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SENSITIVITY AND SPECIFICITY OF THE ASSAYS IN THE COVID-19 PANDEMIC

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Abstract. *Quick identification of coronavirus was an emergency in the COVID-19 pandemic. The most used diagnostic tools were serologic, rapid antigen tests, as fast, easily applicable, and affordable, but with lower sensitivity. The results were usually confirmed with a reverse transcription polymerase chain reaction. This assay requires proper expertise and robust laboratory equipment. It is further, costly and time-consuming, with restricted application in low-income countries. Even so, it is used as a golden standard, since it has high specificity and sensitivity. The serologic antibody-based assays were also applied during this Covid-19 burden. Their application was able two weeks after the Covid-19 onset since that was the period when antibodies might be detected. Here are briefly presented the advantages and disadvantages of these assays. Meanwhile, the majority of the diagnostic tests were developed, with some of them being automated and highly sensitive, but often costly. The general recommendation is the improvement of the sensitivity of the serologic tests and development of the easily applicable, fast, and accurate diagnostic tests.*

Keywords: *Covid-19, RT-PCR, Rapid antigen test, RATs, SARS-CoV-2*

1. INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused worldwide coronavirus disease in 2019. World Health Organization (WHO) declared the pandemic in January 2020 [1, 2]. By now, the variants of SARS-CoV-2, such as Alpha, Delta, and Omicron were identified, reflecting the adaptability in the course of the virus survival. Thus, it is hard to predict the novel mutations in the genes for the spike, or other coronavirus proteins, leading to the new virus variants [3, 4]. In the terms of the rising pandemic, fast and reliable diagnostic tests were an urgent need in the symptomatic but also for the asymptomatic patients.

There is no single test suitable for all the phases of the Covid-19 disease, so the diagnostics, screening, or surveillance requires appropriate assays [2]. Even so, the RATs (rapid antigen tests) and PCR (polymerase chain reaction) were the most common and often complementary diagnostic approaches used during the Covid-19 pandemic. Several systematic reviews and meta-analysis explored their accuracy measuring their sensitivity and specificity [5-9]. The "sensitivity" refers to the accurate identification of the patients that have the disease. On the other side, the test "specificity" refers to the identification of the patients without the disease. Here were briefly presented key issues regarding both topics but with a focus on the diagnostic accuracy.

2. SENSITIVITY AND SPECIFICITY OF THE ANTIGEN AND ANTIBODY BASED ASSAYS

2.1. Sensitivity and specificity of the antigen based assays

The rapid antigen tests were preferable in the context of early diagnostics since they were applicable a few days after the symptom appearance. RATs were mainly referred to the immunochromatographic (ICT) assay and the fluorescence immunochromatographic assay (FIA) [10]. In general, these tests are cost-effective, with 15-30 minutes, fast-obtained results [2, 11]. Usually, these tests are applied before PCR, but they could also detect coronavirus in asymptotic patients. They are relatively portable and applicable in low and middle-income countries with limited staff expertise and laboratory equipment [2].

Several reviews and meta-analysis reported their lower sensitivity as a potential assay disadvantage, but often high specificity. One of them reported the pooled sensitivity of the 68.4% (95% CI: 60.8–75.9; I^2 = 98%) and RATs' specificity of 99.4% (95% CI: 99.1–99.8; I^2 = 90%) [7]. Similar findings were from the meta-analysis performed on 60 studies with real time PCR as a reference test [9]. The pooled sensitivity was 69% (95% CI: 68–70) and specificity was 99% (95%CI: 99–99) [9]. Another meta-analysis of 19 studies, provided results for RATs sensitivity between 28.9% (95% CI 16.4–44.3) and 98.3% (95% CI 91.1–99.7). RATs specificity was between 92.4% (95% CI 87.4–95.9) and 100% (95% CI 99.7–100) [8].

2.2. Sensitivity and specificity of the antibody based assays

The serologic antibody-based assays were also performed, but the evaluation of the antibodies was able two weeks after Covid-19 onset [7, 10, 12]. These antibody-based assays refer to enzyme-linked immunosorbent assays (ELISAs), lateral flow immunoassays (LFIAs), or chemiluminescent immunoassays (CLIAs). They were examined with meta-analysis, although with a great heterogeneity of the studies reported, the pooled sensitivity ranged from 66.0% to 97.8% [12]. Further, similarly to RATs, the pooled specificities ranged from 96.6% to 99.7% [12].

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Another study reported the overall sensitivity from 0%-100% and specificity from 78%-100% [5].

These immunological assays in general, require an improvement of the sensitivity in the context of their diagnostic accuracy.

3. Sensitivity and specificity of the polymerase chain reaction

The nucleic acid amplification tests (NAAT) need well-trained staff with specialized laboratory equipment, particularly for RNA viruses like SARS-CoV-2. One of the recommended NAAT techniques for Covid-19 detection was reverse transcription-quantitative PCR (RT-PCR) [5]. The major advantage of the PCR methods is the applicability to different areas of research after the proper assay optimization [13, 14].

This molecular test could be performed with genetic material isolated from various clinical samples, as reported, from the "sputum, nasopharyngeal or oropharyngeal swabs, bronchoalveolar lavage fluid, nasal or nasopharyngeal aspirate, and lower respiratory tract aspirates" [15]. The basic principle of this method relies on the purified total RNA transformed by an enzyme, reverse transcriptase, into complementary DNA (cDNA). Then, after obtaining the cDNA, an amplification of the target gene is accomplished by quantitative PCR [16]. RT-PCR is very sensitive since even a single copy of the target genomic sequence could be amplified and detected [17], so it might provide an answer to the question if a person has ever been infected with the SARS-CoV-2. Reported sensitivity for the RT-PCR ranged from 79-83%, and specificity was 100% [6]. Another review reported clinical sensitivity that ranged from 75-100% and clinical specificity 80-100% [15]. It is usually used in the patients with the developed symptoms of Covid-19, but with the false-negative RATs to confirm the results.

To prevent contamination and technical errors, fully automated PCR assays were applied [18]. The automation includes nucleic acid extraction, purification, amplification, and detection. These systems shorten the time for obtaining results; they could process a large number of the samples, and minimize the exposure of the personnel to viral samples. The two most common and fully automated assays were the Cobas® SARS-CoV-2 (Roche Molecular Diagnostics, Pleasanton, CA, USA) and Abbott Molecular (Des Plaines, IL, USA). Their pooled sensitivity was high indeed, confirmed by the meta-analysis, so for the Roche Diagnostics/SD Biosensor was 82.4% (95% CI 74.2–88.4) and for the Abbott was 76.9% (95% CI 72.1–81.2) [8]. Also, a study reported sensitivity of 93% and specificity of 100% of Abbott Molecular for detecting SARS-CoV-2 [19]. These assays are certainly beneficial, but with considerable application in low-income countries.

All in all, other conventional and not automated RT-PCRs were costly indeed, time-consuming, but also with reported false negative and false positive findings [20, 21]. Even with the relative disadvantages in the terms of the costs, time to obtain results, and worldwide application, RT-PCR is used as a golden standard in Covid-19 diagnostics due to its high sensitivity and specificity.

4. Publicly available database for Covid-19 statistics

A comprehensive and accessible database, Our World in Data [22] provides interactive graphics of the several important Covid-19 pandemic issues, i.e. deaths, tests, hospitalizations, vaccinations, mortality risks, excess mortality, policy responses e.tc. In the context of Covid-19, it provides statistics of the 207 country profiles with briefly explained metrics and the sources of the data, but above all, it is updated daily. Based on this database, here were presented Covid-19 testing policies, for May 19, 2022 (Figure 1).

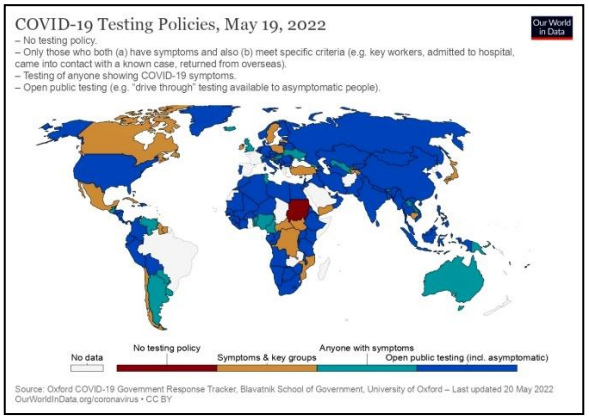

Figure 1. Covid-19 testing policies, for May 19, 2022 (Accessed 20.05.2022)

This interactive graphic provides information by country, increasing from "no testing policy" to "open public testing (including asymptomatic persons)" for January 1, 2020, by May 19, 2022 (Figure 1). So, over time majority of the countries performed open public testing in the first three months of 2022, with a reasonably decreasing in the recent days.

All the Covid-19 testing provided by this database mainly refers to the PCR alone, or the PCR in combination with RATs, depending on the country. The same database provides a seven-day rolling average in the interactive graphic termed "Daily new Covid-19 tests", but here was presented by the day of assessment (Figure 2).

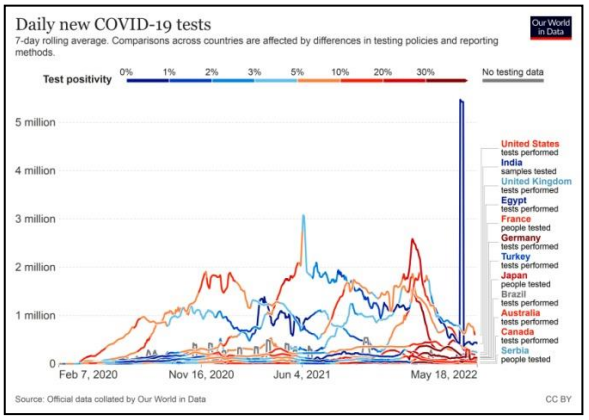

Figure 2. Daily new Covid-19 tests in different countries (Accessed 20.05.2022)

Data obtained from the Our World in Data for the Republic of Serbia on a 7-day rolling average are presented in Figure 3. At the beginning of December 2020, there were around 20,000 tested people, and test positivity was more than 30%. Further, at the beginning of February 2022, there were more than 33,000 tested people, and test positivity was more than 50%. Exact numbers were presented on the interactive graph on the Our World in Data web page. Here the data were presented for the date of assessment (01.06.2022).

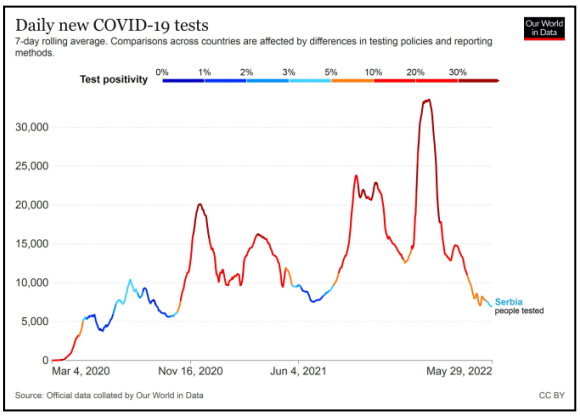

Figure 3. Daily new Covid-19 tests in Serbia, for June 01, 2022 (Accessed 01.06.2022)

The data from this database probably should be interpreted with caution, since the certain heterogeneity, for example in the different PCR or RAT tests applied in combination or alone, asymptomatic, and undetected patients, differences in reporting systems (test number in some countries refers to the persons tested, while in others refers to the number of the tests performed, meaning that the single person could have more repeated test). On the other side, some countries reported the number of tests performed is unclear. Regardless, the database provides a clear explanation of the data collected for each of the 187 counties briefly for the source, tests, cases, positive rates, and description in detail.

Even the certain limitations of the database, the interactivity of the graphics provide a source for conclusions on the variety of the issues concerning Covid-19.

4. Conclusion

The summarized findings of the overview of serological and PCR tests were presented here underlining their main advantages. The RATs were used as the first line of detection since the relatively fast-obtained results. Antibody tests were applicable two weeks after Covid-19 onset that respond with the time of the antibody appearance. Even RT-PCR has certain robustness, referring to the costs, long time to access the results, laboratory, and expertise requirements; it is often used as a confirmatory and complementary test to the RATs. An improvement of the diagnostic tools in the course of increment of their speed and accuracy is suggested.

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