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Frontiers in Public Health, section Infectious Diseases – Surveillance, Prevention and Treatment

Recent advances in the evaluation of serological assays for the diagnosis of SARS-CoV-2 infection and COVID-19

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The use of a unique and large clinical samples panel to perform the head-to-head comparison of the different serological assays is the strength of our study.

A total of **337 plasma** samples collected in the period April-June 2020 fromSARS-CoV-2 RT-PCR positive (n=207) and negative (n=130) subjects were investigated.

We evaluated sensitivity and specificity of five different widely used commercial serological assays for the detection of SARS-CoV-2–specific IgG, IgM and IgA antibodies using reverse transcriptase-PCR assay in nasopharyngeal swab as reference standard test.

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Table 1. Overall sensitivity and specificity of the serological assays for the diagnosis of SARS-CoV-2 infection using RT-PCR as reference standard.

Serological assays			No. of samples	RT-PCR positive	RT-PCR negative	True Positive	False Positive	True Negative	False Negative	Sensitivity % (95%CI)	Specificity % (95%CI)
IgG	LFIA	POCT	336	206	130	173	1	129	33	84.0 (78.2– 88.7)	99.2 (95.8 – 100)
	CLIA	iFlash	337	207	130	186	2	128	21	89.9 (84.9 – 93.6)	98.5 (94.6 – 99.8)
		LIAISON® XL	337	207	130	169	3	127	38	81.6 (75.7 – 86.7)	97.7 (93.4 – 99.5)
	ECLIA	Elecsys®	337	207	130	179	0	130	28	86.5 (81.0 – 90.8)	100 (97.2 – 100)
IgM	LFIA	POCT	336	206	130	98	2	128	108	47.6 (40.6 – 54.6)	98.5 (94.6 – 99.8)
	CLIA	iFlash	337	207	130	113	5	125	94	54.6 (47.5 – 61.5)	96.2 (91.3 – 98.7)
IgA	ELISA	EUROIMMUNE I	294	185	109	156	20	89	29	84.3 (78.3 – 89.2)	81.7 (73.1 – 88.4)

RT-PCR: reverse transcriptase-PCR;CI: confidence interval.

LFIA	PoCT IgG	Proteina N
CLIA	iFlash IgG	Proteine N & S
	LIAISON® XL IgG	Proteine ricombinanti S1 & S2
ECLIA	Elecsys Ig tot	Proteina N
LFIA	PoCT IgM	Proteina N
CLIA	iFlash IgM	Proteine N & S
ELISA	EUROIMMUNE IgA	Subunità S1

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Table 2. Sensitivity and specificity of the serological assays for the diagnosis of SARS-CoV-2 infection in symptomatic and asymptomatic individuals.

Symptomatic individuals											
Serological assays			No. of samples	RT-PCR positive	RT-PCR negative	True Positive	False Positive	True Negative	False Negative	Sensitivity % (95%CI)	Specificity % (95%CI)
IgG	LFIA	POCT	283	187	96	160	1	95	27	85.6 (79.7 – 90.3)	99.0 (94.3 – 100)
	CLIA	iFlash	284	188	96	170	2	94	18	90.4 (85.3 – 94.2)	97.9 (92.7 – 99.7)
		LIAISON® XL	284	188	96	154	2	94	34	81.9 (75.7 – 87.1)	97.9 (92.7 – 99.7)
	ECLIA	Elecsys®	284	188	96	164	0	96	24	87.2 (81.6 – 91.6)	100 (96.2 – 100)
IgM	LFIA	POCT	283	187	96	94	2	94	93	50.3 (42.9 – 57.6)	97.9 (92.7 – 99.7)
	CLIA	iFlash	284	188	96	107	5	91	81	56.9 (49.5 – 64.1)	94.8 (88.3 – 98.3)
IgA	ELISA	EUROIMMUNE I	244	168	76	144	19	57	24	85.7 (79.5 – 90.6)	75.0 (63.7 – 84.2)
Asymptomatic individuals											
Serological assays			No. of samples	RT-PCR positive	RT-PCR negative	True Positive	False Positive	True Negative	False Negative	Sensitivity % (95%CI)	Specificity % (95%CI)
IgG	LFIA	POCT	53	19	34	13	0	34	6	68.4 (43.4 – 87.4)	100 (89.7 – 100)
	CLIA	iFlash	53	19	34	16	0	34	3	84.2 (60.4 – 96.6)	100 (89.7 – 100)
		LIAISON® XL	53	19	34	15	1	33	4	78.9 (54.4 – 93.9)	97.1 (84.7 – 99.9)
	ECLIA	Elecsys®	53	19	34	15	0	34	4	78.9 (54.4 – 93.9)	100 (89.7 – 100)
IgM	LFIA	POCT	53	19	34	4	0	34	15	21.1 (6.0 – 45.6)	100 (89.7 – 100)
	CLIA	iFlash	53	19	34	6	0	34	13	31.6 (12.6 – 56.6)	100 (89.7 – 100)
IgA	ELISA	EUROIMMUNE I	50	17	33	12	1	32	5	70.6 (44.0 – 89.7)	97.0 (84.2 – 99.9)

LFIA	PoCT IgG	Proteina N
CLIA	iFlash IgG	Proteine N & S
	LIAISON® XL IgG	Proteine ricombinanti S1 & S2
ECLIA	Elecsys Ig tot	Proteina N
LFIA	PoCT IgM	Proteina N
CLIA	iFlash IgM	Proteine N & S
ELISA	EUROIMMUNE IgA	Subunità S1

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Table 3. Sensitivity of the serological assays for the diagnosis of SARS-CoV-2 infection by onset of symptoms.

Serological assays			Time elapsed from symptoms onset and blood sample collection					
			≤ 14 days			> 14 days		
			No. of RT-PCR positive	True Positive	Sensitivity (95%CI)	No. RT-PCRpositive	True Positive	Sensitivity (95%CI)
IgG	LFIA	POCT	65	48	73.8 (61.5 – 84.0)	122	112	91.8 (85.4–96.0)
	CLIAs	iFlash	65	52	80 (68.2 – 88.9)	123	118	95.9 (90.8–98.7)
		LIAISON® XL	65	46	70.8 (58.2 – 81.4)	123	108	87.8 (80.7–93.0)
	ECLIA	Elecsys®	65	47	72.3 (59.8 – 82.7)	123	117	95.1 (89.7–98.2)
IgM	LFIA	POCT	65	23	35.4 (23.9 – 48.2)	122	71	58.2 (48.9–67.1)
	CLIA	iFlash	65	35	53.8 (41 – 66.3)	123	72	58.5 (49.3–67.3)
IgA	ELISA	EUROIMMUNE I	57	39	68.4 (54.8–80.1)	111	105	94.6 (88.6–98.0)

RT-PCR: reverse transcriptase-PCR;CI: confidence interval.

LFIA	PoCT IgG	Proteina N
CLIA	iFlash IgG	Proteine N & S
	LIAISON® XL IgG	Proteine ricombinanti S1 & S2
ECLIA	Elecsys Ig tot	Proteina N
LFIA	PoCT IgM	Proteina N
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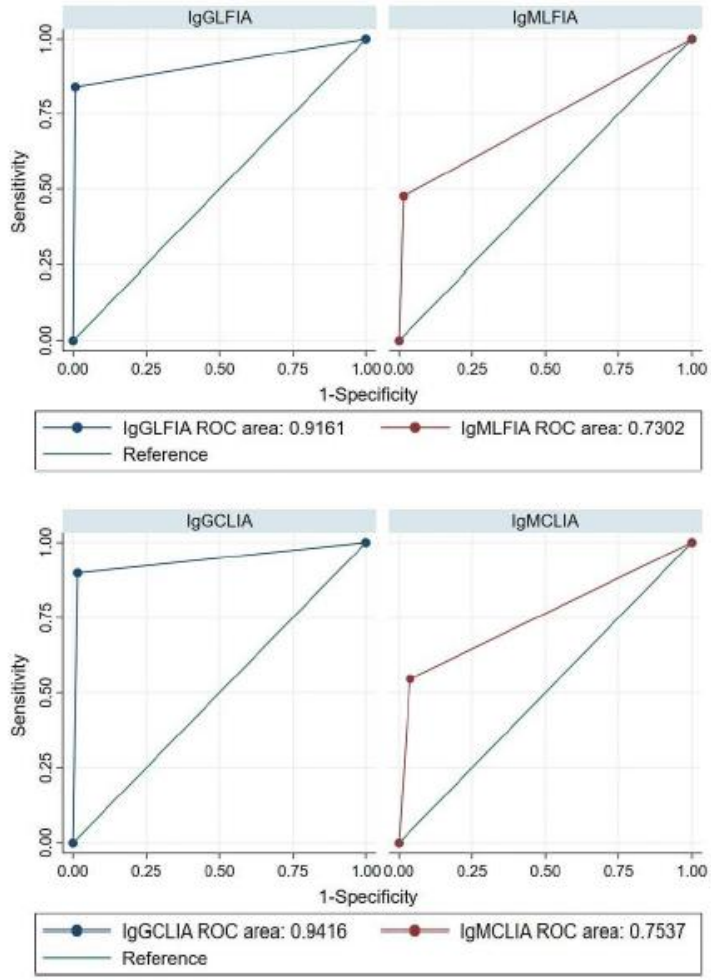
Figure 1. Receiver operating characteristic (ROC) curves for the diagnosis of SARS-CoV-2 infection by IgG and IgM LFIA (A) and CLIA-iFlash (B) using RT-PCR as reference standard. ROC area IgG vs IgM LFIA, p<0.0001; ROC area IgG vs IgM CLIA-iFlash, p<0.0001.



IgG serological assays seem to be a reliable tool for the diagnosis of SARS-CoV-2 infection.

IgM assays seem to have a low sensitivity and

IgA assay is limited by a substantial rate of indeterminate results.

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- ✓ the high number of false-negatives obtained by the IgM assays seems to limit the use of IgM detection as a marker of acute infection

- ✓ the high number of indeterminate results obtained by ELISA IgA makes it difficult to clearly define the application area of the search of this class of immunoglobulin.

- ✓ the very good analytical performances in terms of sensitivity, particularly in sera from convalescent phase, and specificity observed for the fully automated high throughput assays for IgG detection, indicate that the search of IgG may represent a reliable tool for epidemiological serosurveys and for retrospective diagnosis of SARS-CoV-2 infection in targeted populations.

- ✓ the ability of the serological assays to detect antibodies in probable COVID-19 group, serological testing could be an important complement to molecular assay for the diagnosis of SARS-CoV-2 infection in this type of patients.

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- ✓ Future studies are certainly required since many questions remain currently unanswered such as the role, pathogenic or protective, of antibody responses during infection,
- ✓ how long antibodies persist after infection and
- ✓ if the infection results in an immune response that protects individuals from future infections or illness.

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