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METHODOLOGICAL ARTICLE

COMPARISON OF THREE METHODS FOR DETECTION OF ANTI-SARS-COV-2 ANTIBODIES

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Abstract: A novel positive-sense RNA virus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified in December 2019 in China. It is a systemic disease that includes severe respiratory distress, coronavirus disease 19 (Covid-19). The primary way of transmitting this virus is person-to-person contact via respiratory droplets, but it can also be transmitted by contaminated surfaces. Symptoms range from mild to severe, and the virus spreads quickly. On 11 March 2020 Covid-19 was declared a pandemic by the World Health Organization. The standard way to identify the presence of the virus is to detect its genome using real-time reverse transcriptase polymerase chain reaction (RT-PCR). It can be applied to respiratory tract samples such as nasopharyngeal swabs, sputum and bronchoalveolar lavage. In order to identify contact with the virus and immunological response of the individual, tests based on immunoassays were developed. Many of those tests were produced in short periods of time and they mostly differ on the sample that can be used (serum, plasma or whole blood), complexity and/or expense, and the class of the antibody they detect. The reliability of such tests is of high importance for epidemiological surveys as well as for the development of a vaccine. The aim of this study was to compare three commercially available immunoassay tests. Our results show that different serological tests have different sensitivity and specificity, and that the rapid option, which is the easiest to perform and has the lowest cost, provides the least reliable results.

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INTRODUCTION

The novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified in December 2019 in Wuhan City, Hubei Province of China. It is a positive-sense RNA virus which causes respiratory distress, a disease that has been called coronavirus disease 19 (Covid-19). It is believed that the virus spread from China all over the world, so on 11 March 2020 Covid-19 was declared as a pandemic by the World Health Organization (WHO).[1] Since the beginning of the pandemic until today (28/08/2020), Croatia has had 9,192 cases of SARS-CoV-2 infection, 6,595 people have fully recovered and 177 people died.[2] Contact person-to-person via respiratory droplets is the primary way of transmitting the SARS-CoV-2, but it can also be transmitted by contaminated surfaces. The most common symptoms for Covid-19 are fever, dry cough, fatigue and muscle pain, loss of taste or smell or sometimes also headache and lymphopenia. They appear after an incubation period of 2 - 14 days, but there are many asymptomatic cases in which people are also contagious. The patient's condition later depends on their immunity, health, other comorbidities, and age, and can, for example, vary from breathing difficulties and an acute respiratory distress syndrome (ARDS), pneumonia and other functional failure to death.[3]
Real-time RT-PCR is the best method of detecting the RNA of the virus in respiratory tract samples such as nasopharyngeal swabs, sputum and bronchoalveolar lavage of infected patients.[4] Positive detection rates are different for those sample types, and Yang et al. recommend sputum or nasal swabs for accurate diagnosis, while they stated that throat swabs were the least accurate for diagnosis. They also pointed out that, for severe cases, most required is detection of virus in

bronchoalveolar lavage.[5] Serological tests detect antibodies to SARS-CoV-2 and in that way identify individuals who were in contact with virus. It was even suggested that combining RT-PCR with serological tests is optimal for diagnosis of suspected patients.[6] Since SARS-Cov-2 has spread worldwide, many serological tests developed in a short time with the idea of designing an easy and fast tool for confirming SARS-CoV-2 infection, for epidemiological serological surveys, and also for possible future development of a vaccine.[7]

One of the rapid serological tests for the detection of IgG and IgM antibodies to SARS- CoV-2 in different samples (human serum, plasma or whole blood) is the Keul-o-test SARS-CoV- 2 IgG/IgM chromatographic test (Günter Keul GmbH, Germany). This test is based on the principle of lateral flow immunoassay chromatography and is available in cassette form. It takes 10-20 minutes for the test to give results for IgG and IgM antibodies detection on the same cassette.

One of the tests based on an electrochemiluminescence immunoassay that can use human serum and plasma as samples and detect antibodies to SARS-CoV-2 regardless of their immunoglobulin class is the serological Elecsys Anti-SARS-CoV-2 (Roche Diagnostics International Ltd, Switzerland). The test is based on the sandwich principle, and it is intended for use on immunoassay analyzers. In a validation study performed by Roche Diagnostics International and their partners, this test demonstrated an overall specificity of 99.80% and an overall sensitivity of 99.5% for past infection in patients at ≥ 14 days after PCR confirmation.8 Test results are done in about 1,5 hours, and it provides data about total antibodies (IgG and IgM) to SARS-CoV-2.

EDI Novel Coronavirus COVID-19 IgG and IgM ELISA tests detect IgG or IgM antibodies to SARS-CoV-2 only in human serum. Several studies validated this test and reported high “true“ vs. low “false“ positivity rates demonstrating that these immunoassays are suitable for clinical routine, identifying individuals with past SARS-CoV-2 infection with sufficient specificity and sensitivity.[9, 10] Because this method is more demanding than those previously mentioned, around 4 hours are needed to get a results for specifically IgG or IgM antibody class.

The aim of this study was to compare three immunoassays for the detection of IgG and other classes of antibodies raised against SARS-CoV-2 that were made for different samples.

MATERIAL AND METHODS

Patients

In the period from May to July 2020, 1874 patients were tested for antibodies to SARS-CoV-2 in the laboratory of Breyer Polyclinic, of which 32 using a Keul-O-TEST SARS-CoV-2 test, 1724 using Elecsys anti-SARS-Cov-2 and 118 using the EDI Novel Coronavirus COVID-19 IgG ELISA test. Thirty of them were chosen for this study based on the confirmed presence of disease, clinical data and presence of IgG antibodies to SARS-COV-2 detection using the ELISA-based test (Table 1). All patients signed an informed consent form.

After blood was drawn and centrifuged, serum samples from those 30 patients were tested using the rapid Keul-0-test SARS-CoV-2 test and the Elecsys Anti-SARS-CoV-2 test.

Methods

All three tests represent immunoassay-based methods for the detection of anti-SARS-CoV-2 antibodies. Their main differences are the type of sample they use, the complexity of the method itself and the cost.

EDI NOVEL CORONAVIRUS COVID-19 IgG ELISA

ELISA is done in microtiter wells of a microplate that is coated with COVID-19 recombinant nucleocapsid protein. Assay controls and 1:100 diluted human serum samples are added to the wells. After the first incubation period, the unbound protein matrix is removed with a wash step. Then, a horseradish peroxidase (HRP) labeled polyclonal goat anti-human IgG tracer antibody is added to each well. After an incubation period, an immunocomplex is formed if there is specific coronavirus IgG antibody present in the tested specimen. The unbound tracer antibody is removed by the subsequent washing step. HRP-labeled tracer antibody bound to the well is then incubated with a substrate solution and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the anti-SARS-CoV-2 IgG on the wall of the microtiter well is proportional to the amount of the anti-SARS-CoV-2 IgG antibody level in the tested specimen.

ELECSYS ANTI-SARS-CoV-2

Elecsys anti-SARS-Cov-2 assay uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of anti-SARS-CoV-2 antibodies. The test consists of two main parts: during first incubation, 20 µl of sample forms a sandwich complex with biotinylated SARS-CoV-2-specific recombinant antigen and SARS-CoV-2-specific recombinant antigen labeled with a ruthenium complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. Then, the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of voltage to the electrode then induces chemiluminescent emission

Table 1. Patient data

Patient	Anti-SARS-CoV-2 IgG antibody (ELISA)	Symptoms	Other known diseases	PCR test for SARS-CoV-2
1	P	fever < 38 °C, nasal discharge, fatigue, weakness, loss of taste and smell	No	P
2	P	fever < 38 °C, loss of taste and smell	No	P
3	P	fever > 38 °C, loss of taste and smell, diarrhea and headache	No	/
4	P	fever > 38 °C, loss of taste and smell, diarrhea	No	/
5	P	fever < 38 °C, fatigue, weakness, headache	No	P
6	P	No	Myocardial infarction	P
7	P	fever > 38 °C, cough, nasal discharge	No	P
8	P	fever < 38 °C	No	P
9	P	fever < 38 °C, nasal discharge, loss of taste and smell	No	N
10	B	No	No	/
11	N	No	No	/
12	N	No	No	/
13	N	No	No	/
14	N	No	No	/
15	N	No	No	/
16	N	No	No	/
17	N	No	No	/
18	N	No	No	/
19	N	No	No	N
20	N	fever < 38 °C, cough, shortness of breath, nasal discharge, fatigue, weakness, vomit, diarrhea	Hashimoto's disease	N
21	P	fever > 38 °C, cough, shortness of breath, nasal discharge, fatigue, weakness	No	/
22	N	fever < 38 °C, cough, shortness of breath, nasal discharge, fatigue, weakness	Asthma, COPD, immune system disorders	N
23	N	fever < 38 °C, cough, shortness of breath	No	N
24	N	fever < 38 °C, cough, shortness of breath, fatigue, weakness	No	N
25	P	No	No	/
26	P	fever < 38 °C, cough, fatigue, weakness, loss of taste and smell, headache	No	P
27	P	fever < 38 °C, cough, shortness of breath, fatigue, weakness, chest pain		N
28	P	fever < 38 °C, cough, shortness of breath fatigue, weakness	Asthma	/
29	P	fever > 38 °C, cough, shortness of breath, loss of taste and smell, fatigue, weakness	No	P
30	P	fever > 38 °C, cough, shortness of breath, fatigue, weakness	Pneumonia during infection	P

Legend: P – positive result; B – borderline result; N – negative result, / - not tested

which is measured by a photomultiplier. Results are determined by the software by comparing the signal obtained from the sample with the signal of the cutoff value previously obtained by calibration. The result of a sample is given either as reactive (positive for anti-SARS-CoV-2 antibodies) or non-reactive (negative for anti-SARS- CoV-2 antibodies).

KEUL-O-TEST SARS-CoV-2 (IgG/IgM)

The Keul-o-test consists of fixed anti-human IgG and IgM antibodies on the surface of the cassette in the IgG and IgM test regions. To start a test, two drops of buffer and 10 µl of human serum are put one after another in the sample region on the surface of the cassette. The mixture immediately starts to flow through the membrane of cassette due to capillary action. If the patient sample contains anti-SARS-CoV-2 antibodies, then these antibodies will bind to the antigen present in the conjugation pad of the cassette and the complex formed will migrate to the membrane-bound anti-human IgG and/or IgM. A colored band will appear so the test results can be read 10 to 20 minutes after adding the sample.

RESULTS AND DISCUSSION

Based on literature data and the reliability of described methods, we used the ELISA test as a reference.[11]
The ELISA-based method produced the following results: 16 positive samples, 13 negative samples, and borderline results for 1 sample were detected.
The Keul-o-test showed 14 positive samples, 13 negative samples, and 3 borderline results, while the Elecsys anti-SARS-Cov-2 test showed 15 positive, 15 negative, and no borderline cases (Table 2.).
Among ELISA positive cases, 13 were confirmed with other two tests, while 3 cases showed different results using different tests: Patient no. 5 showed positive results with both the ELISA and autoanalyzer test while

Table 2. Comparison of immunoassay-based tests results for antibodies to SARS-Cov-2.

Patient	Manual ELISA test (IgG)	Automated lab- based immunoassay (IgG and IgM)	Rapid manual test (IgG)
1	P	P	P
2	P	P	P
3	P	P	P
4	P	P	P
5	P	P	B
6	P	P	P
7	P	P	P
8	P	P	P
9	P	P	P
10	B	N	N
11	N	N	N
12	N	N	N
13	N	N	B
14	N	N	N
15	N	N	N
16	N	N	N
17	N	N	N
18	N	N	B
19	N	P	N
20	N	N	N
21	P	N	P
22	N	N	N
23	N	N	N
24	N	N	N
25	P	N	N
26	P	P	P
27	P	P	P
28	P	P	P
29	P	P	P
30	P	P	P

Legend: P – positive result; B – borderline result; N – negative result,

the rapid test showed a borderline result. This patient had an infection confirmed by PCR and showed mild symptoms. Such data indicate that the rapid test, in this, case, showed lower sensitivity when compared with the other two tests. Patient no. 21 showed positive results with both ELISA and the rapid test, while autoanalyzer results were negative. Clinical data showed symptoms associated with COVID-19, and PCR-detection of COVID-19 was not done. In this case, the autoanalyzer test showed lower sensitivity than the other tests. For patient no. 25 results were ELISA positive and negative with the other 2 tests. Given that this patient had no symptoms, the PCR-test was not done, and that the patient was considered healthy overall, this case could show both higher ELISA sensitivity than the other two tests, or lower specificity than the other two tests.

Patient no. 10, who was borderline based on the ELISA test, showed negative results when tested using the other two methods and had no symptoms. This case suggests lower specificity of ELISA than the other two tests, especially because the autoanalyzer test cannot discriminate between different classes of anti-SARS-Cov-2 immunoglobulins.

Out of 13 ELISA-based negative cases, 8 patients had no symptoms and the PCR-test was not done, while 4 patients had minor respiratory distress, a fever < 38 °C and the PCR test was negative. Among asymptomatic patients who had a negative ELISA test, two (no. 13 and 18) showed borderline results using the rapid test. In those cases, the rapid test showed lower specificity than the other two tests. Patient no. 19. was asymptomatic, had a negative PCR test and was ELISA and rapid test negative. In this case the autoanalyzer showed positive results that can only be interpreted as false positive.

Although this study included only 30 cases, it showed that serological tests like ELISA and electrochemiluminescence immunoassays might give false negative or false positive results, and therefore should be interpreted only in relation to clinical data and a PCR-test that was performed during the acute phase of the disease. Taken together, our results suggest that the rapid test is the least reliable.

Moreover, two important points need to be taken into account when choosing the optimal immunoassay-based test for e. g. epidemiological studies: sensitivity and specificity of the tests that depends on antibody classes it detects as well as various expected immunological response to a virus in different individuals. [12, 13]

REFERENCES

1. Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far. Pathogens. 2020;9(231). doi.org/10.3390/pathogens9030231
2. https://www.koronavirus.hr/en (28.08.2020)
3. Sharma A, Tiwari S, Deb MK, Marty JL. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): A global pandemic and treatments strategies. Int J Antimicrob Ag. 2020;56(106054). doi.org/10.1016/j.ijantimicag.2020.106054
4. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DGJC, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MPG, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):pii=2000045. doi.org/10.2807/1560-7917.ES.2020.25.3.2000045
5. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, Zhang M,Wang Z, Xing L, Wei J, Peng L, Wong G, Zheng H, Wu W, Liao M, Feng K, Li J, Yang Q, Zhao J, Zhang Z, Liu L, Liu Y. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. The Innovation.2020;1(100061) doi.org/10.1016/j.xinn.2020.100061
6. Wang P. Combination of serological total antibody and RT-PCR test for detection of SARS-COV-2 infections. J Virol Methods. 2020; 283(113919). doi.org/10.1016/j.jviromet.2020.113919
7. La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic

review and clinical guide to molecular and serological in-vitro diagnostic assays. RBMO. 2020;41(3):483-499. doi.org/10.1016/j.rbmo.2020.06.001

8. Muench P, Jochum S, Wenderoth V, Ofenloch-Haehnle B, Hombach M, Strobl M, Sadlowski H, Sachse C, Riedel A. Development and validation of the Elecsys Anti-SARS-CoV-2 immunoassay as a highly specific tool for determining past exposure to SARS-CoV-2. J Clin Microbiol. 2020;58(10): e01694-20. doi.org/10.1128/JCM.01694-20
9. Krüttgen A, Cornelissen CG, Dreher M, Hornef M, Imöhl M, Kleines M. Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG. J Clin Virol. 2020; 128(104394). doi.org/10.1016/j.jcv.2020.104394
10. Bundschuh C, Egger M, Wiesinger K, Gabriel C, Clodi M, Mueller T, Dieplinger B. Evaluation of the EDI enzyme linked immunosorbent assays for the detection of SARS-CoV-2 IgM and IgG antibodies in human plasma. Clin Chim Acta. 2020;509:79-82. doi.org/10.1016/j.cca.2020.05.047
11. Venter M, Richter K. Towards effective diagnostic assays for COVID-19: a review. J Clin Pathol. 2020;73(7):370-377. doi.org/10.1136/jclinpath-2020-206685
12. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, Hu JL, Xu W, Zhang Y, Lv FJ, Su K, Zhang F, Gon J, Wu B, Liu XM, Li JJ, Qiu JF, Chen J, Huang AL. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections [published online ahead of print, 2020 Jun 18]. Nat Med. 2020;10.1038/s41591-020-0965-6. doi.org/10.1038/s41591-020-0965-6
13. Yongchen Z, Shen H, Wang X, Xudong Shi, Li Y, Yan J, Chen Y, Gu B. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. Emerg Microbes Infect. 2020;9(1):833-836. doi.org/10.1080/22221751.2020.1756699