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Coronavirus Pandemic

Clinical validation of two immunochromatographic SARS-CoV-2 antigen tests in near hospital facilities

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Abstract

Introduction: Rapid antigen tests to detect SARS-CoV-2 virus need to be validated. The purpose of clinical validation is to place the test into the everyday working process in health care institutions.

Methodology: The clinical validation of Alltest Covid19 antigen test (Alltest, China) and Vivadiag Pro SARS- CoV-2 antigen tests (Vivacheck, China) started in four Slovenian health care institutions in December as a point-of-care test. Institutions compared the results of antigen tests to Seegene Allplex™ 2019-nCoV rt-PCR assay (SeeGene, South Korea) and Cobas 6800 SARS CoV-2 rt-PCR (Roche, USA).

Results: Sensitivity (90.6%, 95% CI = 84.94%-94.36%) and specificity (100%, 95% CI = 99.41%-100%) of Vivadiag Pro SARS CoV-2 Ag test were observed. While validating Alltest Covid19 Ag assay we got similar results (sensitivity 94.37%, 95% CI = 89.20% – 97.54%), specificity 100% (95% CI = 98.83% - 100%).

Conclusions: Vivadiag Pro SARS CoV-2 Ag test and Alltest Covid19 test proved to be a good screening tool to detect SARS-CoV-2. The accurate information about the patient's status was available almost immediately and there was no need to wait for rt-PCR results. We could prevent further spread of the SARS-CoV-2 in primary care and hospital settings.

Key words: SARS-CoV-2 antigen test, clinical validation.

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Introduction

The COVID-19 pandemic continues to pose a major threat to public health in European countries. On 28 October 2020 EU Commission Communication stated that robust testing strategies and sufficient testing capacities are essential aspects of preparedness and response to COVID-19 [1]. Fast antigen tests can give quick results and can be used massively by efficiently trained non-laboratory health care personnel as point-of-care tests (POCTs). Such results could be used as a mitigation step to stop the uncontrolled spreading of the virus in hospitals. [2]. Rapid antigen tests to detect SARS-CoV-2 virus need to be validated [3]. The purpose of clinical validation is to places the test into the everyday working process in health care institutions [4]. Antigen tests tend to have lower sensitivity than rt-PCR tests (Reverse Transcription Polymerase Chain Reaction). However, in the high prevalent situation when SARS-CoV-2 virus is widespread, antigen test could be used as a mitigation measure to control the spread of a virus. World Health Organization (WHO) set the standards for how antigen tests should be used according to their sensitivity and specificity [5]. In this article, we present multicentric prospective clinical validation of two SARS-CoV-2 antigen tests in four Slovenian health care institutions, which tested both assays as POCT from fresh samples.

Methodology

The clinical validation of Alltest COVID-19 Ag test (Cataloge Number INCP-502, Alltest, Hangzhou, China) and VIVADIAG PRO SARS-COV-2 AG RAPID TEST (Cataloge number not available, Vivacheck, Hangzhou, China) antigen tests started in four Slovenian health care institutions in December 2020. All institutions used antigen tests as POCTs. The first institution, a primary health care drive-in testing point started validation at the beginning of December 2020. The second institution, a cardiology clinic, started validation in the middle of December 2020. Both institutions tested just the Vivadiag antigen test. Two secondary care hospitals performed clinical validation of the Alltest test in December 2020. All health care institutions experienced uncontrolled spread in their

Table 1. VivaDiag Pro antigen assay versus rt-PCR.

Vivadiag Pro SARS COV-2 antigen result vs. rt-PCR	n/N total	%	95% Confidence Interval
Sensitivity	136/150	90.6%	84.94%-94.36%
Specificity	644/644	100%	99.41%-100%
Overall Agreement Rate	780/794	98.2 %	97.06%-98.95%

region (14-days incidence was above 1,000/100,000 inhabitants) and the pressure on the health care institutions was extreme [6].

The 1st, 3rd and 4th institution compared the results of antigen tests to Seegene Allplex™ 2019-nCoV rt-PCR assay (SeeGene, South Korea). The 2nd institution compared the results of antigen tests to Cobas 6800 SARS-CoV-2 rt-PCR (Roche, USA) and Biofire Respiratory panel 2.1 plus (Catalogue number 423883, BioFire, Salt Lake City, USA).

All institutions followed the same algorithm. They tested patients with at least one sign of covid-19, fever above 37.5 °C, tiredness, runny nose, dry cough, dyspnoea, loss of taste and smell, or gastrointestinal problems with at least one sign of upper respiratory tract disease. We took two swabs. A second contralateral nasopharyngeal swab was performed by the same health care worker to be used for the rt-PCR test, which was run by a dedicated team of laboratory technicians in the local public health microbiology laboratory. We excluded patients that had symptoms and signs of covid-19 for more than 7 days according to strict manufacturer's instructions. To optimize performance, testing with antigen test was conducted by trained operators on fresh samples in all institutions.

For statistical analysis, we used MedCalc Statistical Software version 18.11.6 (MedCalc Software bvba, Ostend). The study protocol was approved by the Institutional review board of General hospital Jesenice (Nr. 2020-02) for all institutions. Study participants were enrolled by a dedicated medical doctor and provided written informed consent.

Results

Vivadiag Pro SARS-CoV-2 test was validated in three institutions. COVID-19 laboratory in a secondary care hospital analysed 436 samples, primary health care institution analyzed 158 samples, and cardiology clinic analysed 200 samples. Two secondary care hospitals validated additional 333 samples with the Alltest Covid19 Ag test.

We present validation of the Vivadiag Pro SARS-CoV-2 test in Table 1. The sensitivity of the assay was above 90%. Specificity was 100%. We had 14 cases of false-negative results. In all the cases cycle threshold (Ct) was above 30. People who visited primary care institution and had false-negative antigen test results were either presymptomatic or had one mild symptom with an average Ct of 35 for gene E. Seven cases of false-negative antigen results were found in a hospital. A detailed description of symptoms of the hospital patients is presented in Table 2.

We present validation of Alltest Covid19 Ag test versus rt-PCR test in Table 3. The sensitivity of the test was 94% with 100% specificity. We found seven cases of false-negative results. In all cases of false-negative results, patients came to the hospital between the 5th and 7th day with signs and symptoms of COVID-19 in development and had Ct values above 30.

Table 2. Symptoms and cycle thresholds of patients admitted to hospital with false negative antigen test.

Sample number	Date of test	Days since the first symptom - main problem	Cycle Threshold value in rt-PCR
1	7.12	5 - dyspnoea	37.9
2	9.12	6 - dyspnoea	37.3
3	14.12	6 - gastrointestinal problems	32.9
4	16.12	7 - dyspnoea	31.8
5	18.12	5 - tiredness and chest pain from day 4 – ischemic heart attack after Covid- 19	34.5
6	21.12	came to hospital because non COVID related problems	39.9
7	22.12	came to hospital because non COVID related problems	39.8

Table 3. Alltest Covid19 antigen assay versus rt-PCR.

Alltest Covid-19 antigen result vs. rt-PCR	n/N total	%	95% Confidence Interval
Sensitivity	134/142	94.37%	89.20%-97.54%
Specificity	312/312	100%	98.83%-100%
Overall Agreement Rate	446/454	98.23%	96.56%-99.24%

Discussion

This is the first study that compared immunochromatographic SARS-CoV-2 antigen test versus rt-PCR test in different health institutions, from primary care institution to secondary care covid-19 hospital. Both tests, Vivadiag Pro SARS-CoV-2 Ag test and Alltest Covid19 Ag test, fulfilled WHO criteria for clinical performance [5]. Both tests had excellent specificity. The sensitivity of both tests was very good in all tested environments. An expected drop of sensitivity happened in the case of the Vivadiag Pro SARS-CoV-2 Ag test because validation was done in three very heterogeneous situations, by many health care workers, with heterogeneous patients' populations where some of them had symptoms for more than a couple of days. In one case of the presumably false-positive Alltest Covid19 Ag test, the rt-PCR test was a false negative. In the beginning, the laboratory used GeneExpert rt-PCR that gave an invalid result. Laboratory then tried to confirm the results using the Seegene PCR test and got a positive result. A possible explanation for that might be the presence of a mutation as previously described in the literature [7].

Our results are comparable to other studies. In meta-analysis antigen tests sensitivity varied considerably across five studies with 943 samples (from 0% to 94%). The best test, the Shenzen Bioeasy Ag test, had a sensitivity of 89% [8]. One study compared two antigen tests to single gene direct SARS-CoV-2 rt-PCR. BD Veritor SARS-CoV-2 Ag and Sofia SARS FIA Ag tests showed a high degree of agreement for SARS-CoV-2 detection vs. direct single gene rt-PCR [9].

Another study compared several immunochromatographic antigen tests to rt-PCR. Sensitivity was between 16% to 85%. They explained the difference by the use of a non-validated sample material [10]. In one study, antigen test identified 70.6 % of an rt-PCR positive sample. A major limitation of the study was that they diluted the sample in transport media and did the comparison off-site [11]. Our study was done from fresh samples as a POCT. That can explain much better results in comparison to other studies where they used stored frozen samples to validate in an off-site laboratory.

FindDx platform published several validations of the antigen SARS-CoV-2 assays. Both tests used in our comparison have similar performances as the best SARS-CoV-2 Ag immunochromatographic tests in other comparisons (sensitivity between 76.6% and 90.8%, specificity between 99.3% and 100%) [12-14].

Our study has some limitations. We could not collect Ct data for all samples. The study in hospitals began in high prevalence SARS-CoV-2 conditions, which resulted in perfect specificity. We could not pinpoint the exact start of the symptoms for some hospital patients.

Conclusions

Our results show that the validated SARS-CoV-2 antigen tests fulfil the criteria as defined by WHO with 80% sensitivity and 97% specificity in different health care settings. We had some false-negative cases that were explicable with the low viral burden on mucosal barriers and mild disease, or with more than 5 days since the start of first symptoms when SARS-CoV-2 viral proteins already disappear from the mucosal barrier of the upper respiratory tract due to efficient immune response.

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