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Coronavirus Pandemic

A comparison of a SARS-CoV-2 rapid-test and serological-test in a Public Health Hospital

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Abstract

Introduction: Nowadays, with the start of the vaccination campaign is very important to assess the extent of exposure of the population and identifying rapid, sensitive and accurate test to quickly identify new cases of SARS-CoV-2. The rapid test, cheap and easy to perform, is therefore very useful in developing countries, where the vaccination campaign has not yet reached adequate coverage.

Methodology: We compared the VivaDiag COVID-19 IgM/IgG Rapid Test (VivaCheck Biotech Co., Ltd) with the Roche Elecsys Anti-SARS-CoV-2 (Roche Diagnostics, Rotkreuz, Switzerland) to recognize past infections and to compare VivaDiag COVID-19 IgM/IgG Rapid Test (VivaCheck Biotech Co., Ltd) with Abbott Real Time PCR SARS-CoV-2 assay to recognize infection during its acute phase so that it's possible to evaluate the use of commercially available assays in clinical practice.

Results: Of the 1,100 patients tested with serological and rapid test, 1,085 were negative both to serological and rapid test, 4 patients were positive at rapid (2 for IgM and 2 for IgG) but negative serological test, 11 patients were positive at serological test but negative to rapid.

Of the 300 tested with oropharyngeal swab and rapid test, 294 were negative both to swab and rapid test, 2 positives both to swab and rapid test, 3 positives at swab but negative at rapid test, 1 negative at swab but positive at rapid test.

Conclusions: the combined use of these tests according to the specific needs of users, allows a reliable identification of infected patients in the acute phase, distinguishing them from subjects with an antibody response from a previous infection.

Key words: SARS-CoV-2; rapid test; serological test; asymptomatic subjects; antibody response.

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Introduction

SARS-CoV-2 is a novel RNA virus from the coronavirus family that emerged at the end of December 2019 in Wuhan, China. The World Health Organization (WHO) declared Coronavirus disease 2019 (COVID-19) a pandemic on March 11, 2020 [1-3]. According to phylogenetic analysis, this severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the B lineage of Betacoronavirus genus and the Sarbecovirus subgenus and has more than 85% nucleotide sequence identity with a bat SARS-like CoV [4]. Fever, cough and respiratory distress are the dominant symptoms, associated with sore throat, muscle pain, joint aches, emesis, diarrhea, anosmia dysgeusia as minor symptoms [5,6]. Large scale testing, rapid diagnosis and immediate isolation of cases coupled with rigorous tracking and preventive self-isolation of close contacts are essentials measures to reduce the burden of the COVID-19 pandemic [7]. Since the beginning of the pandemic, medical companies and research institutes have been looking for developing and approving tests to detect current and past viral infection [8].

Diagnosis of suspected cases is confirmed by nucleic acid assays with real-time PCR, using respiratory samples like nasopharyngeal or oropharyngeal swabs [9]. The gold standard test for diagnosis of SARS-COV-2 infection involves detection of viral RNA using nucleic acid amplification tests (NAAT), such as reverse transcription RT-PCR [10], used in the diagnosis of COVID-19 in those with respiratory illness, as well as screening of contacts. These tests can detect infection during its acute phase because SARS-CoV-2virus can initially be detected 1 or 2 days prior to the onset of symptoms in the upper respiratory samples and can persist for 7 to 12 days in moderate cases and up to 2 weeks in severe cases [11]. Even though analytical sensitivity is generally known

to be very high, detection is dependent on several crucial factors such as sampling timing related to the day of illness, sample types [12]. To be performed this test requires facilities to be set up and instruments, with appropriate biosafety measures and skilled laboratory technicians, at a significant cost. Other issues that need to be addressed are the safety of healthcare personnel collecting, storing and transporting the samples and laboratory personnel handling and processing the potentially infectious samples [13-15]. Both, VivaDiag COVID-19 IgM/IgG Rapid Test and Roche Elecsys Anti- SARS-CoV-2can detect specific antibodies for SARS-CoV-2in blood samples. Seroconversion is observed after a median of 3-6 days after symptom onset for IgM and 8-12 days for IgG [16,17].

The aim of this study is to compare the VivaDiag COVID-19 IgM/IgG Rapid Test (VivaCheck Biotech Co., Ltd, Hangzhou, China) with the Roche Elecsys Anti- SARS-CoV-2 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) to recognize past infections and to compare VivaDiag COVID-19 IgM/IgG Rapid Test (VivaCheck Biotech Co., Ltd, Hangzhou, China) with Abbott Real Time PCR SARS-CoV-2assay to recognize infection during its acute phase so that it's possible to evaluate the use of commercially available assays in clinical practice.

Methodology

Study population

To compare the rapid and the serological test, 1,100 healthy subjects, 494 males and 616 females, were enrolled in this study between 15 May 2020 and 09 October 2020 in Naples, Italy. The participants were aged between 20 and 82 years, with a median age of 48 years, and were enrolled on a voluntary basis undergoing both the rapid and serological test, prior triage, after signing informed consent forms. The participants belonged to a homogeneous and casual group of Federico II University employees. These subjects were evaluated for suspicious symptoms (cough, fever, respiratory distress, sore throat, muscle pain, joint aches, emesis, diarrhea, anosmia dysgeusia), foreign travels and connections with infected cases, in the two weeks before the test, through informed consensus. All subjects underwent, in the U.O.C. of Maxillo Facial Surgery of Federico II University of Naples, a rapid and serological test at the same time and, in case of positivity of one of them, a nasopharyngeal oropharyngeal swab was performed within 24 hours. The serological samples and the swabs were analyzed in the Laboratory of Molecular Virology of the AOU Federico II in Naples.

To compare the rapid test with the oropharyngeal swab, 300 subjects with maxillo-facial pathologies, 135 males and 165 females, were enrolled in this study between 1 October 2020 and 29 January 2021 in Naples, Italy. All these participants were aged between 20 and 82 years, with a median age of 48 years, and underwent both rapid test and oropharyngeal swabs, prior triage, after signing informed consent forms, to get access to the AOU of Maxillo Facial Surgery of Federico II University of Naples. These subjects were evaluated for suspicious symptoms (cough, fever, respiratory distress, sore throat, muscle pain, joint aches, emesis, diarrhea, anosmia dysgeusia), foreign travels and connection with infected cases, in the two weeks before the test, through informed consensus. All subjects underwent a rapid test and an oropharyngeal swab at the same time in the U.O.C. of Maxillo Facial Surgery of Federico II University of Naples, analyzed in the Laboratory of Molecular Virology of the AOU Federico II in Naples.

Methods

VivaDiag COVID-19 IgM/IgG Rapid Test (VivaCheck Biotech Co., Ltd, Hangzhou, China), is for the rapid and qualitative detection of IgM and IgG antibodies to SARS-CoV-2in human whole blood (fingertip or veins). This test is a qualitative test and for its rapidity is optimal for mass screening. The Rapid test is based on immunoassay technology. The test device contains: 1) conjugate pad: recombinant SARS-CoV-2 Antigen labeled with colloidal gold which linked FITC, FITC Antibody and quality control antibody gold marker; 2) NC membrane: coated with two detection lines (IgG and IgM line), and one control line (C line). The IgM and IgG detection lines coated with mouse anti-human IgM and IgG antibody, respectively. The quality control line C is coated with quality control antibody. If the sample contains the SARS -CoV2 antigen, it is labeled with colloidal gold to form a sandwich complex with the coated anti-human IgM or IgG monoclonal antibody. The IgM or IgG line will appear purplish-red indicating the positivity for SARS-CoV-2 antibody. In the well added 10 µL sample whole blood and 60-80 µL of buffer. Within 15 minutes the result is ready.

The Elecsys Anti- SARS-CoV-2 is an electrochemiluminescence immunoassay (ECLIA) detecting total antibodies (including IgG) to SARS-CoV-2 in human serum and plasma, using a recombinant protein representing the nucleocapsid antigen (N antigen). Results are reported as a cut off index (COI) and interpreted as negative (COI < 1.0) or

positive (COI > 1.0). Positive and negative controls were prepared using pooled patient samples according to manufacturer's instructions. Controls and patient samples were analyzed on a fully automated Cobas e411 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) according to manufacturer's instructions. Swabs were analyzed for the presence of SARS-CoV-2 RNA using Abbott Real Time PCR SARS-CoV-2 assay.

Abbott Real Time PCR SARS-CoV-2 assay is qualitative assay, CE-IVD marked and FDA approved, and it detects a dual target: RdRp and N-genes. A real time PCR assay detects a positive sample by the accumulation of a fluorescent signal indicating the amplification of the target sequence. The cycle threshold is defined by the number of cycles required for the fluorescent signal to cross the background level (threshold). Ct levels are inversely proportional to the amount of target nucleic acid in the sample, the lower the Ct number, the greater the amount of target sequence. In particular, this assay has a Limit of Detection (LoD) of 100 copies/mL and its sensibility and specificity are 93% and 100%, respectively. The samples were stored at -80 °C for further assays.

Results

Of the 1,100 patients tested with serological and rapid test, 1,085 were negative both to serological and rapid test, 4 patients were positive at rapid (2 for IgM and 2 for IgG) but negative serological test, 11 patients were positive at serological test but negative to rapid.

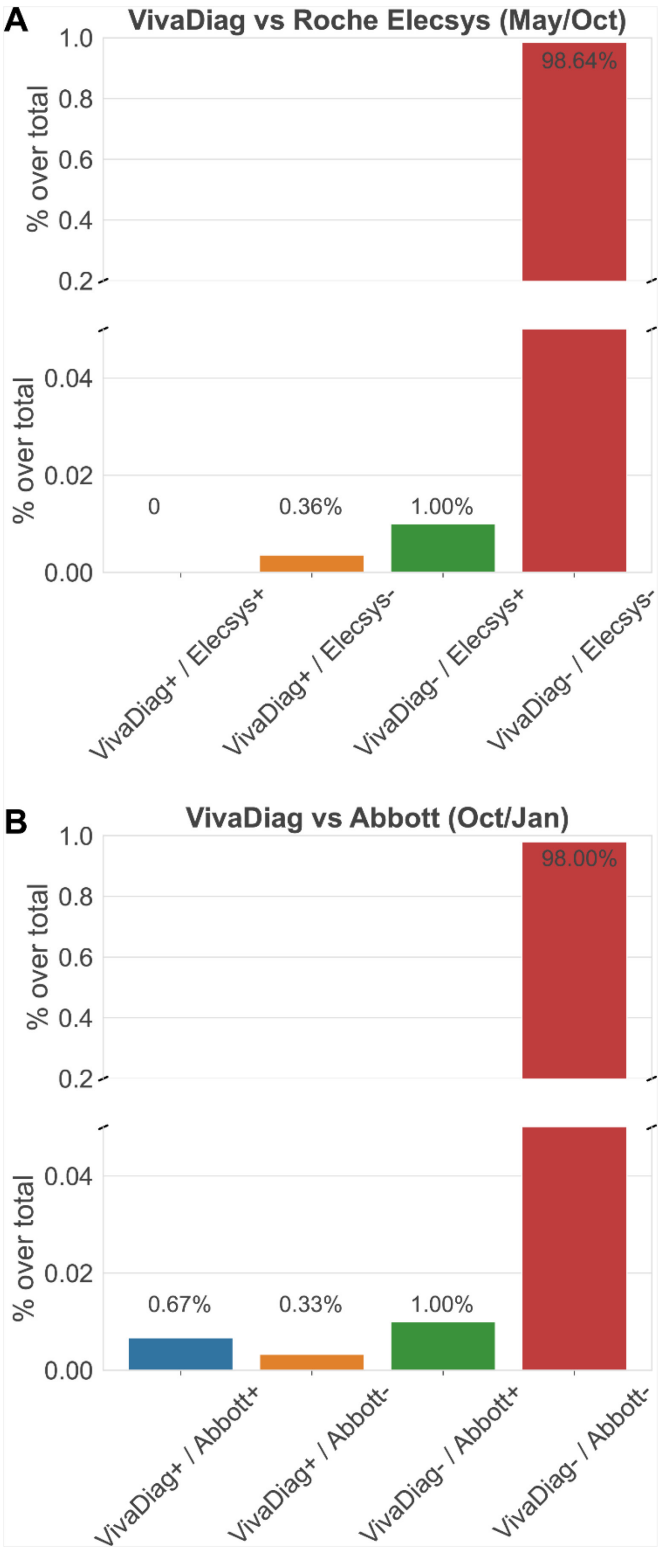
Of the 300 tested with oropharyngeal swab and rapid test, 294 were negative both to swab and rapid test, 2 positives both to swab and rapid test, 3 positives at swab but negative at rapid test, 1 negative at swab but positive at rapid test.

By calculating the percentage of agreement and / or discrepancy of the two tests, therefore comparing the number of positive and negative occurrences of different tests, the percentage obtained represents the number of times in which the two tests have given an agreement or disagreement (Figure 1).

Discussion

The gold standard for the research of SARS-CoV-2 is currently the nasopharyngeal swab, which, if performed with appropriate timing, allows for the identification of subjects with an ongoing infection [9,10]. It's usually performed on symptomatic patients or subjects who had unprotected contact with an established case [12]. Both the rapid and the serological test, on the other hand, allow for the identification of subjects who have come into contact with the virus in the past and have developed antibodies, the IgM and

Figure 1. A. Percentage of results obtained in both VivaDiag. **B.** Abbott tests and Percentage of results obtained in both VivaDiag and Roche Elecsys tests.



IgG immunoglobulins, identifying individuals exposed to the virus [18-24].

In the study carried out 11 subjects tested positive, of the 1,100 serological samples carried out, but negative for both IgG and IgM on the rapid test. The oropharyngeal swab performed on these subjects at 24 hours from the result of the serological test was found to be negative, which is why the percentage of subjects positive to the serological test in our test population was less than 1% (Figure 2).

The entire population who has resulted positive to the serological test was composed of individuals who before the closure of the regional borders in Campania, had been in contact with people from different regions and nations, in crowded places.

Our result of 1% of positive subject at the serological test is in agreement with the ISTAT 0.7% statistical prevalence of positive serological test in the Campania population examined [25].

In the study 2 subjects positive for both IgM and IgG and 2 subjects positive only to the IgM for the rapid test (neither were included in the 11 cases resulted positive to the serological test), were not found to be positive either in the serological test, performed simultaneously and nor in the rapid test nor in the swab performed in the 24h after the test. Based on these data, the specificity of the rapid test is 0.36% (Figure 3).

Figure 2. Percentage of results obtained in both rapid and serologic test.

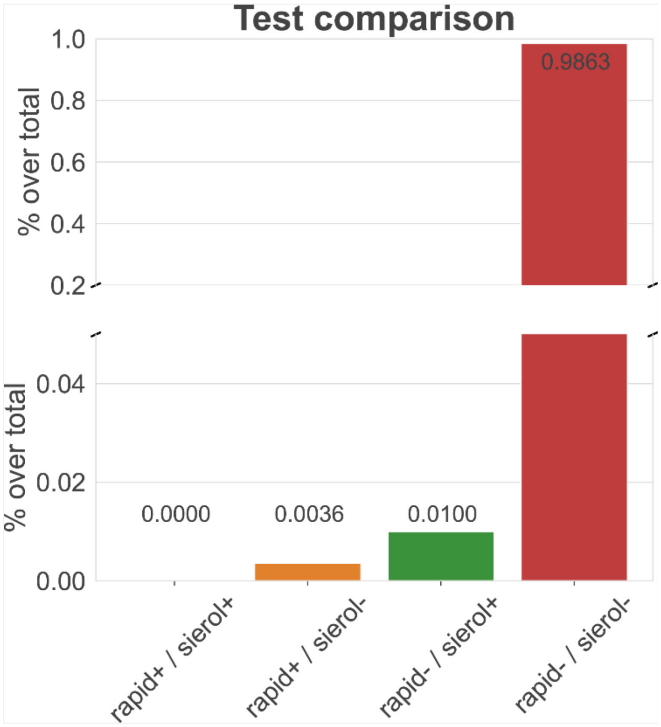
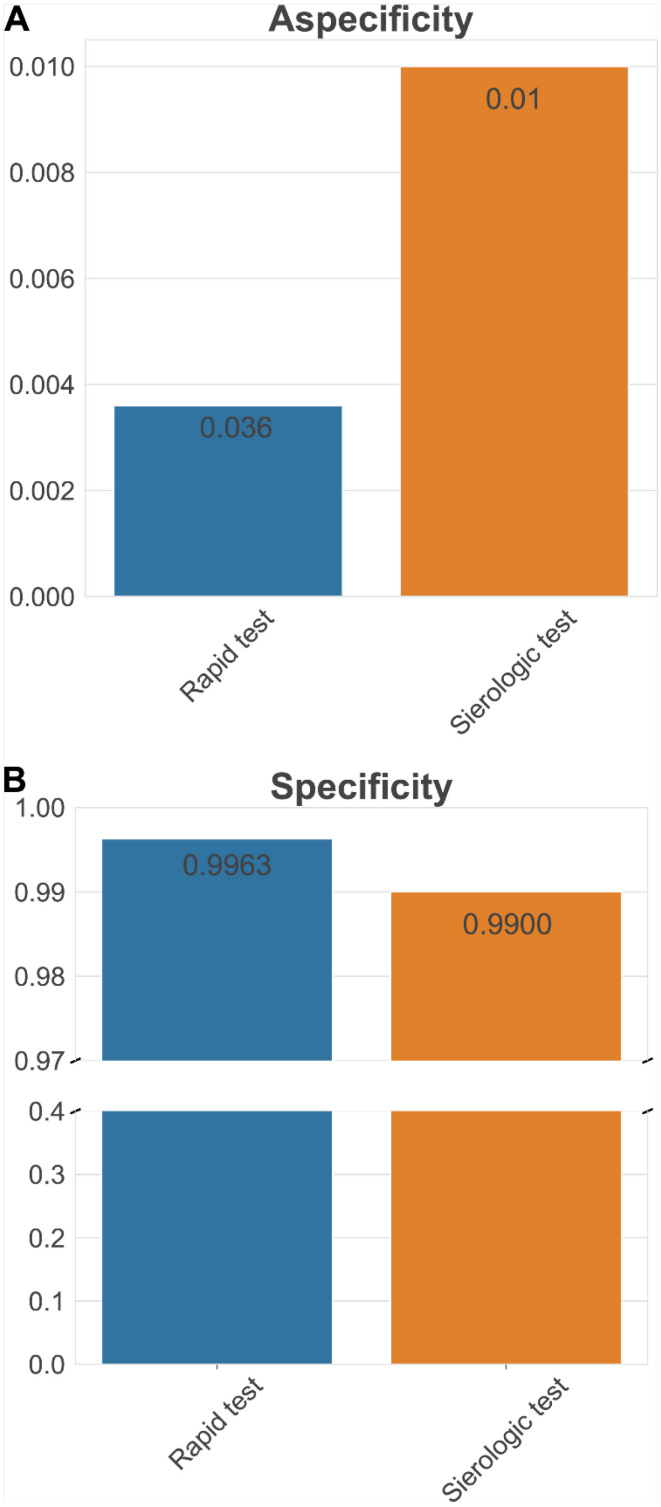


Figure 3. A. Comparison between rapid vs. serologic test, i.e. the possibility of committing error in testing subjects positive to SARS Cov2 when they are actually healthy. **B.** Specificity of the rapid vs. serologic test. Both tests have a high specificity, whereas rapid test results better in identification of true negatives.



In the other study where 300 oropharyngeal swabs were performed, 2 were found to be positive in accordance to the rapid test done at the same time positive for IgM, 3 oropharyngeal swabs were found to be positive in discordance with the rapid test negative both for IgM e IgG, 1 negative oropharyngeal swab was performed on a patient with rapid test positive for IgM. The oropharyngeal swab performed on these subjects at the same time as the rapid test, and was processed in the Laboratory of Molecular Virology of the AOU Federico II in Naples in the next 12h. Based on these data, a specificity of the rapid test is 0.36%. (Figure 4) Both tests have a high specificity, whereas rapid test results are better in the identification of true negatives (Figure 5). Further studies on false-positive and false-negative rates should be performed in the future to determine the specificity of these types of tests as Lisboa Bastos M et al underline in their review of 40 studies [26]. In their systematic review with meta-analysis was verified a sensitivity of ELISAs measuring IgG or IgM of 84.3% (95% confidence interval 75.6% to 90.9%). To our knowledge, our study is one the first to analyze compare both tests.

In this moment, when the vaccination campaign has begun but we do not know when it will be able to involve a sufficient percentage of the population, and at the same time daily activities, including medical activities, must resume at full speed, it is necessary to identify a method that allows health professionals as well as patients who belong to public or private health facilities to protect themselves from possible contagion. The rapid test, cheap and easy to perform, is therefore very useful in developing countries, where the vaccination campaign has not yet reached adequate coverage.

Based on the need to identify a quick but at the same time safe method, we compared the rapid test, which has the advantage of giving an immediate result and can be performed without the need for specific equipment with more complex methods such as the gold standard, buffer nasopharyngeal, which, however, requires time, not always compatible with medical needs, dedicated personnel and instrumentation and with the serological test, which also requires adequate health personnel and specific machinery and which, moreover, allows for the evaluation of the antibody response of an infection not necessarily yet in the acute phase.

Figure 4. Comparison between VivaDiag vs Roche Elecsys test in May-October subjects, i.e. the possibility of committing error in testing subjects positive to SARS Cov2 when they are actually healthy.

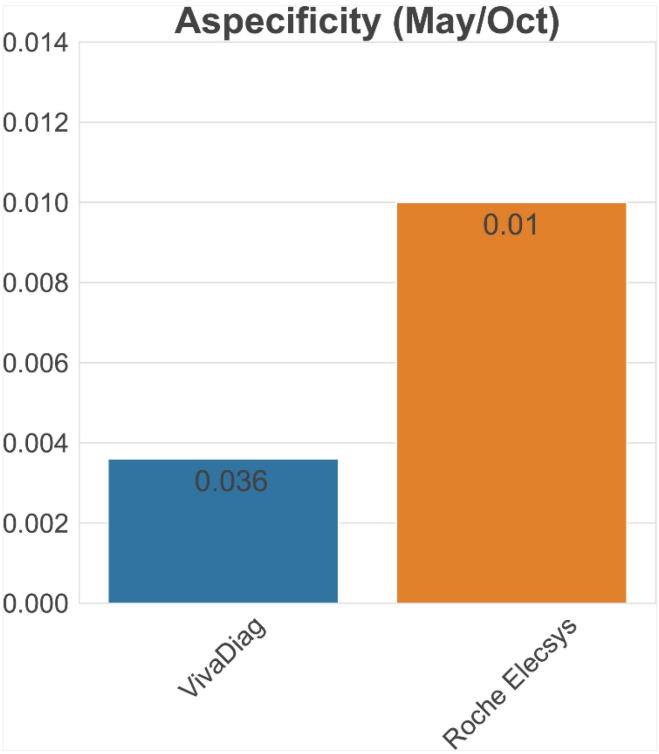
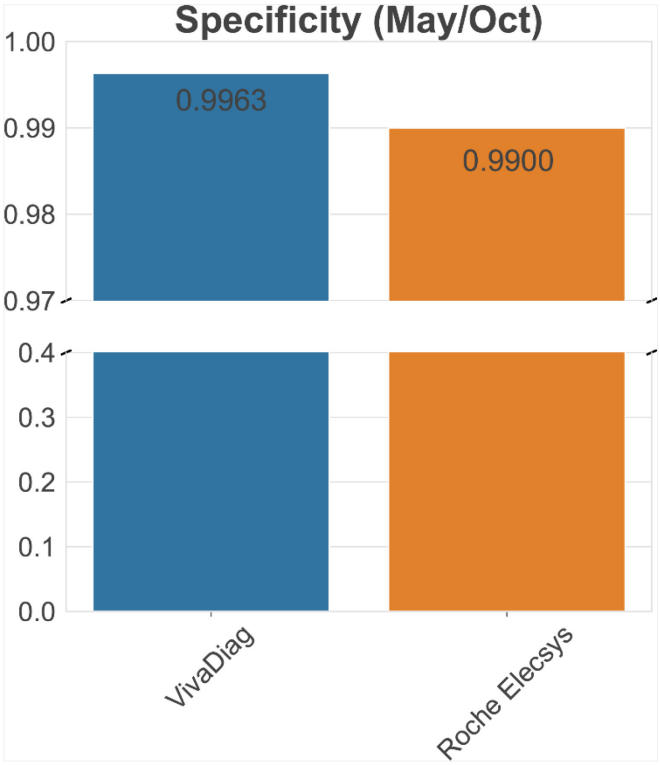


Figure 5. Comparison between VivaDiag vs Roche Elecsys test in May-October subjects. Both tests have a high specificity, whereas rapid test results are better in identifying true negatives.



Conclusions

Of the 1,100 patients tested with serological and rapid test, in 1,085 patients the results of the two tests agreed, only 4 patients were positive at rapid (2 for IgM and 2 for IgG) but negative serological test and 11 patients were positive at serological test but negative to rapid.

Of the 300 tested with oropharyngeal swab and rapid test, in 294 patients the results of the two tests agree, only 3 patients were positives at swab but negative at rapid test and 1 patient was negative at swab but positive at rapid test.

There is much to determine regarding the value of serological and rapid testing in COVID-19 diagnosis and monitoring. At this moment, it is very important to contain a possible new outbreak, assessing the extent of exposure of the population and identifying rapid, sensitive and accurate test to quickly identify new cases of SARS-COV-2. It is of crucial importance to differentiate COVID-19 cases, symptomatic and asymptomatic ones, from healthy people. In a population as the same as the one in the analysis, the speed of the diagnosis is essential for the normal workflow. For this reason, is needful to use a rapid test that is in agreement with the standard test.

It is important to keep in mind that, although this study has shown the high reliability of the rapid test, the patient's symptoms should not be underestimated if the rapid test is negative. For this reason according to our results the combined use of VivaDiag COVID-19 IgM/IgG Rapid Test and Elecsys Anti- SARS-CoV-2 yields a 98.64% probability of correct prognosis, while the combined use of VivaDiag COVID-19 IgM/IgG Rapid Test and Abbott Real Time PCR SARS-CoV-2 assay yields a 98.00% chance of correct prognosis, therefore, the combined use of these tests according to the specific needs of users, allows a reliable identification of infected patients in the acute phase, distinguishing them from subjects with an antibody response from a previous infection.

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