COVID-19 IgG/IgM Rapid Test Cassette

ANALYTICAL VALIDATION

BIOLAN HEALTH

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1. INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a novel coronavirus that causes the disease COVID-19, a respiratory illness that can range from a common cold to more serious illnesses that can lead to death. It was first detected in December 2019 in the Chinese city of Wuhan and has since spread rapidly around the world[1].

It has been reported that infected individuals can generate antibodies against the virus for days or weeks after infection. In most cases, IgG and IgM levels increase markedly within the first few weeks after symptom onset, and peak between the second and the third week. IgM antibodies usually appear in the initial phase of infection and start to decrease considerably after the peak, while IgG antibodies persist for several months.

It has also been shown that the antibodies that target the Receptor Binding Domain (RBD) of the spike (S) protein have a potential neutralising activity against SARS-CoV-2 and, therefore, may prevent future infections[2, 3].

In this context, the COVID-19 IgG/IgM Rapid Test Cassette of BIOLAN HEALTH S.L., a lateral flow immunoassay that allows the rapid (5-10 min) and user-friendly (using a drop of blood) detection of IgG and IgM antibodies that target the RBD, can be a highly useful tool for:

- Identifying people with a current or past COVID-19 infection.
- Identifying people who already have neutralising antibodies against SARS-CoV-2 and are therefore "potentially" immune.
- Performing population-based epidemiological studies to better understand the incidence and spread of the virus.
- Evaluating the effectiveness of any potential vaccine.

[1] https://www.who.int/es/emergencies/diseases/novel-coronavirus-2019

[2] Suthar MS, Zimmerman M, Kauffman R, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. *Cell Reports Medicine*, vol. 1, 3 (2020). https://doi.org/10.1016/j.xcrm.2020.100040

[3] Premkumar, Lakshmanane et al. "The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients." *Science immunology* vol. 5,48 (2020). https://doi.org/10.1126/sciimmunol.abc8413

2. OBJECTIVE

To evaluate the sensitivity and specificity of the COVID-19 IgG/IgM Rapid Test Cassette of BIOLAN Health S.L. for detecting SARS-CoV-2 neutralising antibodies in clinical samples.

3. METHOD

3.1. Sample type

53 serum samples from subjects diagnosed by PCR as COVID-19 positive and COVID-19 negative, provided by the Basque Biobank, and 14 samples from Access Biologicals' seroconversion panel (CVD19SCP), were used.

3.2. Assay Test

BIOLAN HEALTH's COVID-19 IgG/IgM Rapid Test Cassette was used.

3.3. Experimental method for clinical evaluation of the test

The sensitivity of the method for detecting IgG and IgM antibodies against SARS-CoV-2 was determined in samples of COVID-19 positive patients confirmed by PCR, and the specificity was determined in samples of COVID-19 negative patients confirmed by PCR.

First, the positive, negative and overall percent agreement were calculated by comparing the results obtained with the tested product and the ones obtained with the reference test (PCR).

- Positive Percent Agreement (specificity): the percent of observed agreement on positive values between the tested product and the reference test.
- Negative Percent Agreement (sensitivity): the percent of observed agreement on negative values between the tested product and the reference test.
- Overall Percent Agreement: the percent of observed agreement on total values between the tested product and the reference test.

To ensure the accuracy of the product and its applicability in clinical practice, the Positive, Negative and Overall Percent Agreement must be greater than 90%.

Method		Reference Test		Total
		Positive	Negative	
Tested product	Positive	a	b	a+b
	Negative	c	d	c+d
Total		a+c	b+d	a+b+c+d

In general, the formula used to calculate the degree of positive/negative/overall agreement is:

- Positive Percent Agreement = a/(a + c)*100%
- Negative Percent Agreement = d/(b + d)*100%
- Overall Percent Agreement = (a + d)/(a + c + b + d)*100%

If the percent agreement meets the clinical requirements, the two methods are considered equivalent.

Finally, a seroconversion panel of 14 samples provided by Access Biologicals (CVD19SCP) was analysed using the COVID-19 IgG/IgM Rapid Test Cassette from BIOLAN HEALTH and the Negative and Positive Percent Agreement of the tested product was determined by comparing the results obtained with the ones reported by 4 commercial kits for detecting SARS-CoV-2 antibodies.

4. RESULTS

4.1. Clinical agreement

Among the 53 samples included in the study, 23 were from patients confirmed as COVID-19 positive by PCR, and 30 were from patients confirmed as COVID-19 negative. The results obtained using the COVID-19 IgG/IgM Rapid Test Cassette are shown below:

Table 1. Results of the PCR and the COVID-19 IgG/IgM Rapid Test Cassette.

Sample No.	Reference Method PCR	BIOLAN HEALTH COVID-19 IgG/IgM Rapid Test Cassette	
		Anti-SARS-CoV-2 IgM	Anti-SARS-CoV-2 IgG
1	+	+	+

2	+	-	-
3	+	+	+
4	+	+	+
5	+	+	-
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	+	+	+
12	+	+	+
13	+	+	+
14	+	+	+
15	+	+	+
16	+	+	+
17	+	+	+
18	+	+	+
19	+	+	+
20	+	+	+
21	+	+	+
22	+	+	+
23	+	+	+
24	-	-	-
25	-	-	-
26	-	-	-
27	-	-	-
28	-	-	-
29	-	-	-
30	-	-	-
31	-	-	+
32	-	-	-
33	-	-	-
34	-	-	-
35	-	-	-
36	-	-	-
37	-	-	-
38	-	-	-
39	-	-	-
40	-	-	-
41	-	-	-
42	-	-	-
43	-	-	-
44	-	-	-

45	-	+	-
46	-	-	-
47	-	-	-
48	-	-	-
49	-	-	-
50	-	-	-
51	-	-	-
52	-	-	-
53	-	-	-

Table 2. Results obtained for anti-SARS-CoV-2 IgG antibodies.

Method		PCR		Total
		Positive	Negative	
BIOLAN HEALTH COVID-19 IgG/IgM Rapid Test Cassette	Positive IgG	21	1	22
	Negative IgG	2	29	31
Total		23	30	53

Table 3. Results obtained for anti-SARS-CoV-2 IgM antibodies.

Method		PCR		Total
		Positive	Negative	
BIOLAN HEALTH COVID-19 IgG/IgM Rapid Test Cassette	Positive IgM	22	1	23
	Negative IgM	1	29	30
Total		23	30	53

Table 4. Results obtained for anti-SARS-CoV-2 Total antibodies. (IgG and/or IgM).

Method		PCR		Total
		Positive	Negative	
BIOLAN HEALTH COVID-19 IgG/IgM Rapid Test Cassette	Positive IgG and/or IgM	22	2	24
	Negative IgG & IgM	1	28	29
Total		23	30	53

Table 5. Percent Agreement for anti-SARS-CoV-2 IgG, IgM and Total antibodies.

	IgG	IgM	Total
Percent of Negative Agreement (%)	96,67	96,67	93,33
Percent of Positive Agreement (%)	91,3	95,65	95,65
Percent of Overall Agreement (%)	94,34	96,23	94,34

Table 6. Sensitivity for anti-SARS-CoV-2 IgG, IgM and Total antibodies.

	IgG	IgM	Total
Sensitivity (%)	91,3	95,65	95,65
95%CI	90,24 - 100%	87,31 - 100%	87,31 - 100%

Table 7. Specificity for anti-SARS-CoV-2 IgG, IgM and Total antibodies

	IgG	IgM	Total
Specificity (%)	96,67	96,67	93,33
95%CI	79,78 - 100%	90,24 - 100%	84,4 - 100%

Among the 23 positive samples included in the study, 22 samples were positive for IgM and 21 for IgG. On the other hand, among the 30 PCR-confirmed negative samples, 29 were negative for both, IgM and IgG.

As shown in Tables 6 and 7, the test had a **sensitivity of 95.6% and a specificity of 93.3% for the detection of total neutralising anti-SARS-CoV-2 antibodies.** Both, the positive and the negative percent agreement (sensitivity and specificity) and the overall percent agreement, are above 90%, demonstrating that the test meets the requirements for its application in clinical practice.

4.2. Seroconversion panel

This panel consists of 14 samples of human plasma collected in a longitudinal serie from a single donor during the progression of a SARS-CoV-2 infection over a period of time (87 days). The samples have been analysed with 4 commercial anti-SARS-CoV-2 IgG or IgG and IgM detection methods: LIAISON SARS-CoV-2 S1/S2 IgG assay by DiaSorin, Inc., SARS-CoV-2 IgG ELISA Test Kit by Gold

Standard Diagnostics, VITROS Anti-SARS-CoV-2 IgG test by Ortho-Clinical Diagnostics, Inc. and Anti-SARS-CoV-2 (IgG) ELISA kit by Grifols.

As shown in Table 8, the results obtained using the COVID-19 IgG/IgM Rapid Test Cassette starts providing positive results for IgG on the same collection day (50) as the other commercial kits. However, positive values for IgM appeared one day earlier (day 36) and up to one day later (day 87). The coloured bands that appeared for IgM on these two days (day 36 and day 87) are very weak and could indicate a very low concentration of these antibodies.

The Positive Percent Agreement of the COVID-19 IgG/IgM Rapid Test Cassette with commercial kits for the detection of anti-SARS-CoV-2 IgG or IgG and IgM antibodies is **100% for IgG, IgM and total antibodies.**

The Negative Percent Agreement of the COVID-19 IgG/IgM Rapid Test Cassette with commercial kits for the detection of anti-SARS-CoV-2 IgG or IgG and IgM antibodies is **100% for total antibodies and for IgG, and 78% for IgM.**

Table 8. Resuls from the seroconversion panel.

Panel member	Day	Gold Standard Daignostics[a]		Diasorin, Inc.[b]	Progenika Biopharma SA[c]	VITROS[d]		BIOLAN Health	
		SARS-CoV-2 IgG	SARS-CoV-2 IgM	LIASON SARS-CoV-2 S1/S2 IgG	Anti-SARS-CoV-2 (IgG)	Anti-SARS-CoV-2 IgG	Anti-SARS-CoV-2 TOTAL	Anti-SARS-CoV-2 IgG	Anti-SARS-CoV-2 IgM
1	1	0,6	1,5	8,34	0	0,01	4	-	-
2	3	0,50	1,10	8,22	0,00	0,01	0,07	-	-
3	10	0,40	1,40	8,36	0,00	0,01	0,05	-	-
4	15	0,50	1,50	8,14	0,00	0,01	0,05	-	-
5	17	0,50	0,90	7,79	0,00	0,01	0,05	-	-
6	24	0,40	1,90	8,32	0,00	0,01	0,05	-	-
7	31	0,40	1,30	8,63	0,10	0,01	0,06	-	-
8	36	0,40	2,50	6,18	0,10	0,01	0,53	-	+
9	50	22,70	33,50	20,80	2,80	2,16	17,89	+	+
10	64	39,40	21,40	40,90	6,00	9,14	41,18	+	+
11	71	30,80	15,10	45,10	6,90	7,53	47,66	+	+
12	78	27,00	11,50	42,60	4,80	6,96	56,74	+	+
13	80	25,90	10,70	37,70	4,50	5,65	53,57	+	+
14	87	20,70	7,20	32,30	3,90	4,98	62,12	+	+

[a]Units are qualitative. They are defined by the manufacturer; Units < 9.0 are considered negative; Units 9.0 – 11.0 are considered equivocal; Units > 11.0 are considered positive. [b]Arbitrary units (AU/mL) Units < 15.0 are considered negative; Units ≥ 15.0 are considered positive. [c] Units <0.8 are considered negative; units ≥ 0.8 to <1.1 are considered equivocal, units ≥ 1.1 are considered

positive. [d] (S/C) Signal-to-cutoff values. S/C < 1.0 is considered non-reactive for anti-SARS-CoV-2; S/C ~ 1.0 is considered reactive for anti - SARS-CoV-2.

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