Translation of the (Centre National de la Recherche, France) evaluation of the BIOSYNEX COVID-19 BSS (IgG/IgM) serological test.

Name of Kit: BIOSYNEX COVID-19 BSS (IgG/IgM)

Supplier: Biosynex

Detection: anti-SARS-CoV-2 IgM and IgG in whole blood, serum, plasma

Type of test: lateral flow immunochromatography

I. Evaluation of performance by comparison with the CNR (Centre National de la Recherche, France) reference data.

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OBJECTIVES

The objective of the evaluation is to test the performance of the above-mentioned test for the detection of antibodies against SARS-CoV-2 on sera qualified by the reference techniques used at the CNR of the Institut Pasteur.

The reference techniques selected for the present evaluation of the performance of the BIOSYNEX COVID-19 BSS (IgG/IgM) test are ELISA-type tests detecting total immunoglobulins (Ig tot) or IgG against SARS-CoV-2 N antigen.

LIMITATIONS OF THE EVALUATION

- The evaluation was performed on serum or plasma and does not prejudge performance on whole blood.

- Selectivity and in particular cross-reactivity with antibodies to other coronaviruses is not evaluated.

- Analytical sensitivity was evaluated on sequential dilutions of a limited number of plasma or sera.

SUPPLIER DETAILS (from the IFU)

Detection: anti-SARS-CoV-2 IgM and IgG in whole blood, serum, plasma

Type of test: lateral flow immunochromatography

Volume used: 10µl

Reading time: 10 min (do not read after 20 minutes)

Performance: Vendor information from the IFU

Sensitivity and specificity: the study was performed by comparison with the clinical diagnosis of COVID-19 confirmed by RT-PCR. It involved 446 samples for IgG and 456 samples for IgM. The results are summarized in the table below.

IgM Results over 81 positive and 375 negative patients are:

Sensitivity: 91.8 %(95%CI: 83.8%-96.6%)*	Specificity: 99.2 %(95%CI: 97.7%~99.8%)*
Accuracy: 97.8%(95%CI: 96.0%~98.9%)*	*Confidence interval

IgG Results over 77 positives and 369 negative patients are:

Sensitivity: 100% (95%CI: 96.1%~100%)*	Specificity: 99.5% (95%CI: 98.1%~99.9%)*
Accuracy: 99.6%(95%CI: 98.4%~99.9%)*	*Confidence Interval

Note that there is no information regarding the date after symptom onset for the sera of COVID-19 + patients included in this study.

Cross-reactivity

Cross-reactivity was studied on a total of 68 specimens and showed an absence of cross-reactivity for a wide range of antibodies against a variety of viral or bacterial pathogens: influenza A virus, influenza B virus, RSV, Adenovirus, HBsAg, Syphilis, H. Pylori, HIV, HCV or for HAMA.

A slight cross-reactivity was observed for rheumatoid factor.

For other coronaviruses, slight cross-reactivity was observed with SARS-CoV-1 antibody positive specimens and the possibility of cross-reactivity with MERS-CoV positive specimens is indicated.

Cross-reactivity with antibodies to seasonal coronavirus HKU1, OC43, NL63, 229E has not been formally studied, independent of the study of sera from COVID19 negative subjects.

Interference

Various potentially interfering substances were tested and none showed interference in the test at the concentration evaluated (list provided in the supplier's package insert).

MATERIALS AND METHODS

Panel of tested sera:

- COVID-19+ patient sera positive for RT-PCR collected at different times after symptom onset (n=43)

- Pre-pandemic sera (n = 40)

- Plasma/serum dilutions (n=36)

- TOTAL: n=119

CNR Reference Techniques for Sera Qualification

Indirect ELISA, SARS-CoV-2 N antigen, total Ig detection LuLISA, SARS-CoV-2 N antigen, IgG detection

The positivity threshold for dilution was established at ≈ 490 pre-epidemic sera

Sensitivity has been evaluated for ≈ 250 COVID-19+ patient sera (RT-PCR positive) ELISA N

Total Ig: specificity 99%, sensitivity 78%; specificity 95%, sensitivity 81% LuLISA N IgG: specificity 99%, sensitivity 84%; specificity 95%, sensitivity 94

Indirect ELISA, SARS-CoV-2 N antigen, IgM detection (for part of the sera)

Pseudo-neutralization test using lentiviral pseudo-types carrying the SARS-CoV-2 S-spicule protein (for part of the sera)

Kit technique evaluated according to the supplier's instructions

Test sample: 10 µL serum or plasma

Reading time : 10 min

RESULTS

Instructions for use and operation

Instructions for use: as intended

Test interpretation: easy to read

Validation of controls: 100%.

Comparison of results

The characteristics of the 96 sera selected for analysis are shown in Table I-1.

	Patients	Number of serums	Total serums
COVID19+	4	3	12
	6	2	12
	19	1	19
sub-total	*18*		*43*
Pre pandemic	40	1	40
Plasma dilutions	2		17
Serum Dilutions	2		19
Total Samples	105		96

Table I- 1: *Origin of the serums and plasmas used*

For COVID-19+ patients, the results by symptom onset date and overall concordance are summarized in Table I-2 for total Ig

Days	**Total**	**POS CNR**	**%POS CNR**	**POS KIT**	**%POS KIT**	**TP**	**FP**	**FN**	**TN**	**Concordance (%) POS**	**NEG**	**TOT**
0-5	1	1	100%	1	100%	1	0	0	0	100%	-	100%
6-10	3	3	100%	2	67%	2	0	1	0	67%	-	67%
11-15	13	13	100%	12	92%	12	0	1	0	92%	-	92%
16-20	9	9	100%	9	100%	9	0	0	0	100%	-	100%
>20	10	10	100%	10	100%	10	0	0	0	100%	-	100%
sub-total	*36*	*36*	*100%*	*34*	*94%*	*34*	*0*	*2*	*0*	*94%*	*-*	*94%*
unknown	7	7	100%	6	86%	6	0	1	0	86%	-	86%
Total	**43**	**43**	**100%**	**40**	**93%**	**40**	**0**	**3**	**0**	**93%**	**-**	**93%**
Pre pandemic	40	0	0%	0	0%	0	0	0	40	-	100%	100%
TOTAL	**83**	**43**	**1**	**40**	**93%**	**40**	**0**	**3**	**40**	**93%**	**100%**	**96%**

Table I-2: *Percentage positivity and concordance between the kit results (IgM+IgG) and the results of the CNR reference test (Igtot/IgG): .TP, true positive; FP, false positive; FN, false negative; TN, true negative.*

For total IgM/IgG, overall, the kit sensitivity is 93% and concordance was found for 93% (40/43) of the positive sera and 100% (40/40) of the negative sera.
In COVID-19+ patients the concordance is 93% (40/43).

False negatives were observed for sera from COVID-19+ patients relatively early (D10; D13) after symptom onset. It should be noted that the serum at D10 was strongly positive in Igtot/IgG but negative in the pseudo-neutralization test used by the CNR.
No false positives were observed among the sera tested showing excellent specificity.

For IgM, the sensitivity evaluated on sera from COVID-19+ patients is 91%. Negative results were obtained with a serum at D10 that was also low in Igtot/IgG, a serum at D13 that was also a false negative in IgG, and a late serum at D29 that was clearly positive in Igtot/IgG.

Concordance could be analyzed for 11 sera qualified for the presence of IgM, the distribution of which according to days after symptom onset is given in Table I-3 below.

Days	Total	POS CNR IgM
0-5	1	**1**
6-10	0	**0**
11-15	1	**1**
16-20	2	**2**
>20	1	**1**
sub-total	***5***	***5***
unknown	6	**6**
Total	**11**	**11**

Table I-3: *Breakdown by symptom onset date of sera tested for IgM concordance.*

The concordance was 91% (10/11) for positive sera. The false negative was for a COVID-19+ patient serum with unknown onset of symptoms also found to be IgG negative by the Biosynex test, whereas it was positive for both CNR N protein tests.

A subset of sera was also tested for pseudo-neutralization using lentiviral pseudotypes carrying the SARS-CoV-2 S protein.

These sera (n=20; NEG 1; POS 19) were collected from COVID-19+ patients from D10 to D24 after symptom onset.

The concordance in COVID-19+ patients was 95% (18/19) positive and 1/1 (100%) negative.

The false negative was a serum at D13 which is also a false negative for IgG, and negative for IgM. It is possible that there may have been negative interference with the Biosynex test for this serum which is not observed with the CNR tests.

Plasma Dilution Analysis:

In an initial approach to analytical sensitivity, plasma and serum dilutions were used (see Table I-3).

Plasma	Dilution	Igtot CNR	IgM CNR	IgG Kit	IgM Kit
A	2	POS	POS	POS	NEG
	4	POS	POS	POS	NEG
	8	POS	NEG	POS	NEG
	16	POS	NEG	POS	NEG
	32	POS	NEG	POS	NEG
	64	NEG	NEG	POS	NEG
	128	NEG	NEG	POS	NEG
	256	NEG	NEG	POS	NEG
B	2	POS	NEG	POS	POS
	4	POS	NEG	POS	POS
	8	POS	NEG	POS	POS
	128	NEG	NEG	POS	POS
	256	NEG	NEG	POS	NEG
	512	NEG	NEG	NEG	NEG
	1024	NEG	NEG	NEG	NEG
	2048	NEG	NEG	NEG	NEG
	4096	NEG	NEG	NEG	NEG

Serum	Dilution	Igtot CNR	IgM CNR	IgG Kit	IgM Kit
1	8	POS	POS	POS	POS
	16	POS	POS	POS	POS
	32	POS	NEG	POS	POS
	64	POS	NEG	POS	POS
	128	POS	NEG	POS	POS
	256	POS	NEG	POS	POS
	512	POS	NEG	POS	NEG
	1024	NEG	NEG	NEG	NEG
	2048	NEG	NEG	NEG	NEG
	4096	NEG	NEG	NEG	NEG
2	4	POS	POS	POS	POS
	8	POS	POS	POS	NEG
	16	POS	NEG	POS	NEG
	32	POS	NEG	NEG	NEG
	64	POS	NEG	NEG	NEG
	128	POS	NEG	NEG	NEG
	256	NEG	NEG	NEG	NEG
	512	NEG	NEG	NEG	NEG
	1024	NEG	NEG	NEG	NEG

Table I-4: *Comparison of Analytical Sensitivity on Plasma Dilutions*

For IgG, the sensitivity of the kit appears to be slightly higher than the CNR total Ig ELISA on plasma dilutions while it is comparable or slightly lower on serum dilutions. Note that the plasma and serum dilutions were not tested with the more sensitive LuLISA N IgG technique which was taken into account for all other analyses.

For IgM, significant variability is noted between serums and plasmas making interpretation difficult. In addition, the comparison of a purely qualitative test (kit) and a semi-quantitative test (N ELISA) would require the analysis of more samples around the detection limit.

Overall, based on this analysis, the sensitivity of the Biosynex test for both IgG and IgM appears satisfactory.

II. Summary of the performance evaluation carried out by other laboratories

Elements from two other studies are summarized here.

Study 1:

It involved sera from COVID-19+ patients at D0 (n=42) or D8 (n=13) of hospitalization (potentially later from the date of symptom onset) and from cured patients (n=22).

This study shows that the sensitivity for both IgM and IgG reaches 100% for the sera of patients at D8 of hospitalization. Overall a better sensitivity for IgM than for IgG is noted in cured patients, but the date after the onset of symptoms is not known, nor the criteria for cure.

The specificity evaluated in COVID-19 negative control patients (n=19) is in the order of 95%.

Study 2:

This study of about 60 COVID-19+ patients in RT-PCR collected at different times after the onset of symptoms shows a faster increase in sensitivity for IgM than for IgG depending on the date after the onset of symptoms, which reaches a value of about 85% after 15 days after the onset of symptoms.

The specificity evaluated is 100% with the exception of a noted interference with the rheumatoid factor. No cross-reactivity was found with antibodies to other respiratory viruses tested, including seasonal coronaviruses.

Information from an international study reports a sensitivity for sera of COVID-19+ patients collected beyond 15 days after symptom onset of approximately 95% and a specificity of 100% for IgG but lower for IgM.

OVERALL CONCLUSION

Overall, the performance evaluation of the BIOSYNEX COVID-19 BSS (IgG/IgM) Assay in serum or plasma indicates that:

- Specificity is very good for IgG (95-100%) according to various studies. It is also good for IgM although it seems to be slightly lower than for IgG. Overall, the specificity data is in line with the performance claims of the supplier.

- The overall sensitivity for detection of IgG/IgM in COVID-19+ subjects is 93% for IgG+IgM. It increases with time to onset of symptoms and reaches 100% beyond 15 days in agreement with other studies.
- The detection of IgM is somewhat less performant as also noted in other studies with an overall sensitivity of 91% in COVID-19+ subjects which also increases with the time since onset of symptoms.
- A point of attention regarding the reading of the control to validate the kit reading was noted.

As a qualitative test, the BIOSYNEX COVID-19 BSS (IgG/IgM) Test does not provide an indication of the level of antibodies detected and therefore the level of immunity. Furthermore, it should be pointed out that at the present state of knowledge there are no data on correlates of immunity.

Done in Paris, 26 April 2020

Translation of “RAPPORT D’EVALUATION DE LA PERFORMANCE POUR LA DETECTION DES ANTICORPS ANTI SARS-COV-2, BIOSYNEX COVID-19 BSS (IgG/IgM) » by Pr Sylvie Van Der Werf, Dr Sylvie Behillil, and Dr Vincent Enouf, 26 April 2020, Paris