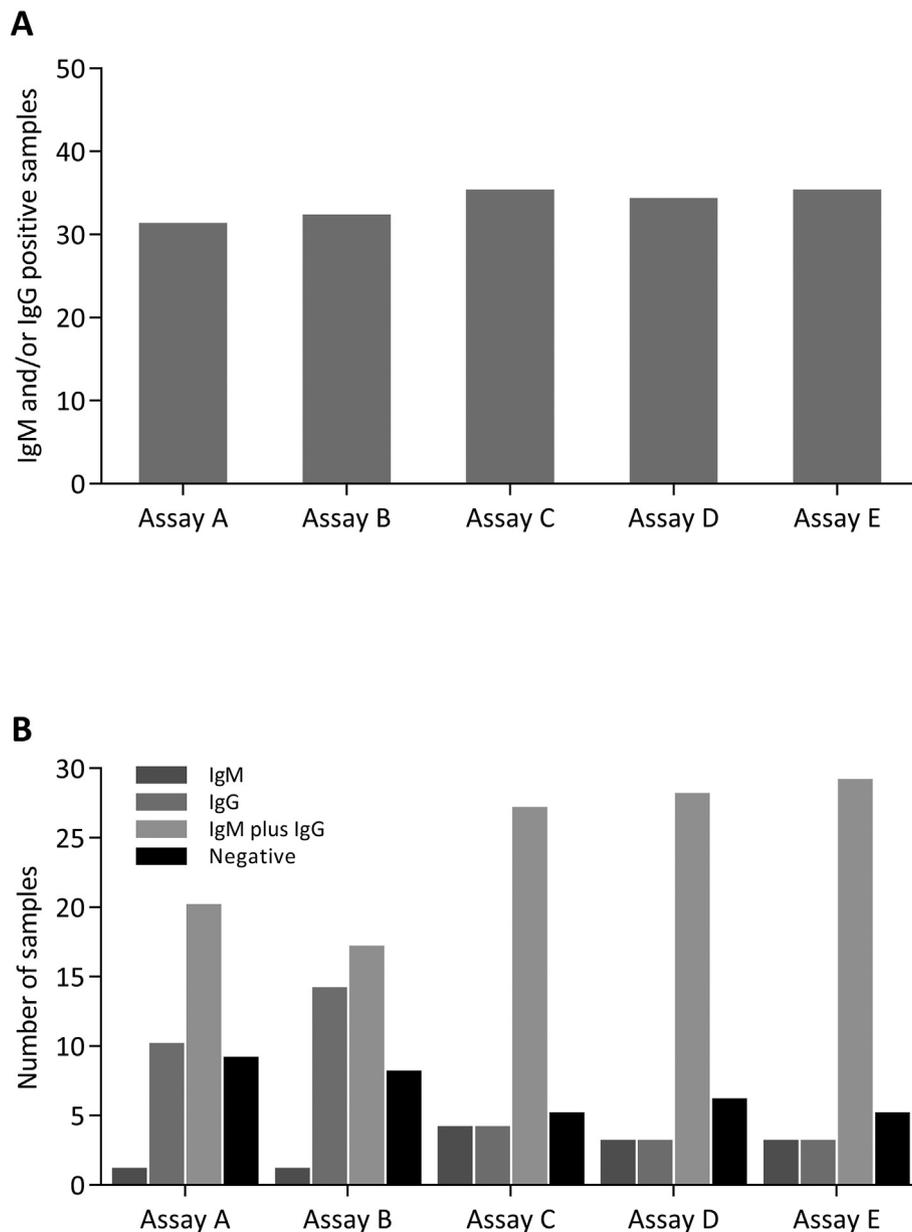
detection IgM positive samples revealed with Assay A and B in comparison with Assay E even though LFIA obviously displays different methodological characteristics with respect to ELISA assays [20].

The serological tests represent an important tool in the viral infection study. The SARS-CoV-2 infection diagnosis is based on RT-PCR assays on respiratory samples such as nasopharyngeal swab [21]. This approach has demonstrated to be effective to recognize acute infection, to isolate the positive patients and to monitor the contacts [22]. However, as

described in other viral infections, the presence of viral genome can be detected during the acute infections and subsequently declines below the detection limit. In addition, is conceivable to observe the failure of SARS-CoV-2 gene detection for several reasons including a low quality of sampling, specimen type, PCR formats, and different sensitivity of various RT-PCR formats [22]. Hence, serological tests can represent a useful tool for the diagnosis and monitoring of infection even though the dynamics of immunoglobulin appearance during the disease

Table 3. Samples challenged with the ELISA and the five different LFIA assays.

Patients	Gender	Age	Assay A		Assay B		Assay C		Assay D		Assay E		ELISA	
			IgM	IgG	IgM	IgG								
1	F	78	-	-	-	-	-	-	-	-	-	-	-	-
2	F	40	+	+	+	+	+	+	+	+	+	+	+	+
3	M	64	+	-	+	-	+	+	+	+	+	+	+	+
4	F	81	+	+	+	+	+	+	+	+	+	+	+	+
5	F	76	-	-	-	-	-	-	-	-	-	-	-	-
6	F	88	-	+	+	+	+	+	-	+	+	+	+	+
7	M	79	-	-	-	-	-	-	-	-	-	-	-	-
8	M	59	-	-	-	-	+	-	+	-	+	-	+	-
9	M	81	-	-	-	-	+	-	+	-	+	-	+	+
10	M	73	-	-	-	-	+	-	+	-	+	+	+	+
11	F	45	-	+	+	+	+	+	+	+	+	+	+	-
12	M	81	+	+	-	+	+	+	+	+	+	+	+	+
13	M	63	+	+	+	+	+	+	+	+	+	+	+	+
14	M	42	-	+	-	+	+	+	+	+	+	+	+	+
15	M	71	-	-	+	+	+	-	-	-	+	-	+	-
16	M	90	-	+	-	+	+	+	+	+	+	+	-	+
17	M	81	+	+	+	+	+	+	+	+	+	+	-	+
18	M	81	-	+	+	+	+	+	+	+	+	+	+	+
19	M	67	-	-	-	-	-	-	-	-	-	-	-	-
20	M	75	-	+	-	+	+	+	+	+	+	+	+	+
21	M	70	-	+	-	+	+	+	+	+	+	+	-	+
22	M	77	-	+	-	+	-	+	-	+	-	+	+	+
23	M	65	+	+	-	+	+	+	+	+	+	+	+	+
24	M	57	+	+	-	+	+	+	+	+	+	+	+	+
25	M	67	+	+	-	+	+	+	+	+	+	+	+	+
26	M	57	+	+	+	+	+	+	+	+	+	+	+	+
27	F	58	+	+	-	+	-	+	+	+	-	+	+	+
28	M	56	+	+	-	+	+	+	+	+	+	+	+	+
29	M	65	+	+	+	+	+	+	+	+	+	+	+	+
30	F	40	+	+	+	+	+	+	+	+	+	+	+	+
31	F	71	+	+	-	+	+	+	+	+	+	+	+	+
32	M	42	-	+	+	+	+	+	+	+	+	+	-	+
33	M	71	+	+	-	+	+	+	+	+	+	+	-	+
34	F	34	+	+	+	+	+	+	+	+	+	+	+	+
35	F	74	-	+	+	+	-	+	-	+	+	+	+	+
36	M	54	+	+	+	+	-	+	+	+	-	+	+	+
37	M	90	+	+	+	+	+	+	+	+	+	+	+	+
38	F	58	-	-	-	-	-	-	-	-	-	-	-	-
39	F	56	+	+	-	+	+	+	+	+	+	+	-	+
40	M	64	+	+	+	+	+	+	+	+	+	+	+	+



**Figure 4.** Analysis of five LFIA assays. In A: Analysis of number of IgM and/or IgG samples. In B: Analysis of IgM+, IgG+, IgM and IgG positive samples displayed by every LFIA assay.

development is not yet fully defined. SARS-CoV-2 ELISA and CMIA-based methods were set up by several manufacturers and its independent evaluation of performances are ongoing [15, 23]. Recently, a comprehensive Cochrane review compared different tests and overall revealed results quite comparable with the one showed in this analysis using the COVID-19 IgG/IgM rapid test cassette LFIA assay [24]. The advantages of these assays are related to high-throughput analysis of samples with the possibility of semiquantitative or quantitative determination in 2–5 h. On the other hand, these serological approaches generally require dedicated laboratory space and some technical training. Moreover, the determination of cut-off assay value and the indeterminate results have to be cautiously evaluated. In addition to these classical serological techniques, some rapid methods were proposed for the SARS-CoV-2 serology. The LFIA assays are rapid methods that can detect the presence of antibodies in 10–30 min and minimal technical training is required. These

methods were used for the evaluation of antibodies and antigen presence in several infections (i.e. Pneumococci, Plasmodium, Legionella, HIV) to obtain a qualitative determination of target [25, 26, 27, 28]. The disadvantages are the lower sensitivity, the lack of quantitative data and the single sample analysis.

Therefore, the LFIA assay may contribute to serological analysis with particular emphasis to its point-of care characteristics. The LFIA antibody testing could play a valuable role for: i) the rapid screening of suspected asymptomatic SARS-CoV-2 infected subjects; ii) the study healthy close contacts in quarantine; iii) the monitoring of RNA positive patients; and iii) the analysis of epidemiological prevalence.

Our data confirmed the role of LFIA in the serological analysis during SARS-CoV-2 infection and, in particular, indicate that COVID-19 IgG/IgM rapid test cassette LFIA assay might represent a valid alternative approach for the antibody to SARS-CoV-2 detection.

## Declarations

### Author contribution statement

Erica Diani: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Pier Paolo Piccaluga: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools and data; Wrote the paper.

Virginia Lotti: Performed the experiments.

Andrea Di Clemente, Marco Ligozzi: Performed the experiments; Analyzed and interpreted the data.

Pasquale De Nardo, Lorenza Lambertenghi, Alice Vianello, Giacomo Marchi: Contributed reagents, materials, analysis tools and data.

Francesca Pizzolo, Simonetta Friso: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools and data.

Giuliana Lo Cascio: Performed the experiments; Contributed reagents, materials, analysis tools and data.

Ercle Concia: Conceived and designed the experiments; Wrote the paper.

Davide Gibellini: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

### Funding statement

This work was supported by the Fondazione Cariverona, ENACT project VIRO-COVID.

### Data availability statement

Data will be made available on request.

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2021.e08192>.

## References

- [1] P. Zhou, X.L. Yang, X.G. Wang, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin, *Nature* 579 (2020) 270–273, 2020.
- [2] N. Chen, M. Zhou, X. Dong, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, *Lancet* 395 (2020) 507–513.
- [3] F. Wu, S. Zhao, B. Yu, et al., A new coronavirus associated with human respiratory disease in China, *Nature* 579 (2020) 265–269.
- [4] update March,9th, WHO Week Epidemiological, 2021, <https://www.who.int/publications/m/item/weekly-epidemiological-update—10-march-2021>.
- [5] WHO Situation Report 51, March 11, 2020. [https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-COVID-19.pdf?sfvrsn=1ba62e57\\_10](https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-COVID-19.pdf?sfvrsn=1ba62e57_10).
- [6] M. Wang, Q. Wu, W.-Z. Xu, et al., Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan, *medRxiv* (2020) published online Feb 18.
- [7] N.-S. Chen, M. Zhou, X. Dong, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, *Lancet* 6736 (2020) 30211–30217.
- [8] C.L. Huang, Y.M. Wang, X.W. Li, et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China, *Lancet* 6736 (2020) 30183–30185.
- [9] D.W. Wang, B. Hu, C. Hu, et al., Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China, *J. Am. Med. Assoc.* (2020) published online Feb 7.
- [10] J.F. Cohen, D.A. Korevaar, S. Matczak, et al., COVID-19-related mortality by age groups in Europe: a meta-analysis, *medRxiv* (2020).
- [11] K.K. To, O.T. Tsang, W.S. Leung, et al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, *Lancet Infect. Dis.* (2020).
- [12] J.-W. Ai, H.C. Zhang, T. Xu, et al., Optimizing diagnostic strategy for novel coronavirus pneumonia, a multi-center study in Eastern China, *medRxiv* (2020).
- [13] National COVID Testing Scientific Advisory Panel\* Evaluation of antibody testing for SARS-CoV-2 using ELISA and lateral flow immunoassays *medRxiv*.
- [14] B. Lou, S. Zheng, Y. Su, et al., Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset, *MedRxiv* (2020).
- [15] J. Zhao, Q. Yuan, H. Wang, et al., Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, *Clin. Infect. Dis.* (2020) ciaa344.
- [16] P.M. Bossuyt, J.B. Reitsma, D.E. Bruns, et al., STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies, *BMJ* 351 (2015) h5527.
- [17] Q. Long, B. Liu, H. Deng, et al., Antibody responses to SARS-CoV-2 in patients with COVID-19, *Nat. Med.* 26 (2020) 845–848.
- [18] F.A. Rabi, M.S. Al Zoubi, G.A. Kasasbeh, et al., SARS-CoV-2 and coronavirus disease 2019: what we know so far, *Pathogens* 9 (2020) 231.
- [19] M. Uddin, F. Mustafa, T.A. Rizvi, et al., SARS-CoV-2/COVID-19: viral genomics, epidemiology, vaccines, and therapeutic interventions, *Viruses* 12 (2020) 526.
- [20] W. Liu, L. Liu, G. Kou, et al., Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2, *J. Clin. Microbiol.* 58 (2020) e00461–e00520.
- [21] Y.W. Tang, J.E. Schmitz, D.H. Persing, C.W. Stratton, The laboratory diagnosis of COVID-19 infection: current issues and challenges, *J. Clin. Microbiol.* 58 (2020) e00512–e00520.
- [22] F. Yu, L. Yan, N. Wang, S. Yang, et al., Quantitative detection and viral load analysis of SARS CoV-2 in infected patients, *Clin. Infect. Dis.* (2020), ciaa345.
- [23] <https://covid-19-diagnostics.jrc.ec.europa.eu/devices/detail/428>.
- [24] Deeks Jj, Dinnes J, Takwoingi Y, et al., Antibody tests for identification of current and past infection with SARS-CoV-2, *Cochrane Database Syst. Rev.* 6 (2020), CD013652.
- [25] I. Nakagiri, T. Tasaka, M. Okai, et al., Screening for human immunodeficiency virus using a newly developed fourth generation lateral flow immunochromatography assay, *J. Virol. Methods* 274 (2019) 113746.
- [26] P.F. Mens, A. van Amerongen, P. Sawa, et al., Molecular diagnosis of malaria in the field: development of a novel 1-step nucleic acid lateral flow immunoassay for the detection of all 4 human *Plasmodium* spp. and its evaluation in Mbita, Kenya, *Diagn. Microbiol. Infect. Dis.* 61 (2008) 421–427.
- [27] C.S. Jørgensen, S.A. Uldum, P.L. Elverdal, Application of a lateral flow test as an additional serological tool for diagnosis of *Legionella* infections, *J. Microbiol. Methods* 96 (2014) 12–15.
- [28] A. Boulos, D. Fairley, J. McKenna, P. Coyle, Evaluation of a rapid antigen test for detection of *Streptococcus pneumoniae* in cerebrospinal fluid, *J. Clin. Pathol.* 70 (2017) 448–450.