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► **To cite this version:**

Julien Exinger, Cédric Hartard, Fanny Lafferrière, Christelle Fenninger, Loic Charbonnière, et al.. Evaluation of a Lateral Flow Immunoassay COVIDTECH® SARS-CoV-2 IgM/IgG Antibody Rapid Test. Japanese Journal of Infectious Diseases, 2022, 75 (4), pp.334-340. 10.7883/yoken.JJID.2021.273 . hal-03859205

HAL Id: hal-03859205

<https://hal.science/hal-03859205>

Submitted on 16 Nov 2023

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Evaluation of a Lateral Flow Immunoassay COVIDTECH® SARS-CoV-2 IgM/IgG Antibody rapid test

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Abstracts.

Key word. SARS-CoV-2, COVID-19, serological diagnosis, Flow lateral immunoassay

Highlights.

- Lateral flow immunoassay permits rapid detection of anti-SARS-CoV-2 IgM and IgG
- The specificity reached 100% for IgM and IgG detection
- Sensitivity reached 100% after day+14 post-symptoms, for IgM/IgG combined interpretation

Abstract (150 words)

The new epidemic coronavirus SARS-CoV-2 is responsible for severe respiratory illness (i.e. COVID-19). RT-PCR is the gold standard in COVID-19 diagnosis and is performed on respiratory samples, while serological tests may contribute to diagnosis and permit seroprevalence studies. The lateral flow immunoassay (LFIA) PolyDTech simultaneously detects anti-SARS-CoV-2 IgM and IgG using a S-protein recombinant antigen and has been independently evaluated in two laboratories. The specificity evaluated on 53 pre-pandemic samples reached 100% for IgM and IgG. Analyzing samples from patients with RT-PCR-confirmed infection, IgM/IgG antibodies were detected in 18/26 (69%)

and 58/58 (100%) samples before day 13th and from the 14th day post-symptom onset respectively. These results were concordant with those of another LFIA method at 89% success rates (BioSynex BSS IgM/IgG) and at 94% with a chemiluminescent Immunoassay (Maglumi Snibe). Overall, this LFIA method detecting anti-SARS-CoV-2 IgM and IgG is suitable for SARS-CoV-2 serological diagnosis, when the patient is, as recommended, > day+14 after onset of symptoms.

1. Introduction

In December 2019, a new coronavirus epidemic, caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) started in Wuhan, China. This new pathogen responsible for COVID-19 has rapidly spread across the globe and resulted in a pandemic affecting more than 53 million people (confirmed cases) and leading to 1,300,576 confirmed deaths on the 13 November 2020 (A ACTUALISER au dernier moment (bulletin hebdomadaire) et penser à ajouter la REF). France has been impacted significantly by the « first wave », with a mean of 4,500 daily confirmed cases during the last week of March 2020 and up to 950 daily confirmed deaths during the first fortnight of April 2020 (<https://geodes.santepubliquefrance.fr/>). The analysis of the dynamics of mortality from COVID-19 in French hospitals showed that the Grand-Est region was one of the most affected in France (beginning week 9, peak during the week 13), thus it represents an ideal locale for the investigation of the effects of the aforementioned virus

Coronaviruses belong to the *Coronaviridae* family and are classified into four genera including α -, β -, γ -, and δ -coronaviruses (Kirtipal et al., 2020). They consist of 60-220 nm diameter enveloped particles presenting the largest known RNA viral genome (26-32 kb in length).

Coronaviruses primarily affects the respiratory system; among the 7 pathogenic coronaviruses currently described, many of them (i.e. CoV-NL63, CoV-229E, CoV-OC43, and CoV-HKU1) cause mild clinical symptoms leading to flu-like illness, while SARS-CoV, MERS-CoV, and the newly described SARS-CoV-2 cause severe respiratory illness and result in death in patients, primarily in comorbid patients with underlying health issues. In addition to severe acute respiratory syndrome, COVID-19 is also associated to systemic inflammation, leading to sepsis, acute cardiac injury, heart failure and multi-organ dysfunction in patients at high risk (Wang et al., 2020).

The SARS-CoV-2 genome shares 82% sequence identity with SARS-CoV and MERS-CoV and > 90% sequence identity for essential enzymes and structural proteins (Naqvi et al., 2020). SARS-CoV-2 comprises four major structural proteins, including the surface spiculated glycoprotein (S) which presents a cell receptor binding domain and the capsid protein (N) (Liu et al., 2020). Protein N is highly antigenic and widely expressed during infection (Liu et al., 2020). The antibodies directed against protein N do not show cross-reactivity with antigens of seasonal coronaviruses i.e. NL63, 229E, OC43 and HKU1 (Guo et al., 2020). The Spike protein is the molecular determinant of viral attachment to the primary cell host receptor Angiotensin converting enzyme-2 (ACE-2), permitting fusion and entry into cells (Zost et al., 2020). Thus antibodies directed against the S protein are thought to neutralize virus entry into the cell (Dogan et al., 2020). Hospitalized patients with severe infection have higher antibody levels and higher neutralization capacity than outpatients (Ma et al., 2020).

The gold standard of COVID-19 diagnosis is RT-PCR on nasopharyngeal swab, or lower respiratory tract samples in severely ill patients. Serological tests help determine whether a person with negative RT-PCR or without RT-PCR diagnosis has been previously or currently infected with SARS-CoV-2. The detection of IgM and / or IgG is optimal from day +15 of the infection. The seroconversion rate is higher for total Ab, than for IgM or IgG (93.1%, 82.7% and 64.7% respectively, (Zhao et al., 2020). The median seroconversion is also shorter for total Ig (11 days) than for IgM (12 days) and IgG

(14 days) (Zhao et al., 2020). The level of IgM and IgA is stable from after infection, whereas the rate of IgG continues to increase after D15 (Guo et al., 2020), and the increase in IgG levels seems to correlate with the decrease in viral load in the respiratory tract (Jin et al., 2020).

The lateral flow immunoassay (LFIA) PolyDTech test is based on an immunochromatographic method that detects simultaneously anti-SARS-CoV-2 IgM and IgG using a SARS-CoV-2 recombinant antigen; the full principle for IgM antibodies is depicted in Figure 1. Typically, when IgM antibodies are present in the blood/serum sample, they react with gold nanoparticles (GNPs) labeled at their surfaces with a SARS-CoV-2 recombinant antigen. After the buffer is added into the well, the IgM/GNP complex elutes to combine with a mouse anti-human IgM monoclonal antibody set at the surface of the test (T) line. The adduct formed develops a brown-red color at the T line, in which corresponds to a positive test. As a control, in the absence of IgM SARS-CoV-2 antibodies the complex is not formed, resulting in no color at the T line.

The principle of the SARS-CoV-2 IgG detection kit is similar, except that the GNPs for the detection are labeled with a mouse anti-human IgG monoclonal antibody which forms a complex in the presence of SARS-CoV-2 IgG antibodies. The complex migrates upon buffer addition and is set at the surface of the T line labeled with the SARS-CoV-2 recombinant antigen, resulting in a red-brown color at the T line.

As a control (C) experiment, GNPs labeled with a rabbit IgG antibody (for IgM control), and GNPs labeled with a mouse anti-human IgG monoclonal antibody (for IgG control) are also present in the kit. Upon elution with the buffer, these control GNPs migrate and set at the surface of the C line which are coated with a goat anti-rabbit IgG antibody and a goat anti-mouse IgG antibody, revealing a brown-red color at the C line. The absence of a red C line indicates an invalid test.

The method was evaluated on clinical samples in two different hospital centers (namely H and N).

The specificity was evaluated on blood samples collected before the SARS-CoV-2 pandemic arrived in France. The sensitivity was evaluated using blood samples from patients with confirmed COVID-19,

diagnosed using molecular methods. The PolyDTech method was compared to another commercial LFIA (BioSynex BSS IgM/IgG) as well as a commercial chemiluminescent Immunoassay (CLIA) method (Maglumi Snibe).

2. Material and Methods

2.1. Negative samples

The negative samples consisted of serum sample collected in two hospital Centers before the SARS-CoV-2 pandemic was reported in France. All serum samples were stored at -20°C before use.

These pre-pandemic serum samples have been used to evaluate the specificity of the PolyDTech LFIA method. The panel of negative serum sample comprised 23 samples from April 2019 for Center H and 30 samples from December 2019 for Centre N.

Additionally, to complete the evaluation of specificity, eight sera from patient previously tested Influenza positive and four with rheumatoid factor have been tested in Center H.

2.2. Samples from COVID-19 patients

For sensitivity evaluation, first, sequential samples of two SARS-CoV-2 positive patients have been tested. Sera were sampled at day (D+)17, D+24, D+38, D+66 and D+112 post-infection for one patient (EL) and at D+10, D+16, D+31 and D+108 for the other one (CH).

In center H, the positive control group consists of 63 laboratory-confirmed COVID-19 patients or hospital staff (method?) that were sampled at least seven days after their first clinical signs. For each participant, information on clinical situation and the disease's kinetics were collected from a survey.

In center N, positive control group consists of 21 laboratory-confirmed COVID-19 patients (Viral RNA was extracted using the NucliSens® technology (bioMerieux France) and amplified by RT-PCR protocols targeting RdRp gene developed by Institut Pasteur Paris). For each, the day of the initial appearance of clinical indicators was collected from medical records.

Thus, 93 samples from PCR-confirmed positive patients, ranging from D+5 to D+112 post-symptoms onset, were tested.

2.3. Lateral Flow Immunoassay for detection of IgM and IgG antibodies for SARS-CoV-2

The PolyDTech LFIA method permits the qualitative detection of IgM and IgG anti-SARS-CoV2.

According to the manufacturer's instructions, the serum sample (20 μ L) was deposited in the well of the device. In a second step, 80 to 100 μ L of the buffer were added into the well. The reading of the test is made after 15 to 20 minutes.

The presence of a red-brown color on C line is required to validate the test. The presence of a brown-red line on T line revealed the detection of IgM and/or IgM SARS-CoV-2 antibodies in the sample.

2.4. Comparison of methods

The comparison was done on 27 samples ranging from D+5 and D+112 post-symptoms. The PolyDTech LFIA Method was compared to another LFIA method, the BIOSYNEX COVID-19 BSS IgG/IgM, and a CLIA IgM+IgG method, Maglumi Snibe.

The antigen used in the Biosynex COVID-19 BSS method corresponds to the RBP domain of S protein. Analyses were realized according to the manufacturer recommendations. Briefly 10 μ L of sera was deposited in the S well and 2 droplets of diluent were deposited in the B well of the test cassette. The result was read after 10 minutes and before 20 minutes after the deposit. The control line must be present to validate the test. The detection of IgM and/or IgG is associated with the presence a colored line in the IgM and/or IgG zone.

The Maglumi SARS-CoV-2 antibodies IgM and IgG CLIA test used two antigens (S and N protein) coated on magnetic beads. The quantity of antibodies in a sample (i.e. 10 μ L) is correlated with the relative light units (RLU) measured by chemiluminescence. A Cut off value was 1.00 UA/mL as recommended by the manufacturer.

3. Results

3.1. Specificity of the assay

The specificity was evaluated on 53 serum specimens collected before the emergence of SARS-CoV-2 in France. All samples were negative for IgG and IgM (Table 1). Additionally, no cross-reaction was observed when tested eight sera from influenza-positive patients, or four sera positives for rheumatoid factor.

3.2. Sensitivity of the assay

Overall, 93 serum samples were collected from laboratory-confirmed COVID-19 patients.

For the 84 individual serum samples, the period from onset to serum samples extends from 5-116 days (Table 2). The global sensitivity for all the samples was 53.6% (45/84) for IgM, 85.7% (72/84) for IgG, and 90.5% (76/84) for IgM/IgG. Serum samples were subdivided in subgroups according to sample collection time. The samples taken before D+7 were all negative. For samples taken between D+7 and D+10, and between D+11 to D+13, the IgM/IgG sensitivity was 75.0% and 81.8% respectively. Before between D+7 to D+10, IgM were detected in 7/12 samples, while IgG were detected in 6/12 samples. Between D+11 to D+13, IgM were detected in 6/11 samples, while IgG were detected in 8/11 samples. From D+14, the sensitivity of IgG detection reached 100.0%, and consequently the overall sensitivity of IgM/IgG reached 100.0%. In these samples, IgM were detected in 11/18 samples before D+21, and 21/41 from D+21.

Overall, the Negative Predictive Value (NPV) was calculated to be 86.9% and the positive predictive value (PPV) reached 100.0% for IgM/IgG detection (Table 3). The NPV reached 93.0% for samples from D+7 and 100% for samples from D+14.

Iterative samples were obtained for two patients (n=5 and n=4). One was negative for IgM for all samples from D+17 to D+112 but positive for IgG for all the 5 samples, while the other one was still negative for IgM and IgG from D+10 to D+108 after symptoms (Table 4).

3.3. Comparison with other LFIA and CLIA method

The FLIA PolyDtech was compared to another FLIA method, Biosynex BSS G/M and to the Maglumi Snibe CLIA method. Eighteen samples were analyzed using the three methods. All samples from D+13 (n=8) were consistently positive for IgM and/or IgG. For earlier samples, 2/9 were discordant between both FLIA methods (sampled at D+8 and D+10 post symptoms onset), and 1/9 was discordant between PolyDtech FLIA and CLIA method, sampled at D+10.

Overall, the concordance of IgM/IgG detection between the two LFIA methods reached (16/18) 89.0% and the concordance of IgM/IgG detection between the PolyDtech LFIA method and the Maglumi Snibe CLIA method reached (16/17) 94.0% (Table 4).

Elsewhere, two patients with iterative samples were analysed (n=5 and n=4). For one patient, all five samples ranging from D+17 to D+112 were positive for IgM and/or IgG whatever the method used. For the other patient, usual serological kinetic was only observed using the Biosynex FLIA.

4. Discussion

The recent outbreak of a novel coronavirus leads to the need for new diagnosis methods.

LFIA has the advantages to be easy to implant, quick to realize and doesn't require device. While RT-PCR is the only diagnostic method currently validated for SAR-CoV-2 infection, serological tests are needed to evaluate the seroprevalence of the infection and to improve the knowledge of COVID-19 spread, thus improving combat of the disease. Serologic tests can also allow confirmation of a SARS-CoV-2 infection if RT-PCR was not able to be realized or was negative despite characteristic symptoms. The viral antigens recommended in the context of serological tests are protein S "spike", especially the RBD domain, or protein N (Liu et al., 2020).

In this study we evaluated a new LFIA, for IgM and IgG anti-SARS-CoV-2 detection based on anti-spike protein immunoglobulins detection. This test was independently evaluated on two hospital laboratories in North-Eastern France, where the epidemic massively developed during the first three months of 2020.

In SARS-CoV-2 serological tests development, Spike protein and the nucleoprotein have been used as antigens because they are highly antigenic. The specificity of anti-Spike antibodies has been shown to be higher than the specificity of N protein (Ayoubia et al., 2020). The specificity of the method was evaluated on pre-pandemic serum from 2019 November and August. All pre-pandemic samples were negative for anti-SARS-CoV-2 IgM and IgG. In the context of new viral emergence, specificity could be impacted by cross-reactivity with other pathogens, circulating in the same place and time, meaning the seasonal coronavirus (CoV-NL63, CoV-229E, CoV-OC43, and CoV-HKU1) or previously emerging coronaviruses like SARS-CoV or MERS-CoV. Previous studies have demonstrated that cross-reaction with seasonal coronavirus are weak. They are more frequent with SARS-CoV and at a lower rate with MERS-COV in accordance with phylogeny of theses coronaviruses (Ayoubia et al., 2020). In countries where MERS-CoV and SARS-Cov have not experienced intensively circulation, this cross-reactivity may not have an impact on the specificity of serological methods. However, the specificity of other

ELISA and CLIA methods have been previously evaluated and reached 99% and 92.2% for IgM, and 99% and 100% for IgG, respectively (Infantino et al., 2020). Elsewhere specificity of another immunochromatographic assay was evaluated to be 98% (Imai et al., 2020). The specificity of the LFIA evaluated here was 100%, in conformity with French recommendations.

According to Liu et al., the sensitivity of protein S-based ELISA for IgM was higher than that of ELISA based on the N protein (Liu et al., 2020). The sensitivity of ELISA S-based assay was found to reach 60.5% between D+6 and D+10 after disease onset, 96% between D+16 and D+20, and 100% after D+31 (Ong et al., 2020). For the method evaluated here, IgM was the most sensitive before D+11 (58% vs 50% for IgG), while from D+11, IgG was the most sensitive (88%). The global sensitivity of IgM/IgG reached 97% from D+10 and 100% from D+14. The French sanitary authority (Haute Autorité de Santé, 2020) recommends the use of automated ELISA tests, detecting IgM and IgG or total Ig, and displaying a minimum sensitivity of 90 to 95% and a minimum specificity of 98%. As serological diagnosis is recommended from D+14 post symptoms onset, the method is meeting the requirements of HAS recommendations. Otherwise, the anti-S antibodies seem to persist longer in serum and thus more accurately detect the presence of past infection (Ripperger et al., 2020), even if it was not evaluated here.

The kinetics of anti-SARS-CoV-2 seroconversion is still not understood completely. Multiple situations have been described, including early IgG seroconversion before D+7 and IgG seroconversion earlier than IgM (Long et al., 2020). These different situations are also observed in the present study.

Surprisingly, one patient was negative at D+10-108 for IgM and IgG; crucially this serum was also negative with numerous other dependable methods, including Maglumi Snibe IgG (Eurobio) and Platelia SARS-CoV-2 Total Ab (bioRad) (data not show), highlighting the advances still to be made in the field. Indeed, it has been described that non-hospitalized patients may have lower antibodies rate (Grzelak et al., 2020) which can adversely affect the probability of detection

Overall, this LFIA method detecting anti-SARS-CoV-2 IgM and IgG displays appreciable specificity and sensitivity. It meets the requirements of recommendations for serological diagnosis of COVID-19,

when RT-PCR is negative or cannot be realized, provided that patient has developed symptoms for more than 14 days.

Author statement.

F Lafferrière: Methodology; data acquisition

C Hartard: Writing - original draft

C Fenninger: Methodology; data acquisition

J. Charbonnière : Writing - review & editing

J Exinger: Conceptualization; Data curation; Methodology; Writing - review & editing

H Jeulin : Conceptualization; Data acquisition; Data curation ; Methodology, Writing - original

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Table 1. Evaluation of the specificity of PolyDTech lateral flow immunoassay for anti-SARS-CoV-2 IgM/IgG detection

Center	Serum	Date	IgM		IgG		IgM/IgG	
			Negative	Positive	Negative	Positive	Negative	Positive
Centre H	n=23	April 2019	23	0	23	0	23	0
Centre N	n=30	December 2019	30	0	30	0	30	0
Total			53	0	53	0	53	0

Table 2. Evaluation of the sensitivity of PolyDTech lateral flow immunoassay for anti-SARS-CoV-2 IgM/IgG detection

case	SEX	Age (years)	Day post-symptoms	IgM	IgG	IgM/IgG	Sensitivity
N-13	H	62	5	-	-	-	Not evaluated
N-1	M	27	6	-	-	-	
N-11	F	33	6	-	-	-	
H-1	M	75	7	-	+	+	75.00%
N-4	f	42	7	+	-	+	
N-2	M	43	8	-	-	-	
N-9	M	8	8	+	-	+	
N-14	H	70	8	-	-	-	
N-17	F	69	8	+	+	+	
H-10	F	58	9	+	+	+	
H-12	F	56	9	+	+	+	
H-5	F	59	10	+	+	+	
H-21	M	83	10	-	+	+	
N-8	F	5	10	+	-	+	
N-16	M	42	10	-	-	-	
H-7	F	84	11	+	+	+	
H-20	F	86	11	+	+	+	
H-24	M	84	11	+	+	+	
H-37	M	32	11	+	-	+	
H-43	M	87	11	-	-	-	
H-26	M	83	12	-	+	+	81.82%
H-45	F	41	12	-	+	+	
N-12	M	40	12	-	-	-	
H-4	F	70	13	-	+	+	100.00%
H-44	M	48	13	+	+	+	
N-7	M	94	13	+	+	+	
H-3	M	73	14	+	+	+	

case	SEX	Age (years)	Day post-symptoms	IgM	IgG	IgM/IgG	Sensitivity
H-22	M	79	14	+	+	+	100.00%
H-62	M	81	14	+	+	+	
N-10	F	85	14	+	+	+	
N-18	M	58	15	-	+	+	
H-51	M	21	16	+	+	+	
H-53	F	39	16	-	+	+	
H-8	M	82	17	+	+	+	
H-27	F	23	17	-	+	+	
H-31	F	44	17	-	+	+	
N-3	M	40	18	+	+	+	
H-16	F	55	19	+	+	+	
H-18	F	26	19	+	+	+	
H-23	F	49	19	-	+	+	
H-11	M	55	20	+	+	+	
H-15	M	44	20	-	+	+	
H-39	F	36	20	-	+	+	
H-42	F	86	20	+	+	+	
H-25	M	25	21	+	+	+	
H-29	F	32	21	-	+	+	
H-40	F	34	21	-	+	+	
H-49	F	23	21	-	+	+	
H-52	M	22	21	+	+	+	
H-59	M	41	22	-	+	+	
H-61	M	65	22	-	+	+	
H-17	F	64	23	-	+	+	
H-30	F	48	23	+	+	+	
H-2	M	43	24	-	+	+	
N-5	M	36	24	+	+	+	
H-6	M	72	25	+	+	+	
H-28	F	28	25	-	+	+	
N-20	M	69	25	+	+	+	
H-35	F	57	26	+	+	+	
H-38	F	49	26	-	+	+	
H-34	F	52	27	-	+	+	
H-36	F	46	28	+	+	+	
H-48	F	40	28	-	+	+	
H-50	F	35	28	-	+	+	
N-19	M	78	28	+	+	+	
H-58	M	61	29	+	+	+	
H-56	F	92	30	-	+	+	
H-60	F	36	32	+	+	+	

case	SEX	Age (years)	Day post-symptoms	IgM	IgG	IgM/IgG	Sensitivity
H-9	F	45	33	+	+	+	
H-14	F	66	33	+	+	+	
H-32	F	42	33	-	+	+	
H-33	F	26	34	+	+	+	
H-41	F	42	36	+	+	+	
H-55	M	80	36	-	+	+	
H-57	F	89	37	+	+	+	
H-19	F	27	38	-	+	+	
H-54	M	58	43	+	+	+	
H-63	M	40	51	-	+	+	
N-21	F	31	51	-	+	+	
N-6	M	53	64	+	+	+	
H-13	M	74	116	-	+	+	
H-46	M	20	NA	+	+	+	
H-47	M	58	NA	+	+	+	
N-15	F	58	NA	+	+	+	

Table 3. Evaluation of the overall positive predictive value and the negative predictive value of Lateral flow immunoassay PolyDTech

	laboratory-confirmed COVID	Pre-pandemic	Total
IgM and or IgG +	76	0	81
IgM and IgG -	8	53	61
Total	84	53	137

Figure 1. Composition and principle of the SARS-CoV-2 IgM detection kit.

1) Composition of the SARS-CoV-2 IgM antibody test

● Gold Nanoparticles ▲ SARS-CoV-2 recombinant antigen Y SARS-CoV-2 IgM antibody

Y Mouse anti-human IgM monoclonal antibody Y Goat anti-rabbit IgG antibody Y Rabbit IgG antibody

2) Principle

