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Diagnosis of SARS-CoV-2 infection: preliminary results of six serology tests

Dijagnostika SARS-CoV-2 infekcije: preliminarni rezultati šest seroloških testova

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Abstract

The most important use of serology in the COVID-19 diagnostics is for determination of the extent of disease in the population. However, immunoassays could represent an additional diagnostic method, especially in patients with exposure history and clinical symptoms compatible with COVID-19 who failed to be confirmed by RT-PCR. We analyzed the preliminary results of six serology tests for the diagnosis of SARS-CoV-2. Three point-of-care lateral flow chromatographic immunoassays (POC): ACRO, AMP and ENCODE and three enzyme immunoassays (ELISA): DiaPro, Vircell and Euroimmun were used. A total of 15 serum samples from COVID-19 patients and 15 serum samples from asymptomatic persons were tested. Time of sampling for COVID-19 patients was 4 – 10 days (N=4), 11 – 19 days (N=6) and 20 – 34 days (N=5) after disease onset. Initially reactive results were confirmed using a virus neutralization test (VNT). In COVID-19 patients (N=15), IgM/IgA positive detection rates were 9/60.0% (ACRO), 11/73.3% (AMP, ENCODE, Euroimmun), 12/80.0% (DiaPro) and 13/86.6% (Vircell). Overall IgG detection rates were 10//66.6% (AMP, Euroimmun) and 11/73.3% (other tests). According to the sampling time, positive detection rates were as follows: a) days 4 – 10: 1/25.0% and 2/50.0% (IgM/IgA and IgG); b) days 11 –19: 4/66.6%-6/100% (IgM/IgA), 4/66.6% and 5/83.3% (IgG); c) days 20 – 34: 4/80.0% and 5/100% (IgM/IgA), 5/100% (IgG). One asymptomatic participant tested IgM/IgA positive using ACRO, DiaPro and Vircell was confirmed seropositive using a VNT. In a group of asymptomatic persons detected seronegative using a VNT (N=14), IgM/IgA negative detection rates were 12/85.7% (ACRO), 13/92.8% (DiaPro, Vircell) and 14/100% (AMP, ENCODE, Euroimmun). IgG negative detection rates were 13/92.8% (ACRO) and 14/100% (other tests). ELISA tests showed a higher overall IgM/IgA sensitivity compared to POC tests in patients with COVID-19, while the IgG sensitivity was similar in both POC and ELISA.

Sažetak

Najznačajnija primjena seroloških testova u dijagnostici COVID-19 je u svrhu procjene proširenosti bolesti u populaciji. Međutim, imunotestovi mogu poslužiti kao dodatni dijagnostički postupak, posebice kod bolesnika s podatkom o izloženosti COVID-19 i prisutnim kliničkim simptomima, kod kojih je rezultat RT-PCR testa bio negativan. U ovome smo radu analizirali preliminarne rezultate šest seroloških testova za dijagnostiku SARS-CoV-2. Korištena su tri 'point-of care' imunokromatografska testa (POC): ACRO, AMP i ENCODE te tri imunoenzimska testa (DiaPro, Vircell i Euroimmun). Testirano je ukupno 15 uzoraka seruma bolesnika s COVID-19 infekcijom i 15 uzoraka seruma asimptomatskih osoba. Vrijeme uzorkovanja kod bolesnika s COVID-19 iznosilo je 4 – 10 dana (N=4), 11 – 19 dana (N=6) te 20 – 34 dana (N=5) od početka bolesti. Svi su početno reaktivni rezultati potvrđeni testom neutralizacije virusa (VNT). Kod bolesnika s COVID-19 (N=15), učestalost detekcije IgM/IgA protutijela iznosila je 9/60,0% (ACRO), 11/73,3% (AMP, ENCODE, Euroimmun), 12/80,0% (DiaPro) te 13/86,6% (Vircell). Učestalost detekcije IgG protutijela iznosila je 10/66,6% (AMP, Euroimmun) te 11/73,3% (ostali testovi). Ovisno o vremenu uzorkovanja, učestalost detekcije protutijela iznosila je: a) 4-10. dana: 1/25,0% i 2/50,0% (IgM/IgA i IgG); b) 11-19. dana: 4/66,6%-6/100% (IgM/IgA), 4/66,6% i 5/83,3% (IgG; c) 20-34. dana: 4/80,0% i 5/100%

(IgM/IgA), 5/100% (IgG). U jedne asimptomatske osobe s dokazanim IgM/IgA protutijelima testom ACRO, DiaPro i Vircell potvrđena su neutralizacijska protutijela. U skupini seronegativnih asimptomatskih osoba dokazanih VNT testom (N=14), negativan nalaz IgM/IgA je nađen u 12/85,7% (ACRO), 13/92,8% (DiaPro, Vircell) i 14/100% (AMP, ENCODE, Euroimmun) uzoraka, dok je negativan nalaz IgG dokazan kod 13/92,8% (ACRO) te 14/100% (ostali testovi) uzoraka. ELISA testovi pokazali su višu osjetljivost detekcije IgM/IgA protutijela u usporedbi s POC testovima, dok je osjetljivost detekcije IgG protutijela bila podjednaka u POC i ELISA testovima.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that emerged in December 2019 in Wuhan, China. Due to its rapid global transmission, the World Health Organization (WHO) declared a pandemic in May 2020. Globally, 30,905,162 cases and 958,703 deaths due to coronavirus disease (COVID-19) in 216 countries were reported to WHO as of September 21, 2020[1]. Person-to-person SARS-CoV-2 transmission is thought to occur among close contacts mainly via respiratory droplets[2]. However, other routes of transmission including feco-oral[3] and through eye secretions are also suggested[4]. Clinical spectrum of COVID-19 varies from asymptomatic infection to severe and fatal pneumonia[5]. SARS-CoV-2 diagnosis is based on detection of viral RNA in respiratory specimens using a reverse-transcriptase polymerase chain reaction (RT-PCR), while serology is useful for determining the extent of disease in the population. However, immunoassays could represent an additional diagnostic method that could provide information on active/recent SARS-CoV-2 infection, especially in patients with exposure history and clinical symptoms compatible with COVID-19 who failed to be confirmed by RT-PCR[6,7]. Different serology tests are commercially available, including point-of-care lateral flow chromatographic immunoassays (POC) and enzyme immunoassays (ELISA)[8]. However, cross-reactivity to other coronaviruses can be challenging.

The aim of this study was to analyse the preliminary results of six serology tests for SARS-CoV-2 diagnosis.

Materials and Methods

A total of 30 serum samples collected from patients with RT-PCR confirmed COVID-19 (N=15) and asymptomatic persons with negative SARS-CoV-2 RT-PCR test (N=15) were tested for the presence of SARS-CoV-2 IgG and IgM and/or IgA antibodies by using six different commercial serology tests: three POC and three ELISA tests (Table 1). Time of sampling for COVID-19 patients was 4 – 10 days (N=4), 11-19 days (N=6) and 20 – 34 days (N=5) after disease onset. The results of ELISA were calculated according to the manufacturer's recommendation and expressed as follows: a) sample/calibration ratio; S/Co (DiaPro), b) antibody index; AI (Vircell), c) absorbance ratio (Euroimmun).

Table 1. Serology tests used for SARS-CoV-2 antibody detection

Tablica 1. Serološki testovi korišteni za detekciju protutijela na SARS-CoV-2

Test	Manufacturer	Antigen	Reference values
Point-of-care lateral chromatographic immunoassay			
ACRO 2019-nCoV IgG/IgM Rapid Test Casette	Acro Biotech, Rancho Cucamonga, CA, USA	NA	
AMP Rapid Test SARS-Cov-2 IgG/IgM	AMEDA Labordiagnostik, Graz, Austria	NA	
ENCODE COVID-19 IgM/IgG	Zhuhai Encode Medical Engeneering, Zhuhai, China	NA	
Enzyme immunoassay			
COVID-19 IgM; IgA; IgG	DiaPro, Sesto San Giovanni, Italy	N, S	IgM/IgG/IgA (S/Co) <0.9 negative; 0.9-1.1 equivocal; >1.1 positive
Covid-19 ELISA IgM+IgA; IgG	Vircell, Granada, Spain	N, S	IgM/IgA (AI) <6 negative; 6-8 borderline; >8 positive
			IgG (AI) <4 negative; 4-6 borderline; >6 positive
Anti-SARS-CoV-2 ELISA IgA; IgG	Euroimmun, Lübeck, Germany	S	IgA/IgG (absorbance ratio) <0.8 negative; 0.8-1.1 borderline; >1.1 positive

N =nucleocapsid; S =spike protein; NA =data not available

All initially reactive IgM, IgG or IgA samples were confirmed by using a virus neutralization test (VNT). SARS-CoV-2 HR1/8933 strain, isolated from the nasopharyngeal swab of COVID-19 patient on Vero E6 cells, was used for VNT. Maximum cytopathic effect was visible on the 4th day after inoculation and the virus replication was confirmed by RT-PCR. Prior to VNT, virus was titrated by 50% TCID ($TCID_{50}$) by using Vero cells and the titer was determined using the Reed and Muench formula. Heat inactivated serum samples (56°C/30 min) were tested in duplicate in 96-well plates. Two-fold serum dilutions starting from 1:2 were prepared and mixed with the equal volume (25 µl) containing 100 $TCID_{50}$ of the virus. After 1 h of incubation at 37°C in CO_2 incubator, 50 µl of Vero E6 cells in a concentration of $2x10^5$ cells/ml, was added to each well and incubated for four days. The antibody titer was defined as the reciprocal value of the highest serum dilution that showed 100% neutralization in at least half of the infected wells. Titer of >8 was considered positive[9].

The study was approved by the Ethics Committee of the Croatian Institute of Public Health.

Results

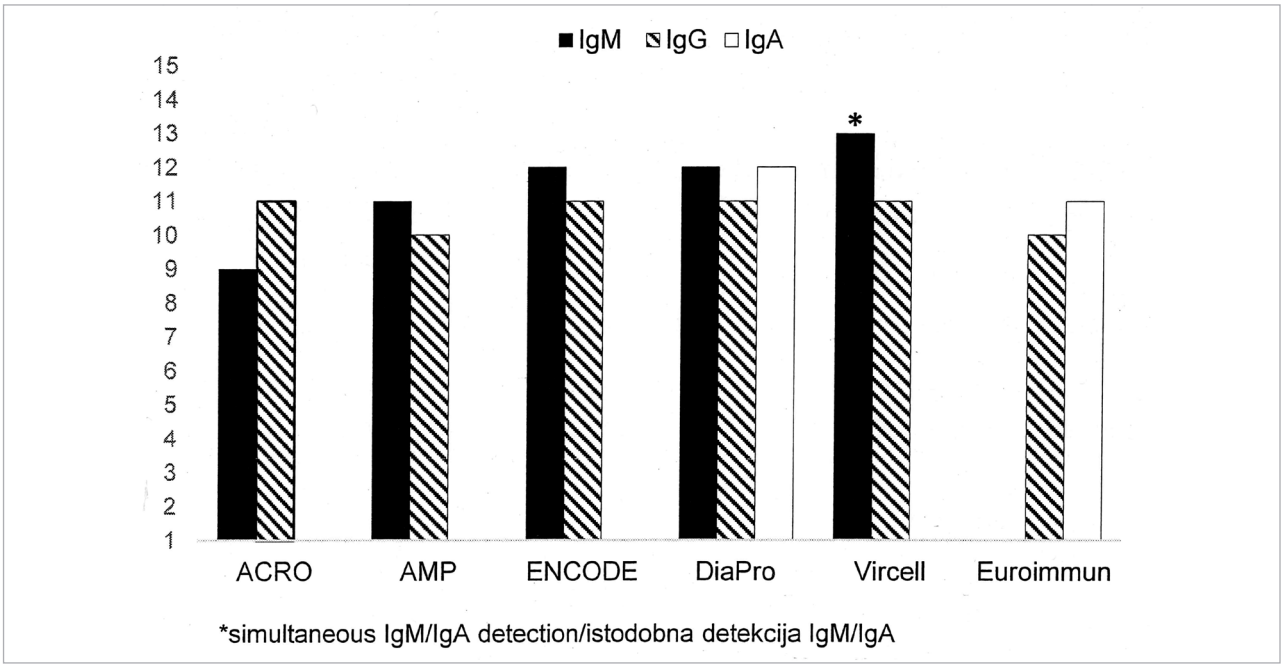
The serology results are presented in Figures 1 – 2 and Table 2. In a group of COVID-19 patients, IgM and/or IgA antibodies were detected in 9/60.0% (ACRO), 11/73.3% (AMP), 12/80.0% (ENCODE), 12/80.0% (DiaPro), 13/86.6% (Vircell) and 11/73.3% (Euroimmun) serum samples. The overall detection rates of IgG antibodies were 11/73.3% (ACRO), 10/66.6% (AMP), 11/73.3% (ENCODE), 11/73.3% (DiaPro), 11/73.3% (Vircell) and 10/66.6% (Euroimmun) (Figure 1).

Table 2. IgM/IgA and IgG SARS-CoV-2 detection rate in COVID-19 patients according to the sampling time
Tablica 2. Učestalost detekcije IgM/IgA i IgG protutijela u bolesnika s COVID-19 ovisno o vremenu uzorkovanja

Day*	N	ACRO		AMP		ENCODE		DiaPro			Vircell		Euroimmun	
	samples	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgA	IgM/IgA	IgG	IgG	IgA
4-10	4	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)
11-19	6	4 (66.6%)	5 (83.3%)	5 (83.3%)	4 (66.6%)	5 (83.3%)	4 (66.6%)	5 (83.3%)	4 (66.6%)	5 (83.3%)	6 (100%)	4 (66.6%)	4 (66.6%)	5 (83.3%)
20-34	5	4 (80.0%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)	5 (100%)

*days after disease onset

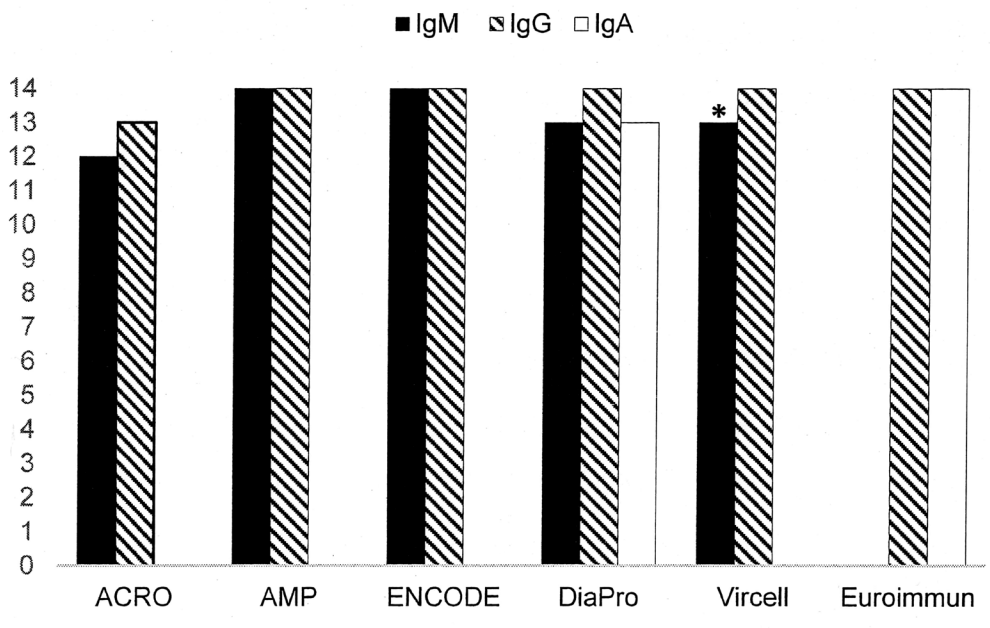
Figure 1. IgM/IgA and IgG SARS-CoV-2 positive detection rate in COVID-19 patients (N=15)
Slika 1. Učestalost pozitivne detekcije IgM/IgA i IgG protutijela u bolesnika s COVID-19 (N=15)



In a group of asymptomatic persons, one participant tested IgM/IgA positive using ACRO, DiaPro and Vircell was confirmed seropositive using a VNT. AMP and ENCODE tests showed a false negative IgM result. Titers of neutralizing antibodies were 8 (asymptomatic person) and 32 – 256 (patients with RT-PCR confirmed COVID-19). In the other 14 samples confirmed negative using a VNT, IgM and/or IgA negative detection rates were 12/85.7% (ACRO), 13/92.8% (DiaPro and Vircell) and 14/100% (AMP, ENCODE, Euroimmun). IgG negative detection rates were 13/92.8% (ACRO) and 14/100% (AMP, ENCODE, DiaPro, Vircell, Euroimmun) (figure 2).

IgM/IgA detection rates in COVID patients according to sampling time after disease onset varied from 1/25.0% to 2/50.0% (days 4 – 10), 4/66.6% to 6/100% (days 11 – 19) and 4/80% to 5/100% (days 21 – 34). IgG detection rates were 1/25.0-2/50.0% (days 4 – 10), 4/66.6%-5/83.3% (days 11 – 19) and 5/100% (days 20 – 34) (Table 1).

Figure 2. IgM/IgA and IgG SARS-CoV-2 negative detection rates in asymptomatic persons (N=14)
Slika 2. Učestalost negativne detekcije IgM/IgA i IgG protutijela u asimptomatskih osoba (N=14)



Discussion

The most important current use of serology in COVID-19 diagnostics is to determine how much community transmission has occurred (seroprevalence in asymptomatic cases and mild infections)[10]. In contrast to RT-PCR, the antibodies reveal evidence of an infection any time from about a week after the infection occurred[11]. ELISA test is the most commonly used screening test for detection of novel coronaviruses. However, due to a possible cross-reactivity with other coronaviruses as well as some other viruses such as Epstein-Barr virus which induces a robust polyclonal antibody response[12], confirmation with more specific test is required. The ELISA modular system to individually detect antibodies against SARS-CoV-2 spike protein 1, spike protein 2 and nucleocapsid seems to be more specific serological test for COVID-19. Additionally, a surrogate VNT designed to detect total neutralizing antibodies in an isotype- and species-independent manner is available which does not require a biosafety level 3 (BSL-3) laboratory[13]. However, detection of neutralizing antibodies by using a cell culture is still the 'gold standard' confirmatory serological test for SARS-CoV-2[8]. Since VNT requires live virus and BSL-3 laboratory, confirmatory testing is usually performed only in the reference laboratories.

In this study, the IgM/IgA (recent infection) and IgG (past infection) detection rates of six commercial tests for serological diagnosis of SARS-CoV-2 were compared. All three ELISA tests used showed generally a higher overall IgM/IgA sensitivity compared to POC tests 73.3-86.6%; 11 – 13/15 samples *vs* 60.0-80.0%; 9 – 12/15 samples) in patients with COVID-19, while IgG sensitivity was similar in all tests ranging

from 66.6%; 10/15 samples (AMP, Euroimmun) to 73.3%; 11/15 samples (other tests). In asymptomatic persons, negative IgM/IgA detection rates varied from 85.7%; 12/14 samples (ACRO) to 100%; 14/14 samples (AMP, ENCODE, Euroimmun) and IgG from 92.8%; 13/14 samples (ACRO) to 100%; 14/14 samples (other tests).

Recently published articles and preprints deposited in MedrXiv and BiorXiv showed that SARS-CoV-2 IgM antibodies could be detected as early as three days and peeks between two and three weeks after disease onset[7,14], while IgG antibodies can be present as early as four days and peak after 17 days[6,7,15]. In a study from Singapore, 25% of the COVID-19 patients had detectable antibodies in the first week of illness, 66.7% by the second week and 100% by the third week of illness[14]. In this study IgM/IgA antibodies were detected in 1 – 2/4 (25.0%-50.0%) patients tested within 10 days, 4 – 6/6 (66.6%-100%) patients tested between 11 – 19 days and 4 – 5/5 (80%-100%) patients tested more than 20 days after disease onset. The ACRO POC test showed lower IgM detection rate in the period 20 – 34 days (4/5; 80.0%) compared to other test used (5/5; 100%). IgG detection rates were similar to IgM detection rates in the period less than 10 days (1-2/4; 25.0%-50.0%) and 11-19 days (4-5/6; 66.6%-83.3%), while all tests detected IgG antibodies 21 to 34 days after disease onset (5/5; 100%).

In a Chinese study, the positive rate for IgG reached 100% around 20 days after symptoms onset[7]. Similarly, in this study all tested POC and ELISA test detected IgG antibodies in 100% (5/5) patients tested 20 and more days after onset of symptoms. In China, three types of seroconversion were observed: synchronous seroconversion of IgG and IgM; IgM seroconverted earlier than that of IgG; IgM seroconverted later than that of IgG[7]. In patients tested in this study, majority of them had detectable both IgM and IgG antibodies at the time of testing.

One IgM positive/IgG negative sample from asymptomatic person detected by ACRO, DiaPro and Vircell was confirmed using a VNT. ENCODE and AMP POC tests did not detect IgM antibodies (false negative result). Among 14 samples from asymptomatic persons confirmed negative using a VNT, negative detection rates of IgM and/or IgA were 12/85.7% (ACRO), 13/92.8% (DiaPro, Vircell) and 14/100% (AMP, ENCODE, Euroimmun).

In conclusion, among POC tested, ENCODE test showed the highest both IgM and IgG positive detection rates in COVID-19 patients. Vircell ELISA test showed the highest IgM positive detection rate compared to other ELISA tests. In asymptomatic persons, ACRO POC showed the lowest IgM and IgG negative rates, while AMP, ENCODE and Euroimmun showed the highest negative detection rates (14/14; 100%).

This study has some limitations. Although similar with other studies, due to the small number of of samples tested, the results should be interpreted with caution. Further investigation on large number COVID-19 patients as well as asymptomatic persons should be performed to determine the sensitivity and specificity of serology tests in SARS-CoV-2 diagnostics.

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