Comparative analysis of three immunoassays for SARS-CoV-2 detection

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Abstract. As the 2019 coronavirus disease (COVID-19) spread rapidly around the world starting in late 2019, researchers were intrigued by the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. In many nations, the real-time reverse transcription polymerase chain reaction (RT-PCR) is the accepted "gold standard" technique to detect COVID-19 because of its rapid process with high-level specificity. To solve the false-positive results caused by real-time RT-PCR test, more immunoassays in serological diagnosis need to be developed such as enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA) and lateral flow immunoassays (LFIA). When it comes to IgG antibodies, ELISA has excellent specificity and sensitivity, and it has higher accurately resulted in the detection of patients after 7 days symptom, but the process is lengthier. LFIA is the fastest approach but has lower sensitivity with limit detection duration of early stage, and CLIA has short procedure with high sensitivity with required high-costly equipment to test samples. Highly sensitive and specific SARS-CoV-2 infection diagnosis was made achievable by serology-based immunoassay, and ELISA is the best tool to confirmed COVID-19 in patient, and LFIA and CLIA would provide useful additional instruments for SARS-CoV-2 analysis. This research will compare and analyze the application performance of these three detection methods in detecting viruses.

Keywords: COVID-19; ELISA; immunoassays.

1. Introduction

Coronavirus disease 2019 (COVID-19) presents the tendency of quickly spread and large-crowed infection and it poses a serious threat to people's health [1]. Sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shares strikingly identical genome and set of symptoms with 2002 SARS-CoV outbreak [2]. SARS-CoV-2 is not only to induce serious disease in respiratory system, but also to trigger the inflammatory response by infecting virus, encompassing the body's innate and adaptive immunological responses, or so far as to kill T lymphocyte cells and facilitate its apoptosis. Indeed, in some grievous COVID-19 patients, systemic inflammation induced by a range of cytokines rise, and destroy distant organs and leads to multiorgan failure or death finally [2].

The method based on the nucleic acid to detect COVID-19 is widespread approach during the epidemic period in many countries, because of its quickly-detection with high-level sensitivity and great specificity. To this end, the polymerase chain reaction (PCR) can be used in the virus detection [3]. So that, real-time reverse transcriptase PCR (RT-PCR) can be used as the primary approach to detect COVID-19 attract scientists interesting, due to it specific and simple qualitative results. Nevertheless, expensive machine of testing, long time-consuming of testing, and professional lab skills necessary made RT-PCR test to be more complicated and difficulty to test by ordinary people own. Except for the problem of false-negative and false-positive findings in RT-PCR test for COVID-19 cases, issues in process of RT-PCR test cause undetectable COVID-19 in patients. For example, the RT-PCR approach is hard to explore patients with couple of weeks post-symptom due to high-pot of viral load in the upper respiratory occurs within the first 7 days of symptom [4]. Detection antibodies in serum of samples may solve this problem. Serological tests are quickly, cheaper, high-throughput, and easy procedure that contract with molecular method, and the limitation of RT-PCR assay for detection of COVID-19 make serological diagnosis to be more necessary and worthier.

Enzyme-linked immunosorbent assay (ELISA) is wildly-known method to detect disease as an important member of immunoassays and serological tests, and early stage of analysis of HIV is well

known in the application of ELISA, because its high-selectivity and high-sensitivity. The fourth generation ELISA method decreases the window period down to two weeks and early phase detection of HIV seroconversion, making it efficiently reduce the spread of HIV [5]. HIV as a famous member of single-stranded RNA virus is well-known, it has led scientists to reflect on whether ELISA, a "gold standard" for detection protein method, as a high-level selectivity approach to identify SARS-CoV-2 infection on the early phase of infection. Additionally, the antigen-antibody reaction is used in various serological test procedures to identify SARS-CoV-2 infection. This paper will compare three common immune detection methods in the detection of COVID-19, including ELISA, lateral flow immunoassays (LFIA) and chemiluminescent immunoassays (CLIA).

2. Comparative analysis of immunoassay methods

Because the use of nucleic acid is hard to detect after 7 day of symptoms or even longer precisely by RT-PCR, detection of antigen-antibody reaction method to be a sally port in detection of COVID-19. B cells product IgM, IgG and IgA antibodies during the immunity. IgM is the antibodies against virus developed at the initial phase of immunity after illness with short duration time. IgG has long duration and slow disappearance character with response at the latter phase of immunity, and it is easy to detect due to its high concentration than other antibodies in the immunology system. IgA is the major part of antibodies in immunity that found in mucous or saliva usually, where the most sample sources. Both antibodies IgM and IgG could detect when patient re-infect virus, which significantly raise the significance of IgM and IgG antibody. In addition, connecting the antibodies IgM, IgG and IgA titers with serology-based assays is necessary, because it can not only detect both symptomatic and asymptomatic patients' specificity, but also can enhance the sensitivity and accuracy for SARS-CoV-2 detection.

Jonathan et al. provides results from 16 commercially antibody tests that confirmed by Brazilian Health Regulatory Agency, and it described there has good sensitivity (82%) and specificity (97%) for IgM antibodies, and also have high sensitivity (97%) and specificity (98%) for IgG antibodies from the naso and oropharyngeal swabs samples, and the sensitivity (97%) and specificity (98%) in the pools for those samples are advanced [6]. Thus, the testing antibodies support the possible to increase the sensitivity and specificity for SARS-CoV-2 analysis.

As the "gold standard" in immunoassays to detection various protein, ELISA is a good tool to quantify measure the specific antibodies in COVID-19 detection. Antibodies bind to it to detect the patient samples after the capture antigen is immobilized on the plate, and then a complex is formed by enzyme-labeled detection antibody with captured antibodies, and colorimetric reaction occurs by enzyme interact with its substrate, which quantified and measured the presence and/or concentration of the specific antibodies [4, 7].

Table 1. Analysis of specificity and sensitivity in Euroimmun ELISA [8].

Parameters	Samples with negative PCR test		Samples with positive PCR test		Samples ≥4 days after positive PCR	
Antibodies	IgA	IgG	IgA	IgG	IgA	IgG
No. Samples	86	86	82	82	42	42
Negative	76	84	14	27	4	0
Positive	10	2	68	55	38	42
Borderline	4	1	6	2	-	-
Agreement (%) (95 %CI)	88.4%	97.7%	82.9%	67.1%	90.5%	100%

Euroimmun anti SARS-CoV-2 assay (Euroimmun ELISA), is one received method by Emergency Use Authorization (EUA), assesses human IgA and IgG antibodies against SARS-CoV-2 in serum or EDTA plasma [8]. The results from Euroimmun ELISA by measuring a ratio of extinction of samples

to the extinction of the calibrator, and the ratio is explained in negative, borderline and positive [8]. There are different results from samples with negative or positive PCR tested in Euroimmun ELISA for IgA and IgG, as shown in Table 1 [8]. Euroimmun ELISA determined better specificity (97.7%) for detection of IgG in samples with negative PCR test, and has high specificity (82.9%) for IgA detection in cases with positive PCR test. However, both of samples with negative or positive PCR test have related borderline results, which fall in between negative and positive that unsure if more closely negative or positive, and it makes results doubtful. Moreover, Euroimmun ELISA in IgG had excellent sensitivity (100%) in samples, because IgG have longer duration after infection than IgA antibodies.

LFIA is a type of rapid diagnostic tests. The theory of the method is that a liquid sample with the target analyte diffuses different parts of the polymer strip through capillary action, and the immobilized trapping antibody interacts with the target analyte [4]. The whole procedure is different with ELISA. The first step is to place a liquid sample containing the target analyte into an adsorbent sample pad, and the sample migrates to the assay area where fixed IgG and IgM antibodies are present via a coupled release pad containing the target analyte antibodies with colored or fluorescent particles [4]. The target in samples describes the reaction on the test line indicates, and assay determined due to the correct liquid flow through the strip is indicated by a reaction on the control line [4].

Table 2. The sensitivity for various days after symptoms onset in Euroimmun ELISA [7].

Days after onset	Sensitivity of ELISA (95 %CI)			
	Number	IgA	Number	IgG
1-7	22/28	71.4%	15/28	53.5%
8-14	38/39	97.4%	32/39	82.1%
15-28	49/50	98%	48/50	96%
Asymptomatic	13/13	100%	12/13	92.3%
Total	121/130	93.1%	106/130	81.5%

Table 3. The sensitivity analysis after symptoms onset in three LFIA methods [7].

Days after onset	Sensitivity of Lateral Flow Immunoassay (95 %CI)									
	Test A				Test B				Test C	
	N	IgM	N	IgG	N	IgM	N	IgG	N	IgM/IgG
1-7	8/27	29.6	13/27	48.1	16/24	66.7	14/24	58.3	18/27	66.7
8-14	17/39	43.6	28/39	71.8	24/32	75	30/32	93.8	38/39	97.4
15-28	12/49	24.5	41/49	83.7	23/25	92	25/25	100	49/50	98
Asymptomatic	4/11	36.3	7/11	63.6	7/11	63.6	10/11	90.9	11/13	84.6
Total	41/126	32.5	89/126	70.6	70/92	76.1	79/92	85.9	116/129	89.9

Compared with ELISA method, LFIA method is a rapid and simple detection method, which can be widely used in various detection fields, but its sensitivity and specificity are lower than ELISA method. Maria et al. reported the results of COVID-19 detection between Euroimmun ELISA with three different LFIA [5]. All IgG results are better than IgM respectively, whatever LFIA or ELISA, and Euroimmun ELISA showed best specific (100%) in the IgG [7]. Test C had the best specific value (95.2%) amount three LFIA test in the IgM/IgG results, but it may lack credibility because of it lack relevant data of IgM and IgG bands. Moreover, ELISA in IgA had higher sensitive than other tests in first 7 days after onset, as shown in Table 2 and Table 3 [7]. And both ELISA in IgA and Test C in IgM/IgG had better sensitivity (97.4%) results 8 to 14 days following the symptoms. Interestingly, IgG Test B had better sensitivity (100%) after 15 days onset. All results from LFIA and ELISA showed IgG antibodies had more specificity sensitivity and it may due to its high concentration feature. The lowest sensitivity in both Euroimmun ELISA and three LFIA tests in first 7 days onset showed those two methods are not a good choice in early stage of COVID-19 diagnosis.

Ong et al. contrasted Wantai ELISA with Orient Gene Biotech LFIA in COVID-19 detection, and both had high specificity (98%). But ELISA had higher sensitivity (62%) than LFIA (43%) respectively in all patient's samples shown in Table 4 [9]. Orient Gene Biotech LFIA had lowest sensitivity (28%) in the patient in first 7 days onset, and increase its sensitivity (60%) after one-week onset. The same tendency of raising sensitivity occurs in Wantai ELISA, but the sensitivity both related higher than LFIA in the same period, that is 49% sensitivity in early stage and 79% sensitivity in latter stage of diagnosis. Thus, ELISA had higher sensitivity compared with LFIA.

Table 4. The sensitivity and specificity on Wantai ELISA assays and Orient Gene Biotech LFIA in different stage of diagnosis [9].

Assay	Agreement	All patients	<7 days after symptom onset	≥7 days after symptom onset
Orient Gene Biotech LFIA	Sensitivity (95%CI)	43/99 (43%)	11/39 (28%)	31/52 (60%)
	Specificity (95%CI)	126/129 (98%)	39/40 (98%)	48/50 (96%)
Wantai ELISA	Sensitivity (95%CI)	59/95 (62%)	19/39 (49%)	38/48 (79%)
	Specificity (95%CI)	125/128 (98%)	39/40 (98%)	48/50 (96%)

CLIA is a method of immunoassay technique, where a luminescent molecular serve as the "indicator" of the reaction [4]. This approach has high specificity of the immune reaction and good sensitivity for luminescence response, and it analyze immunochemistry for a large number of samples automated and fast [4]. Moreover, CLIA has the similar mechanism with ELISA, but shorter procedure and no reagent required to halt the enzymatic reaction [4]. Using CLIA to detection SARS-CoV-2 infection, specific antibodies IgM, IgG and IgA in serous samples were trapped by N protein or RBD protein-coated magnetic particle in the first basic steps, and tough chemiluminescence read from reaction of acridinium with substrates followed by detection of human IgA, IgM and IgG by a secondary acridinium-conjugated antibody [4].

Table 5. The specificity for two Chemiluminescent assay and three ELISA methods [10].

Parameters	ABBOTT SARS-CoV-2 IgG assay	ROCHE Elecsys Anti-SARS-CoV-2	Euroimmun SARS-CoV-2 IgG assay	GA GENERIC CoV-2 IgG assay	Vircell COVID-19 ELISA IgG assay
No. Samples	69	69	69	69	41
Negative	69	69	67	64	37
Borderline	0	0	1	2	0
Positive	0	0	1	3	4
Specificity (%) (95 %CI)	100%	100%	97.1%	92.7%	90.2%

Matthaios et al. compare two chemiluminescent assays and three ELISA methods in IgG antibodies [10]. All samples were confirmed by real-time PCR using naspharyngeal swab and all results measured by OD value of samples to the cutoff, and the borderline is the value between positive and negative [10]. Both chemiluminescent assays have higher specificity (100%) than ELISA, as shown in Table 5 [10], and Euroimmun ELISA assay have better specificity (97.1%) among three ELISA methods. There are no borderline samples in both two chemiluminescent assays, which described chemiluminescent assay have more valuable and reliable results than ELISA. As shown in the Table 6 [10], GA GENERIC ELISA test and Vircell ELISA test have higher sensitivity (88.9%

and 85.7%, respectively) than ABBOTT assay and ROCHE test (81.8% and 72.7%, respectively) in all 99 samples.

Table 6. The sensitivity for two chemiluminescent assays and three ELISA methods in IgG antibodies for samples confirmed COVID-19 [10].

Parameters	ABBOTT SARS-CoV-2 IgG assay	ROCHE Elecsys Anti-SARS-CoV-2	Euroimmun SARS-CoV-2 IgG	GA GENERIC CoV-2 IgG assay	Vircell COVID-19 ELISA IgG assay
Total samples confirmed COVID-19					
No. Samples	99	99	99	99	70
Positive	81	72	65	88	60
Borderline	0	0	7	2	2
Negative	18	27	27	9	8
Sensitivity (%) (95 %CI)	81.8%	72.7%	65.7%	88.9%	85.7%
Group 1: samples confirmed COVID-19 without symptoms					
No. Samples	29	29	29	29	25
Positive	26	23	20	28	25
Borderline	0	0	4	1	0
Negative	3	6	5	0	0
Sensitivity (%) (95 %CI)	82.8%	62.1%	69%	96.6%	100%
Group 2: samples confirmed COVID-19 with symptoms onset <15 days					
No. Samples	36	36	36	36	31
Positive	25	23	18	27	24
Borderline	0	0	2	1	1
Negative	11	13	16	8	6
Sensitivity (%) (95 %CI)	69.4%	63.9%	50.0%	75.0%	77.4%
Group 3: samples confirmed COVID-19 with symptoms onset ≥15 days					
No. Samples	34	34	34	34	14
Positive	32	31	27	33	11
Borderline	0	0	1	0	1
Negative	2	3	6	1	2
Sensitivity (%) (95 %CI)	94.1%	91.2%	79.4%	97.1%	78.6%

It also occurred at Group 1 and Group 2, but two chemiluminescent assays had higher specificity than Vircell ELISA test (94.1%, 91.2% and 78.6%). It also occurred in the results of sensitivity, both two chemiluminescent assays have no borderline samples, but three ELISA tests were not. It made the results from chemiluminescent assays are more reliable. Euroimmun ELISA test has lower sensitivity in all samples, and the results of Euroimmun ELISA were similar to other research (65.7% vs 67.1%) [8, 10]. It's surprised that two chemiluminescent tests have higher sensitivity than

Euroimmun ELISA assay (94.1%, 91.2% and 79.4%) in Group 3, which is increasing sensitivity than total samples, Group 1 and Group 2.

Ling et al. determined both IgG and IgM rN-base have excellent sensitivity and specificity [11]. The sensitivity (97.9%) and specificity (99.7%) of rN-based IgG is higher than the sensitivity (95.7%) and specificity (85.7%) of rS-based IgG, and the sensitivity (97.9%) and specificity (99.7%) of rN-based IgM is higher than the sensitivity (89.1%) and specificity (97.0%) of rS-based IgM. Moreover, they supported the positive samples have enhanced IgG compared with normal controls in the chemiluminescence assay, and the sensitivity (95.6%) and specificity (96.6%) of IgM is similar with the sensitivity (97.7%) and specificity (95.2%) of IgG antibodies [11]. The rS-based IgM in ELISA is more sensitive than R-based IgM, which may be because the S1 protein, as a transmembrane protein, stimulates the body to produce IgM antibodies early in infection [11]. Thus, ELISA and chemiluminescence have high-level of sensitivity and specificity in both IgG and IgM antibodies with recombination N and S protein of SARS-CoV-2 diagnosis.

3. Conclusion

To solve the problems caused by RT-PCR test in false-positive and false-negative results and hardly-control necessary, and diagnosis effectively the patients with or without symptom whether or not infect SARS-CoV-2 virus, immunoassays test in serum specimens improved. By comparing three immunoassays to detect SARS-CoV-2 infection, ELISA, as the “gold standard” method in detection for antigen-antibody interaction, has higher sensitivity and specificity. However, ELISA has longer procedure than LFIA and CLIA, which could cause patients to know the results in a delay. Even though both LFIA and CLIA only cost no more than 30 minutes in the whole process, ELISA is still the most helpful detection tools. LFIA, as the most rapidly approach among three assays, is only could detect early and mid-infection period with lower sensitivity, and CLIA requires expensive and specific device. Thus, LFIA and CLIA are two good choices to test COVID-19 as the detection tools to screen the huge number of samples in the community, and ELISA method is rather be an excellent tool to confirmed the SARS-CoV-2 infection. Nevertheless, all three immunoassays have low rate in the confirmed positive samples detection. Moreover, almost all of the data used for studying of immunoassays test infection of SARS-CoV-2 are identified by RT-PCR data, which made bias for results. At the same time, the number of serum samples are not enough large to support having precise results from three immunoassays, and those are still need to improve in the future. SARS-CoV-2 is the ssRNA virus, which has high rate of mutation character with other RNA viruses, and scientists are need to looking for a useful with simple and quickly procedure with excellent sensitivity and specificity method to detect COVID-19 in both early and later stage of immune in whatever molecule and protein level methods. The discovery of effective detection assays can more effectively diagnose the spread of the virus caused by delay, and also enable patients to get timely treatment.

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