

RAPID SEROLOGICAL TESTS FOR SARS-COV-2 IgG/IgM – NOT WORTH ATTENTION?

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Abstract

Objectives: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome virus 2 (SARS-CoV-2) had spread worldwide since December 2019 and became a pandemic in March 2020. The diagnosis of an active infection is based on the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) from the nasopharyngeal swab specimen. The aim of the current analysis was to assess the usefulness of the rapid serological tests for diagnosing SARS-CoV-2 infections. **Material and Methods:** The rapid serological tests detecting IgG/IgM antibodies for SARS-CoV-2 were voluntarily performed in asymptomatic employees of 2 companies. The examination was conducted at the date and time selected online by the study participants. The testing team consisted of 2 nurses collecting the samples and 1 doctor who interpreted the results. Each positive rapid test result was verified by an RT-PCR examination from a nasopharyngeal swab. The testing kits named Vazyme: 2019-nCoV IgG/IgM Detection Kit (Colloidal Gold-Based) were provided by the employer along with the manual and certificates. **Results:** The overall interest in testing among employees was below the employer's expectations and reached 30% and 20% in each of the 2 companies, respectively. A total of 516 participants were included in the analysis. Ten positive results of the rapid tests were documented, including 7 for IgM and 3 for IgG antibodies. No positive result was confirmed by the detection of the genetic material of the SARS-CoV-2 by the RT-PCR examination. **Conclusions:** Herein, the authors demonstrated the uselessness of rapid serological tests performed in asymptomatic volunteers for diagnosing SARS-CoV-2 infections. *Int J Occup Med Environ Health.* 2021;34(2)

Key words:

RT-PCR, screening, employees, COVID-19, SARS-CoV-2, rapid serological tests

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome virus 2 (SARS-CoV-2) had spread worldwide since December 2019 and became a pandemic in March 2020. By the end of September, approx. 33 million affected people with >1 million deaths globally due to COVID-19 were reported to the World Health Organization (WHO), whereas in Poland nearly 88 thousand cases were documented of whom more than 2400 persons died.

The majority of the infected persons are asymptomatic or experience mild to moderate respiratory illness and recover without requiring special treatment; however, they may transmit the virus to other susceptible individuals. Therefore, the timely and proper diagnosis of the infection, even in patients with mild disease forms or in asymptomatic persons, is crucial to prevent the transmission of the virus. According to the WHO and national recommendations, the diagnosis of an active SARS-CoV-2 infection is based on the real-time reverse transcriptase-polymerase

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chain reaction (RT-PCR) from the nasopharyngeal swab specimen [1,2].

More than 3 million genetic tests for SARS-CoV-2 infections were performed in Poland from the beginning of the epidemic, with a daily number exceeding 35 000. Since a molecular test takes several hours, and taking into account the decrease in the RT-PCR sensitivity along with the development of the patients' immune response after the 10th day from the onset of the infection, the need for easier-to-perform and faster diagnostic methods has occurred. Among them, rapid serological tests detecting IgG/IgM antibodies for SARS-CoV-2 are available.

The current analysis aimed to assess the usefulness of the rapid serological tests for diagnosing SARS-CoV-2 infections.

MATERIAL AND METHODS

The research was carried out in May and June 2020 in 2 companies located in the Silesian Voivodeship, Poland. The rapid serological tests detecting IgG/IgM antibodies for SARS-CoV-2 were voluntarily performed in asymptomatic employees from these companies. Before any study procedure, the participants were asked to read the information about the examination and then sign the informed consent form (Annex 1). After that, the employees were asked to fast for 6 h before the test.

The examination was conducted at the date and time selected online by the study participants. The dedicated time allowed the participants not to be exposed to gathering and to avoid queuing, as well as to monitor the intensity of traffic. In front of the entrance to the building or tent, a diagram of the participants' movement was displayed with reminding arrows inside.

The testing team consisted of 2 nurses taking the samples and 1 doctor who interpreted the results after 10 min of the time recorded by the nurse on the chart and then issued written recommendations to the employee (Annex 2).

At the time of testing, the staff was equipped with protective clothing, including gloves, safety goggles, surgical or FFP2/3 respiratory masks, caps and protective footwear. A distance of at least 2 m between the participants was maintained in the admission office, whereas the testing staff kept a distance of 3 m from each other. This was achieved thanks to a person from the occupational health and safety section of the company, who coordinated the admission of the employees at the site where they signed informed consent forms (there was a change of the mask to a new one and disposable pens were provided). That person was also responsible for the marking of squares on the floor in the waiting room, which could not be crossed by the participants waiting for the result.

All the study procedures took 4 h, following which there was an hour of break for lunch and rest, and then another 4 h of testing took place. During 1 h of testing, 25 participants were examined and each nurse had a new patient every 5 min. During this time, the nurse had to disinfect her hands, put on new gloves, take 2 drops of blood from her finger with an automatic lancing device, put 1 drop of blood and 2 drops of dilution buffer on the test window for both tests, record the hour in agreement and bring them to the table next to the doctor's place. After the test, the participant was sent to the waiting room, where at a distance, in his/her square, he/she waited for the doctor's call and the result of the test.

Each positive rapid test result was verified by the RT-PCR examination from a nasopharyngeal swab. In such a case, the doctor took a swab from the nose and throat, and obtained the necessary data. After taking the material, the employee received a card advising him/her to self-isolate and stay in the mask, following which he/she left the site using his/her own transport. Every evening the samples of the nasopharyngeal swabs were taken to the Warsaw Genomics genetic laboratory. The tests were provided by the employer along with the operating manual and certificates (Vazyme: 2019-nCoV IgG/IgM Detection Kit).

RESULTS

Approximately 30% of the employees in the first company and 20% in the second one were interested in the study, accounting for 516 participants tested during 4 days. The level of interest among employees was significantly below the employer's expectations. The male predominance was observed in the analyzed population consisting of 340 males (65.8%). All the participants were adults (age range: 18–65 years). None of the participants reported a high-risk contact with a person with confirmed COVID-19. None of them presented any clinical symptoms of COVID-19.

Ten positive results of the cassette tests, including 7 of IgM and 3 of IgG, were documented. There was no case where both IgM and IgG were positive. In all 10 positive cases, the results of nasopharyngeal swabs for the genetic material of SARS-CoV-2 were negative.

A large amount of disposable equipment was used and a lot of effort was made to ensure the safety of the procedure. However, the employees felt taken care of, the employer fulfilled the recommendations of the global manager, and the epidemiological situation in the plant was partially recognized.

Noteworthy, the testing procedure was associated with some risk for the employer because taking into account the epidemiological situation in the neighboring mines, he did not know what to expect and whether he would maintain production continuity.

DISCUSSION

The outbreak of COVID-19 with an increasing number of SARS-CoV-2 infections worldwide has revealed the need for timely and reliable diagnostic tests to identify affected patients, even asymptomatic, and to prevent the transmission of the disease [3].

Antibody assays seem to be an attractive diagnostic strategy for the detection of the SARS-CoV-2 infection, especially rapid semi-quantitative and qualitative ones. The low cost, the easiness of performing and interpreta-

tion, and fast turn-around time make this option attractive as regards quick diagnosis and mass screening [5]. Serological diagnosis allows for the detection of the IgM/IgG antibodies class and could be important particularly in asymptomatic persons or those with mild symptoms. Determining the optimal time for serological tests is related to the phenomenon of the so-called "serological window" which, in the case of COVID-19, lasts for 7–14 days [3]. There is some evidence suggesting that antibodies for SARS-CoV-2 are produced within the range of 5–14 days after the onset of the disease symptoms [3,5,6].

Hence, serological tests detecting the presence of antibodies have a limited diagnostic value in the early stage of the SARS-CoV-2 infection. Testing specific antibodies for SARS-CoV-2 allows achieving sensitivity at a level similar to RT-PCR at 2–3 weeks after the onset of the COVID-19 symptoms when the patient no longer has SARS-CoV-2 RNA, which is tantamount to not being infectious [7]. So, demonstrating the presence of antibodies does not identify infected people and, therefore, cannot be the basis for the isolation of potential sources of the infection. Serological tests detecting the specific antibodies may be useful in population studies to assess the rate of the people that have been exposed to the virus, or in epidemiological investigations, especially in the case of a history of feverish symptoms [6,8,9].

Many companies offer rapid immunochromatographic tests to detect antibodies for SARS-CoV-2, the so-called cassette tests. The predictive value of the currently available cassette tests is mainly based on manufacturers' registration data; therefore, there is no complete information to assess its' clinical usefulness.

Despite the simplicity of performing, and a very short time needed to obtain the results of, the antibody assays, the poor sensitivity and specificity limit their accuracy and value, and, according to guidelines, they are useful for research and epidemiological studies only [1,2]. The analysis of point-of-care (POC) serological assays for SARS-CoV-2 case identification in the prospective multicentre cohort

study conducted in the UK demonstrated the usefulness of rapid serological tests in estimating SARS-CoV-2 seroprevalence among health-care workers [9].

Noteworthy, a negative result of the rapid serological test does not rule out the SARS-CoV-2 infection, e.g., due to the serological window or the lack of antibody production in some people; the illness process and host immune factors could contribute to a false-negative result [10]. Positive results of the antibody tests should not be used as a basis for diagnosing SARS-CoV-2 infections since they may be caused by a previous or ongoing infection with other coronaviruses, such as HKU1, NL63, OC43 or 229E coronavirus, or other viruses, including adenoviruses, EBV, CMV, or the presence of autoantibodies, the rheumatoid factor and vaccine antibodies (flu) [10–12]. To conclusively confirm or exclude the SARS-CoV-2 infection, a molecular technique test has to be performed.

The current analysis confirmed that rapid serological tests should not be considered as a diagnostic tool in the detection of the SARS-CoV-2 infection. The authors of this study demonstrated that none of the positive results of the rapid antibody test was confirmed by the molecular test. Hence, these findings of the low value of rapid serological tests are in line with the results demonstrated by other researchers [8,13–16]. Cassaniti et al. [15] performed the rapid serological tests detecting IgM/IgG antibodies and molecular tests concurrently in the cohort of patients admitted to the emergency room department. A comparative analysis conducted among 50 patients revealed that 1 in 8 positive IgM and/or IgG was negative in the RT-PCR examination and sensitivity was very poor (<20%). The study performed by Döhla et al. [16] in 49 patients with symptoms of a respiratory tract infection documented 11 positive results of rapid serological tests of which 8 were true-positive, 3 were false-positive, whereas 24 were true-negative and 14 were false-negative, when verified by molecular tests.

Loconsole et al. [13] documented the low value of rapid serological tests in 819 Italian patients admitted to hos-

pital with or without symptoms of COVID-19. In all the study participants, rapid serological tests were done and, in the case of positivity, the molecular test for the SARS-CoV-2 infection was performed. Antibodies were detected in 8.6% of these patients (both IgM and IgG or in 1 class only) among asymptomatic and symptomatic individuals. The sensitivity and specificity rates were reported at the level of 35–40% and 85–99%, depending on the presence of symptoms and time from the onset of the disease. Similar to this analysis, the authors of this study did not perform molecular tests in all the study participants and this fact seems to be the major weakness of the study which does not allow assessing the rate of the false-negative results.

In contrary, Wu et al. [17] demonstrated a reliable performance in the detection of SARS-CoV-2-specific antibodies of 4 POC serological tests. However, it should be emphasized that the tests were performed only in symptomatic patients and detection sensitivity was time-dependent, so the results of these POC tests should be interpreted in the context of the clinical picture. This analysis was performed in persons without any clinical symptoms of COVID-19 for the assessment of the usefulness of the rapid cassette tests in mass screening [17].

CONCLUSIONS

The results of the current study confirmed the uselessness of the rapid antibody tests for mass screening and detection of SARS-CoV-2 infections in asymptomatic persons. Due to low sensitivity and specificity, they should not be considered as a diagnostic method alternative to molecular tests.

REFERENCES

1. Flisiak R, Horban A, Jaroszewicz J, Kozielowicz D, Pawłowska M, Parczewski M, et al. Management of SARS-CoV-2 infection: recommendations of the Polish Association of Epidemiologists and Infectiologists as of March 31, 2020. *Pol Arch Intern Med.* 2020;130(4):352–7, <https://doi.org/10.20452/pamw.15270>.

2. Flisiak R, Horban A, Jaroszewicz J, Koziulewicz D, Pawłowska M, Parczewski M, et al. Annex #1 as of 8 June 2020 to: Management of SARS-CoV-2 infection: recommendations of the Polish Association of Epidemiologists and Infectiologists as of March 31, 2020. *Pol Arch Intern Med.* 2020;130:557–8, <https://doi.org/10.20452/pamw.15424>.
3. Ji T, Liu Z, Wang G, Guo X, Akbar Khan S, Lai C, et al. Detection of COVID-19: A review of the current literature and future perspectives. *Biosens Bioelectron.* 2020;166:112455, <https://doi.org/10.1016/j.bios.2020.112455>.
4. Rastawicki W, Rokosz-Chudziak N. Characteristics and assessment of the usefulness of serological tests in the diagnostic of infections caused by coronavirus SARS-CoV-2 on the basis of available manufacturer's data and literature review. *Przegl Epidemiol.* 2020;74(1):49–68, <https://doi.org/10.32394/pe.74.11>.
5. Loeffelholz MJ, Tang Y-W. Laboratory diagnosis of emerging human coronavirus infections – the state of the art. *Emerg Microbes Infect.* 2020;9(1):747–56, <https://doi.org/10.1080/2221751.2020.1745095>.
6. Hanson KE, Caliendo AM, Arias CA, Englund JA, Hayden MK, Lee MJ, et al. Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19: Serologic Testing. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2020;ciaa1343, <https://doi.org/10.1093/cid/ciaa1343>.
7. Okba NMA, Muller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients. *Emerg Infect Dis.* 2020;26(7):1478–88. <https://doi.org/10.3201/eid2607.200841>.
8. Lisboa Bastos M, Tavaziva G, Abidi SK, Campbell JR, Haraoui L-P, Johnston JC, et al. Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ.* 2020;m2516, <https://doi.org/10.1136/bmj.m2516>.
9. Pallett SJC, Rayment M, Patel A, Fitzgerald-Smith SAM, Denny SJ, Charani E, et al. Point-of-care serological assays for delayed SARS-CoV-2 case identification among health-care workers in the UK: a prospective multicentre cohort study. *Lancet Respir Med.* 2020;8(9):885–94, [https://doi.org/10.1016/S2213-2600\(20\)30315-5](https://doi.org/10.1016/S2213-2600(20)30315-5).
10. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). *Clin Infect Dis.* 2020;71(15):778–85, <https://doi.org/10.1093/cid/ciaa310>.
11. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev.* 2020;6:CD013652, <https://doi.org/10.1002/14651858.CD013652>.
12. Paradiso AV, De Summa S, Loconsole D, Procacci V, Salustio A, Centrone F, et al. Rapid Serological Assays and SARS-CoV-2 Real-Time Polymerase Chain Reaction Assays for the Detection of SARS-CoV-2: Comparative Study. *J Med Internet Res.* 2020;22(10):e19152, <https://doi.org/10.2196/19152>.
13. Loconsole D, Centrone F, Morcavallo C, Campanella S, Salustio A, Quarto M, et al. The Light and Shadow of Rapid Serological Tests for SARS-CoV-2 Infection: Results from a Study in a Large Emergency Department. *Int J Environ Res Public Health.* 2020;17:6493, <https://doi.org/10.3390/ijerph17186493>.
14. Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect.* 2020;9(1):386–9, <https://doi.org/10.1080/22221751.2020.1729071>.
15. Cassaniti I, Novazzi F, Giardina F, Salinaro F, Sachs M, Perlini S, et al. Performance of VivaDiag COVID-19 IgM/IgG Rapid Test is inadequate for diagnosis of COVID-19 in acute patients referring to emergency room department. *J Med Virol.* 2020;92(10):1724–7, <https://doi.org/10.1002/jmv.25800>.
16. Döhla M, Boesecke C, Schulte B, Diegmann C, Sib E, Richter E, et al. Rapid point-of-care testing for SARS-CoV-2 in a community screening setting shows low sensitivity. *Public Health.* 2020;182:170–2, <https://doi.org/10.1016/j.puhe.2020.04.009>.

17. Wu J-L, Tseng W-P, Lin C-H, Lee T-F, Chung M-Y, Huang C-H, et al. Four point-of-care lateral flow immunoassays for diagnosis of COVID-19 and for assessing dynamics of antibody responses to SARS-CoV-2. *J Infect.* 2020;81(3):435–42, <https://doi.org/10.1016/j.jinf.2020.06.023>.

Annex 1. Patient’s declaration (consent to the examination)

<p>I declare that I have been informed about the purposes, effects and risks of testing for the presence of IgM or IgG antibodies in my body, specific for the SARS-CoV-2 virus. I consent to the conduction of this examination by [.....].</p>	
<p>[patient’s first name and last name]</p>	<p>[PESEL No.]</p>
<p>I authorize the following person to obtain information about the result of this examination:</p>	
<p>[first name and last name]</p>	<p>[contact details]</p>
<p>I authorize the following person to access my medical records:</p>	
<p>[first name and last name]</p>	<p>[contact details]</p>
<p>I agree/I do not agree* to share my medical records with a close relative after my death.</p>	

* Delete as appropriate.

[Date and patient’s signature]

Annex 2. Written recommendations to the employee

IgM/IgG status	IgM-	IgM+
IgG-	<ul style="list-style-type: none"> - You most likely have no antibodies and are not protected against the risk of infection. - You should be careful and follow the rules of protection against SARS-CoV-2. 	<ul style="list-style-type: none"> - You probably have antibodies that indicate contact with the virus. To be sure of the diagnosis, a molecular test will be performed based on a swab from the nose and throat. - For safety reasons, leave the workplace with your own transport and stay at home, observing the rules of isolation and protection against SARS-CoV-2. During this time, use a protective mask. - Until the result of the molecular test, you will receive a sick leave or you can work remotely (as instructed by the employer), and if this is not possible, the employer may decide to use your outstanding vacation leave.
IgG+	<ul style="list-style-type: none"> - You probably have antibodies that indicate contact with the virus. To be sure of the diagnosis, a molecular test will be performed based on a swab from the nose and throat. - For safety reasons, leave the workplace with your own transport and stay at home, observing the rules of isolation and protection against SARS-CoV-2. During this time, use a protective mask. - Until the result of the molecular test, you will receive a sick leave or you can work remotely (as instructed by the employer), and if this is not possible, the employer may decide to use your outstanding vacation leave. - Even though you are probably not infected anymore, you should still exercise caution and follow the rules of protection against SARS-CoV-2. 	<ul style="list-style-type: none"> - You probably have antibodies that indicate contact with the virus. To be sure of the diagnosis, a molecular test will be performed based on a swab from the nose and throat. - For safety reasons, leave the workplace with the own transport and stay at home, observing the rules of isolation and protection against SARS-CoV-2. During this time, use a protective mask. - Until the result of the molecular test, you will receive a sick leave or you can work remotely (as instructed by the employer), and if this is not possible, the employer may decide to use your outstanding vacation leave.

Medical recommendations: IgG/IgM antibodies detected/not detected* in the fingerstick blood drop test for SARS Cov2 (see the description above).

[date and doctor's signature]

* Delete as appropriate.