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Reciew

Diagnostic performance of serological assays in the detection of SARS-CoV-2.

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Abstract: *Introduction*. The gold-standard method for the diagnosis of the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or COVID-19) foresees the examination of respiratory tract swabs by real-time reverse-transcription polymerase chain reaction (rRT-PCR). Another group of diagnostic tests, developed to overcome the limitations of RT-PCR includes the serological assays, which have the purpose to detect the antibody response to SARS-CoV-2 infection (IgM and IgG titers). The aim of this review was to establish the diagnostic capability of the existing serological tests in the detection of COVID-19 infection. *Materials and Methods*. Electronic research was conducted in PubMed, Scopus, Science Direct and Cochrane Library, and only 10 articles, testing 10 different types of serological assays, met the inclusion criteria and were consequently submitted to quality assessment and data extraction. Quantitative data about the sensitivity, specificity, positive/negative predictive value and IgM/IgG titer provided by each antibody test were reported in our review. *Results*. Almost all the serological tests used in the included items were recorded to ensure high sensitivity and specificity, identifying the presence of IgM and IgG antibodies against SARS-CoV-2 in patients with COVID-19 certain diagnosis (confirmed by RT-PCR), and in participants with suspected infection (SARS-CoV-2 clinical diagnosis and/or RT-PCR negative subjects). *Conclusion*. Serological tests may represent reliable diagnostic tools in the detection of SARS-CoV-2 infection and they should be implemented complementary to real-time RT-PCR.

Keywords: Sars-Cov-2; COVID-19; RT-PCR; Serological assay; infection

1. Introduction

The current global pandemic caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 or COVID-19), whose initial outbreak was detected in December 2019 in Wuhan (China), represents a real threat to international health [1,2,3]. This new pathogen, which is an enveloped, non-segmented, positive sense RNA virus, belongs to the Coronaviridae family, as well as the Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) [4,5]. Fever, cough expectoration, myalgia, fatigue, dyspnea and gastrointestinal symptoms are the most frequent clinical manifestations induced by SARS-CoV-2 [6,7,8]. In some cases, the progression of the illness results to be relatively asymptomatic, while in other cases pneumonia, acute respiratory distress syndrome (ARDS), sepsis or septic shock may occur [9,10]. The main COVID-19 transmission routes are represented by respiratory droplets

(within a long distance of 2 meters), generated by infected subjects during coughing and sneezing and by contact with contaminated surfaces [11,12,13,14]. No specific antiviral therapy against COVID-19 has been introduced yet: treatment protocols include broad-spectrum antiviral drugs (remdesivir, lopinavir, ritonavir, favipiravir), which should be administrated in the early stage of the infection and antimalarial/autoimmune disease drugs (chloroquine, hydroxychloroquine) [15,16,17]. In order to limit the spread of the infection among the population, isolating the infected persons, adequate diagnostic strategies should be implemented. The most commonly method used to detect SARS-CoV-2 foresees the analysis of nasopharyngeal and throat swabs by real-time reverse-transcription polymerase chain reaction (rRT-PCR) test [18,19,20]. A complementary diagnostic procedure is represented by the chest computerized tomography (TC), tool that may allow the detection of COVID-19 in rRT-PCR false-negative cases. Even if real-time RT-PCR plays a crucial role in SARS-CoV-2 diagnosis, it presents several limitations: it could not be able to identify the virus in the early stage of the infection, it requires a long time to obtain the results, which may be influenced by external factors, such as sampling operation method, performance of detection kits and nucleic acid extraction from clinical material procedure [21,22]. Another group of diagnostic tests used for SARS-CoV-2 identification is that of serological assays. Several different antibodies tests have been developed, which differ depending to the targeted viral antigen (for example nucleoprotein or spike protein); the most common searched biomarkers for COVID-19 detection are IgM and IgG antibodies [23]. The purpose of this type of tests may be the identification of PCR-negative cases and asymptomatic patients or the evaluation of vaccine response during clinical trials [24]. According to the study by Li-Xin Xie [25], in most cases IgM antibodies result to be present 3-5 days after the onset of symptoms while IgG titer is higher in the recovery phase than in the acute one. Patel et al. [26] reported that seroconversion in infected patients may occur between 7 and 11 days after the exposure to the virus. Due to the delayed appearance of IgM and IgG antibodies, serological tests result to be unreliable in the acute phase of the infection. The Infectious Diseases Society of America stated that serological tests still remain clinically unverified and suggested that serology results alone should not be used for diagnostic decisions [27]. Although no clear evidence about the duration of the immunity protection against SARS-CoV-2 have been recorded, the data showed in the research by Bao et al. [28] demonstrated that the primary COVID-19 infection in monkeys may generate an immunity response, which could be able to protect from subsequent exposures.

1.1. Objectives

The aim of this research was to review literature in order to obtain an overview about the existing serological tests for the detection of SARS-CoV-2 infection and to establish their reliability in the diagnosis of this new pathogen.

1.2. Clinical Question (PICO)

- P: SARS-CoV-2 serological tests
- I: effectiveness of antibodies tests in the diagnosis of COVID-19 infected patients
- C: comparison between rRT-PCR tests and serological tests as diagnostic tools for SARS-CoV-2 infection, evaluation of their advantages and disadvantages
- O: overview about the existing serological tests for the diagnosis of COVID-19 infection and evaluation of their reliability

2. . Materials and Methods

2.2.1. Protocol and Registration

In order to provide a transparent and complete protocol for systematic reviews, the PRISMA statement [29] was followed for methods and inclusion criteria selection.

2.2.3. Eligibility Criteria

2.2.4. Search

In order to select the items concerning the utilization of antibodies tests to diagnose COVID-19 infected patients, we conducted an electronic research on PubMed, Scopus, Science Direct and Cochrane Library databases, analyzing papers published by April 2020. No restrictions were imposed with regards to demographics and clinical characteristics of the included patients (age, gender, comorbidities) and both articles with or without negative control groups were considered. Only studies written in English language were selected. We combined the following keywords with the Boolean term "AND": "Serological test", "COVID-19", "Antibodies" and "Immune system".

2.2.5. Study Selection and Data Collection Process

Following the inclusion criteria, eligible studies for this review were selected by two researchers (G.M., D.L.), who independently examined title, abstract and full text of each article found during the electronic searching. The same researchers performed data extraction from the selected items: number of the enrolled patients/blood samples, patients and blood samples source, diagnostic tool used to confirm the presence of SARS-CoV-2 infection in the included patients, type of serological assay that was tested and quantitative data about the sensitivity, specificity, positive/negative predictive value and IgM/IgG titer provided by each antibody test. Only articles that used real-time RT-PCR as diagnostic tool to confirm the positivity to SARS-CoV-2 were considered and rates and percentages were used for the principal outcome measures. The flow chart used for this review is shown in Figure 1.

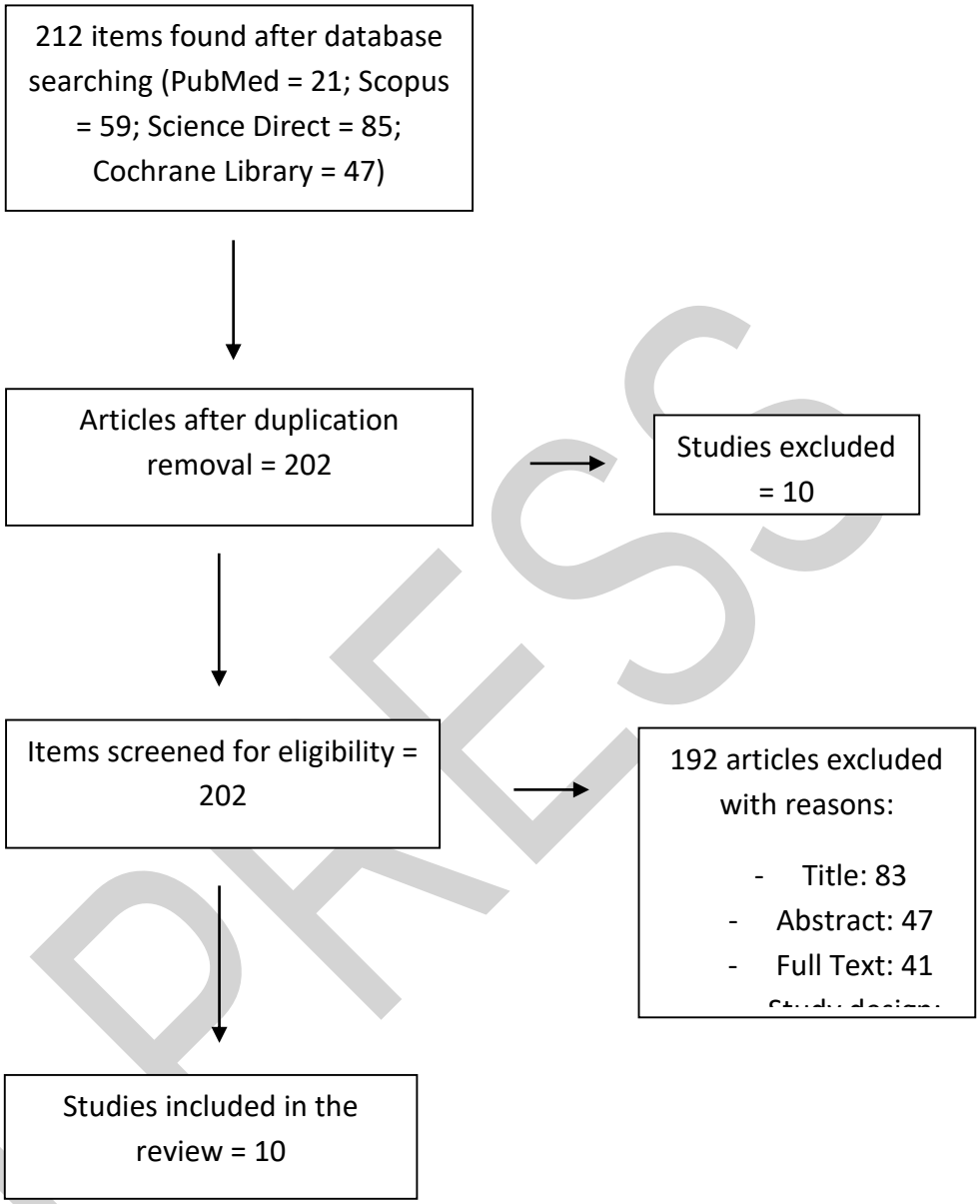

Figure 1. Flow chart of the items assessment.

133 *2.2.6. Quality Assessment*

134 Quality assessment of the selected items was investigated using the Newcastle-Ottawa scale
135 (NOS) [30], recording an high quality level of the included researches: the studies average quality
136 was equal to 6.1, while the highest score was equal to 7 and the lowest one was 5 (Table 1). The
137 majority of studies included case and control groups, in which data were recorded using the same
138 methodology. The presence of SARS-CoV-2 infection in the cases of all the selected articles was
139 confirmed following the guidelines for diagnosis of COVID-19 [31].

140 **Table 1.** Quality evaluation of the included articles.

Studies	Definition of Cases	Representativeness of Cases	Selection of Controls	Definition of Controls	Comparability	Exposure	Total
Cassaniti et al. 2020	+	+	+	+	+-	++-	7
Infantino et al. 2020	+	+	+	+	+-	+--	6
Jin et al. 2020	+	+	+	+	+-	++-	7
Lee et al. 2020	+	+	+	+	+-	++-	7
Li et al. 2020	+	+	+	+	+-	++-	7
Pan et al. 2020	+	+	-	-	+-	++-	5
Xiang et al. 2020	+	+	+	+	+-	++-	7
Yongchen Et al. 2020	+	+	-	-	+-	++-	5
Zhang et al. 2020	+	+	-	-	+-	++-	5
Zhao et al. 2020	+	+	-	-	+-	++-	5

141 + = star assigned; - = star not assigned

142 **3. Results**

143 *3.1. Study Selection and Characteristics*

144 A total of 212 articles were identified after the electronic research in four different databases
145 (PubMed, Scopus, Science Direct, Cochrane Library). Ten studies were excluded based on duplication
146 removal and 202 items were assessed for eligibility. 192 articles were not included with reasons and
147 only 10 articles were included in this review: 83 studies were excluded after examining the title, 47
148 after analyzing the abstract, 41 after reading the full text, while 21 researches were not selected
149 because of their study design (reviews or case reports). The main characteristics of each selected
150 article are summarized in Table 2, which reports the number of enrolled patients and blood samples
151 and their source, the diagnostic tool performed to ascertain the positivity to SARS-CoV-2 (real-time
152 RT-PCR) and the type of serological assay whose diagnostic capability was evaluated. By adding the
153 participants of each study, a total of 1362 subjects were included in this review, of which 945 were
154 cases (COVID-19 positive patients, whose diagnosis was confirmed by real-time RT-PCR), 310 were
155 negative controls and 107 were subjects with suspected infection (SARS-CoV-2 clinical diagnosis,
156 real-time RT-PCR negative patients). Eight of the included articles were conducted in China, while
157 the remaining in Italy. Data about IgM and IgG titer, sensitivity, specificity, positive and negative
158 predictive value provided by each serological test were recorded in this review.

Table 2. List of the included items.

Study	Number of enrolled subjects/blood samples	Diagnosis of SARS-CoV-2	Patients/blood samples source	Serological test
Cassaniti et al. 2020	110 subjects: - 30 healthy volunteers - 30 positive patients - 50 patients with fever and respiratory syndrome	Respiratory samples tested by real-time RT-PCR	Fondazione IRCCS Policlinico San Matteo	VivaDiag COVID-19 IgM/IgG Rapid Test LFIA
Infantino et al. 2020	125 subjects: - 61 positive patients - 64 negative controls	Oropharyngeal and nasopharyngeal swabs tested by RT-PCR	San Giovanni di Dio Hospital (Florence, Italy)	iFlash1800 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China)
Jin et al. 2020	76 subjects: - 43 positive patients - 33 patients with suspected infection (control group)	Oral swab or sputum tested by real-time RT-PCR	XiXi Hospital of Hangzhou (Zhejiang Province, China)	iFlash3000 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China)
Lee et al. 2020	42 subjects: - 14 positive patients/33 serum samples - 28 negative controls/28 serum samples	Oropharyngeal and nasopharyngeal swabs, oral gargling and sputum tested by real-time RT-PCR for SARS-CoV-2	Enrolled patients were treated at six hospitals in Taiwan between January and March 2020	ALLTEST 2019-nCoV IgM/IgG Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd. Hangzhou, China)
Li et al. 2020	525 subjects: - 397 positive patients - 128 negative controls	Guideline for diagnosis and treatment of COVID-19	Eight hospitals and Chinese CDC agencies	SARS-CoV-2 rapid IgM/IgG combined antibody test (LFIA) kit designed and manufactured by Jiangsu Medomics Medical Technologies (Nanjing, China)
Pan et al. 2020	134 blood samples from 105 positive patients	Throat swab tested by real-time RT-PCR for SARS-CoV-2	Zhongnan Hospital of Wuhan University (Hubei, China)	Colloidal gold-based immunochromatographic (ICG) strip targeting IgM/IgG, conducted in Zhongnan Hospital of Whuan University (Hubei, China)
Xiang et al. 2020	169 subjects: - 85 positive	Nasopharyngeal and/or oropharyngeal swabs samples tested	Union Hospital, Tongji Medical College, Huazhong University of Science	ELISA kits, Livzon Inc, Zhuhai, P.R.China, lot number of IgM: 20200308, IgG: 20200308

Study	Number of enrolled subjects/blood samples	Diagnosis of SARS-CoV-2	Patients/blood samples source	Serological test
	patients/216 blood samples - 24 patients with suspected infection - 60 negative controls	by RT-PCR for SARS-CoV-2	and Technology (China)	
Yongchen et al. 2020	21 positive patients: - 11 non-severe - 5 severe - 5 asymptomatic	Throat swabs samples tested by realt-time RT-PCR for SARS-CoV-2	Second Hospital of Nanjing and Affiliated Hospital of Xuzhou Medical University (Jiangsu Province, China)	Gold immunochromatography assay supplied by Innovita Co., LTd, China (CFDA approved)
Zhang et al. 2020	16 positive patients	Oral, anal and blood samples tested by qPCR	Wuhan pulmonary hospital (China)	In-house anti-SARS-CoV IgG/IgM ELISA kits (using a cross-reactive nucleocapsid protein from another SARS-related virus Rp3, which is 92% identical to COVID-2019 nucleocapsid protein)
Zhao et al. 2020	535 serial plasma samples from 173 positive patients	Respiratory tract samples tested by real-time RT-PCR	Shenzhen Third People's Hospital (China)	ELISA kits supplied by Beijing Wantai Biological Pharmacy Enterprise Co.,Ltd,

CLIA = chemiluminescence immunoassay; *CoV* = coronavirus; *ELISA* = enzyme-linked immunosorbent assay; *HCoV* = human coronavirus; *LFIA* = lateral flow immunoassay; *MERS-CoV* = Middle East Respiratory Syndrome; *qPCR* = quantitative polymerase chain reaction; *RT-PCR* = reverse transcription-polymerase chain reaction; *SARS-CoV* = Severe Acute Respiratory Syndrome – Coronavirus;

*Positive patients: patients with confirmed diagnosis of SARS-CoV-2 by testing respiratory tract swabs with real-time RT-PCR

3.2. Results of Individual Studies

The data recorded in this review (Table 3, Table 4, Table 5, Table 6) referred to 10 different types of serological tests, which were performed in the included articles in order to establish whether the antibody response can be considered a reliable diagnostic tool for SARS-CoV-2 infection. The *VivaDiag COVID-19 IgM/IgG Rapid Test lateral flow immunoassay (LFIA)* was used in a sample of 110 patients in the study by Cassaniti et al. [32]: 19 out of 30 cases (63.3%) were positive for both IgM and IgG, 5 of them were weakly positive, while all negative controls were recorded to be negative for both antibodies. The same study also included 50 patients at their first access at emergency department, who were later tested for COVID-19 by RT-PCR, detecting 38 COVID-19 positive patients, among whom only 7 (18.4%) showed positivity for both IgM and IgG. The sensitivity and specificity of *VivaDiag* test were recorded to be 18.4% and 91.7% respectively, results that lead the authors not to recommend its use in the infection diagnostic process. Two of the included items performed the serological test using *iFlash1800* [33] and *iFlash3000* [34] *fully automated chemiluminescence immunoassay (CLIA) analyzer from Shenzhen YHLO biotechnology Co., Ltd (China)* respectively. *iFlash1800 CLIA analyzer* guaranteed an overall sensitivity of 75% and a specificity of 100% for IgG and 92.2% for IgM; *iFlash 3000 CLIA analyzer* reported, among the cases, 41 IgM/IgG positive (64.1%), 3 IgM positive

(16.7%) and 5 IgG positive patients out of 64, with a sensitivity of 48.1% (IgM) and 88.9% (IgG) and a specificity equal to 100% (IgM) and 90.9% (IgG). Lee et al. [35] used the *ALLTEST 2019-nCoV IgM/IgG Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd. Hangzhou, China)* to identify SARS-CoV-2 IgM and IgG in 14 COVID-19 positive patients (6 with symptoms and 8 without symptoms or with mild symptoms). All the symptomatic subjects showed IgG positivity (6/6), while 2 of them were IgM negative (2/6). Three of the asymptomatic patients had positive IgG but none of them had positive IgM. The sensitivity and specificity of the SARS-CoV-2 rapid IgM/IgG combined antibody test kit developed in the study by Li et al. [36] were 88.66% and 90.63% respectively, 256 out of 397 cases and 1 out 128 negative controls were positive for both IgM and IgG. By using a *colloidal gold-based immunochromatographic (ICG) strip*, Pan et al. [37] analyzed 108 blood samples, of which 86 were taken from COVID-19 positive patients and 22 from subjects with suspected infection (with negative RT-PCR): 55.8% and 54.7% of SARS-CoV-2 positive blood samples showed positivity to IgM and IgG respectively, while 36.4% and 59.1% of the blood samples taken from patients with suspected infection were positive for IgM and IgG respectively. In the research by Xiang et al. [38] the antibodies against SARS-CoV-2 were found with an *enzyme-linked immunosorbent assay (ELISA)*: of 66 positive participants 51 were IgM positive and 55 were IgG positive, 21 patients with suspected infection out of 24 were IgM positive and 17 IgG positive; negative controls (60) were all negative for IgM and only 3 were positive for IgG. The *gold immunochromatography assay supplied by Innovita Co., LTd, China (CFDA approved)* tested by Yongchen et al. [39] highlighted that among COVID-19 positive group (21 persons) all the symptomatic patients (17 subjects) were recorded to be seropositive during follow-up period. Zhang et al. [40] developed an in-house *anti-SARS-CoV IgG/IgM ELISA kit* (using a cross-reactive nucleocapsid protein from another SARS-related virus Rp3, which is 92% identical to COVID-2019 nucleocapsid protein), employing it to investigate the antibody response in 16 positive participants: this study demonstrated that IgM and IgG titers were low or undetectable in the day of first sampling (day 0), and that on day 5 an increase of viral antibodies could be observed in nearly all patients: IgM and IgG positive rate increased from 50% (8/16) to 81% (13/16) and from 81% (13/16) to 100% (16/16). The *ELISA kit supplied by Beijing Wantai Biological Pharmacy Enterprise Co.,Ltd* used by Zhao et al. [41] reported that 82.7% and 64.7% of 173 SARS-CoV-2 positive subjects were IgM and IgG positive respectively, ensuring a sensitivity equal to 66.7% in the early phase of the illness.

Table 3. Anti-SARS-CoV-2 IgM and IgG rates in the COVID-19 positive population sample.

Study	Total sample	IgM/IgG positive patients	IgM/IgG negative patients	IgM positive	IgG positive
Cassaniti et al. 2020	30	19/30 (63.3%) 5/30 (16.7%) weakly positive	5/30 (16.7%)	1/30 (3.3%): IgM positive and IgG negative	
Infantino et al. 2020	64	41/64 (64.1%)		3/64 (4.7%)	5/64 (7.8%)
Jin et al. 2020	27		3/27	13/27 (48%)	24/27 (88.9%)
Lee et al. 2020	6 with symptoms			4/6 (with symptoms)	6/6 (with symptoms)
	8 without symptoms/mild symptoms			0/8 (without symptoms)	3/8 (without symptoms)
Li et al. 2020	397	256/397		72/397	24/397
Pan et al. 2020	86 blood samples			48/86 (55.8%)	47/86 (54.7%)
Xiang et al. 2020	66			51/66 positive	55/66

Zhao et al. 2020	173		143/173 (82.7%)	112/173 (64.7%)

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213 **Table 4.** Anti-SARS-CoV-2 IgM and IgG rates in patients with suspected infection/fever + respiratory
214 syndrome (positivity to COVID-19 not confirmed by RT-PCR).

Study	Total sample	IgM/IgG positive patients	IgM/IgG negative patients	IgM positive	IgG positive
Cassaniti et al. 2020	50 patients at their first access at emergency department, later tested for COVID-19 by RT-PCR: - 12 negative - 38 positive	1 out of the 12 negative patients (8.3%) 7 out of the 38 positive patients (18.4%)	31 out of the 38 positive patients		
Pan et al. 2020	22 blood samples			8/22 (36.4%)	13/22 (59.1%)
Xiang et al. 2020	24			21/24	17/24

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216 **Table 5.** Anti-SARS-CoV-2 IgM and IgG rates in negative controls.

Study	Total sample	IgM/IgG positive patients	IgM/IgG negative patients	IgM positive	IgG positive
Cassaniti et al. 2020	30		30 (100%)		
Jin et al. 2020	33			0 (0%)	3 (9.1%)
Lee et al. 2020	28		28 (100%)		
Li et al. 2020	128	1/128		10/128	1/128
Xiang et al. 2020	60			0/60	3/60

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218 **Table 6.** Sensitivity, Specificity, Positive predictive value, Negative predictive values of serum IgM
219 and IgG antibodies to diagnose COVID-19.

Serological test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
VivaDiag COVID-19 IgM/IgG Rapid Test LFIA	18.4%**	91.7%**	87.5%**	26.2%**
iFlash1800 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China)	73.3% (IgM) 76.7% (IgG)	92.2% (IgM) 100% (IgG)	81.5% (IgM) 100% (IgG)	88.1% (IgM) 92.8% (IgG)
iFlash3000 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China)	48.1% (IgM)* 88.9% (IgG)*	100% (IgM)* 90.9% (IgG)*	100% (IgM)* 88.9% (IgG)*	70.2% (IgM)* 90.9% (IgG)*
ALLTEST 2019-nCoV IgM/IgG Rapid Test Cassette (Hangzhou ALLTEST Biotech Co., Ltd. Hangzhou, China)	90.9% (IgM)* 99.9% (IgG)*	97.0% (IgM)* 98.0% (IgG)*		

Serological test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
SARS-CoV-2 rapid IgM/IgG combined antibody test kit designed and manufactured by Jiangsu Medomics Medical Technologies (Nanjing, China)	88.66%*	90.63%***		
ELISA kits, Livzon Inc, Zhuhai, P.R.China, lot number of IgM: 20200308, IgG: 20200308	77.3% (IgM)* 83.3% (IgG)* 100% (IgM)** 96.6% (IgG)**	100% (IgM)* 94.8% (IgG)* 87.5% (IgM) 70.8% (IgG)	100% (IgM)* 83.8% (IgG)* 100% (IgM) 85.0% (IgG)	80.0% (IgM)* 88.9% (IgG)* 95.2% (IgM) 89.1% (IgG)
ELISA kits supplied by Beijing Wantai Biological Pharmacy Enterprise Co.,Ltd	66.7% (early phase of illness)			

CLIA = chemiluminescence immunoassay; *ELISA* = enzyme-linked immunosorbent assay; *LFIA* = lateral flow immunoassay; *ICG* = immunochromatographic; *SN* = sensitivity; *SP* = specificity;

*: values refer to SARS-CoV-2 positive patients

**: values refer to patients enrolled from emergency room department or to patients with suspected COVID-19 pneumonia

***: values refer to negative controls

4. Discussion

This review aimed to provide an overview of the existing serological tests, assessing their capacity to detect the presence of IgM and IgG antibodies against SARS-CoV-2 in the blood samples taken from patients. During the COVID-19 infection an hyperactivation of natural immunity cells, such as macrophages and monocytes has been recorded, which consequently causes the diminution of lymphocytes and the increase of neutrophils, interleukin-6 (IL-6) and reactive protein C (PCR) [42]. With regard to the adaptive immune response, the activation of B lymphocytes of the humoral immunity (which occurs approximately after one week from the infection) leads to the production of specific antibodies against SARS-CoV-2: IgM and IgG [43]. According to literature, IgM antibodies constitute the first defense line during a viral infection and it can indicate the acute phase of the disease; IgG antibodies represent the long-term immunity and immunological memory and their presence highlights that the exposure to the pathogen has occurred several days before [43,44]. The study by Demey et al. [45] tested 4 different immunochromatographic assays, demonstrating that the antibodies detection time was, on average, 8-10 days after the symptoms onset, while the case report of Thevarajan et al. [46] recorded a progressive increment of anti-SARS-CoV-2 IgM and IgG from day 7 until day 20 since the beginning of the illness. Two of the articles selected for this review noticed that the IgM-positive rate tended to increase at first but then decline, while those of IgG was higher than IgM at all times [34] and that IgM and IgG positive rate increased from 50% to 81% and from 81% to 100% respectively [40]. Pan et al. [37] divided the infection into three stages: early (1-7 days from the onset), intermediate (8-14 days) and late (more than 15 days), establishing that the positive rate of IgM raised from 11.1% (early stage) to 78.6% (intermediate stage) and 74.2% (late stage), whereas that of IgG was 3.6% in early stage, 57.1% and 96.8% in the intermediate and late stage respectively. Infantino et al. [33] was able to detect IgM and IgG from day 10 to day 30 and from day 20 onwards after COVID-19 infection respectively. On the basis of the results obtained by Lee et al. [35], the persistence of positive real-time RT-PCR seemed to be shorter in symptomatic patients, who developed IgM antibodies. The median seroconversion time detected by Zhao et al. [41] was day 12 and day 14 for IgM and IgG respectively. Among the 5 patients with severe symptoms analyzed by Yongchen et al. [39], the antibody response was individuated within week 2 and 3 out of 5 of these subjects developed IgG response prior to viral clearance, indicating that high levels of this new pathogen viral load may provide an early antibody response [47,48]. Almost all the included items

agreed on the fact that serological tests could be effective and reliable diagnostic tools in the SARS-CoV-2 infection identification, since they are able to provide high sensitivity and specificity and that their utilisation should be complementary to the execution of real-time RT-PCR. Because of its several limitations, rRT-PCR could report negative results also in infected individuals: very early or late collection of the swabs, poor quality of the specimen containing insufficient material quantity, wrong technical procedures. Furthermore, it takes long time to generate RT-PCR test results, it requires first-rate certified laboratory facilities with ad hoc educated staff and it may provide different results depending on the sampling site (oropharyngeal or nasopharyngeal swabs) [49]. Antibody assays could provide a faster, less expansive and simpler (no laboratory training need) method to diagnose COVID-19 [38]. As Li et al. [36] stated, serological tests may be used to screen the possible asymptomatic carriers [50], knowing that the majority of them develop anti-SARS-CoV-2 antibodies and, since these assays are able to individuate IgM and IgG simultaneously, they could be used for early diagnosis (detecting IgM) and for monitoring during the therapy [37]. The narrative review by Cheng et al. [51] highlighted that serological tests negative results should not be a valuable reason to exclude SARS-CoV-2 infection, considering that the patient may has been recently exposed to the pathogen. Moreover, present or past infections due to other coronaviruses could lead to cross-reactivity of antibody to non-SARS-CoV-2 coronavirus proteins. According to the same authors, these type of assays could be useful for epidemiologic studies, vaccine studies and risk assessment of health care workers. On the contrary, Cassaniti et al. [32] performed the serological assay also on 30 healthy volunteers, 10 of who had been infected in the past with OC43, 229E, HKU1 and NL63 coronavirus, reporting no cross-reactivity with antibodies against these pathogens. Infantino et al. [33] confirmed the absence of cross reaction with other coronaviruses, but demonstrated that cytomegalovirus (CMV) infections and some rheumatic diseases could interfere with the test.

5. Conclusion

The global pandemic caused by the novel SARS-CoV-2 is threatening the international health and the screen of the population on a large scale has became imperative. Besides the use of real-time RT-PCR to individuate the presence of COVID-19, the detection of specific antibodies response to this pathogen thorough serological assays may represents a reliable diagnostic protocol. Serological assays seems to be able to overcome the nucleic acids tests limitations, ensuring the diagnosis in asymptomatic patients and in false-negative RT-PCR results.

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