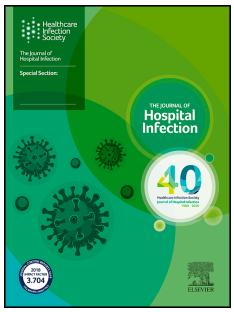

Comparison of five SARS-CoV-2 rapid antigen tests in a hospital setting and performance of one antigen assay in routine practice. A useful tool to guide isolation precautions?

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Comparison of five SARS-CoV-2 rapid antigen tests in a hospital setting and performance of one antigen assay in routine practice. A useful tool to guide isolation precautions?

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Keywords: SARS-CoV-2; rapid antigen detection; rRT-PCR; infection prevention; isolation precautions

Summary

Background

In a hospital setting, there is a need for rapid detection of SARS-CoV-2 to guide isolation measures and targeted admission.

Aim

First, we evaluated the diagnostic performance of five SARS-CoV-2 rapid nucleocapsid protein antigen detection (RAD) assays (Biosynex, Biotical, Orient Gene, Panbio, SD Biosensor). Secondly, we described the performance and impact of the implementation of the SD Biosensor assay at our emergency department.

Methods

Sensitivity and specificity of the five RAD assays were analyzed on 100 respiratory samples: 60 real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) confirmed SARS-CoV-2 positive samples, 24 SARS-CoV-2 RNA negative samples and 16 samples positive for other respiratory pathogens. We adapted the manufacturer's protocol, to validate the antigen tests on transport media used for rRT-PCR in our routine practice.

The SD Biosensor RAD assay was implemented as screening method for rapid diagnosis and targeted admission.

Findings

Sensitivity of the included RAD assays ranged from 88.9% to 100% for samples with Ct <26, specificity from 46.2% to 100%. During the implementation period, 4195 RAD tests were performed. Due to the rapid RAD result, 157 patients were transferred directly to the COVID-19 cohort instead of the regular ward (n=47) or the temporary COVID-19 ward (n=110).

Conclusion

The SD Biosensor, Biotical and Panbio SARS-CoV-2 antigen tests had an acceptable overall performance and seem to be able to detect most of the contagious patients. In the context of high prevalence of SARS-CoV-2, RAD tests can be used as a rapid screening tool, to guide infection prevention measures and aid targeted admission.

Introduction

Since the start of the coronavirus disease 2019 (COVID-19) pandemic in December 2019, the gold standard for the diagnosis of COVID-19 is the detection of SARS-CoV-2 viral RNA in respiratory tract samples by real-time reverse-transcriptase-polymerase chain reaction (rRT-PCR) [1,2]. Despite an excellent sensitivity and specificity, rRT-PCR is a complex analysis and has a long turnaround time (5 to 17 hours) [3]. Several new rapid antigen detection (RAD) tests for the qualitative detection of SARS-CoV-2 antigen on nasopharyngeal swab samples have been introduced. They are easy to perform and results are available after 10-30 min. Specificity is consistently reported to be high. Sensitivity compared to rRT-PCR is highly variable (0-94%) and related to the viral load, with higher sensitivity reported on samples with higher viral load [4-7]. Overall, RAD assays are less sensitive than rRT-PCR. The World Health Organization (WHO) recommends RAD tests to reach a minimum performance of ≥80% sensitivity and ≥97% specificity compared to rRT-PCR [4].

In a hospital setting, taking the appropriate measures to prevent transmission of SARS-CoV-2 is a key priority to protect patients and healthcare workers (HCW) [8]. Assuming that RAD assays have a high specificity, patients with a positive RAD test can be diagnosed with COVID-19 and transferred directly to a COVID-19 ward where appropriate infection prevention precautions are applied. Due to the higher limit of detection of RAD tests compared to rRT-PCR, RAD assays cannot exclude COVID-19 disease but can be used to aid infection prevention and control precautions, as they are expected to detect the most contagious patients and HCW.

In our institution, the COVID-19 pandemic led to the intensification of standard infection prevention precautions with strict application of hand and respiratory hygiene guidelines and social distancing. Additionally, at any time, HCW use a surgical mask, patients and visitors use comfort masks. Strict isolation precautions are applied to patients with suspected or confirmed COVID-19 disease. For a local epidemiological view see Appendix Table I.

All patients admitted to our hospital are tested for SARS-CoV-2 using an in-house rRT-PCR [2]. Patients with symptoms suspected for COVID-19 on admission are admitted to the pre-cohort area (suspected COVID-19 ward) in a single isolation room, while waiting for the rRT-PCR result. If their COVID-19 test is positive, they are transferred to the cohort area (confirmed COVID-19) ward. If COVID-19 is excluded (based on rRT-PCR in combination with clinical presentation and radiology) they are transferred to a

regular ward. Due to the broad clinical presentation of COVID-19 disease, many patients meet the definition of a possible case at initial presentation [9] and are admitted to the pre-cohort ward, delaying specialized care and investigations for their actual medical problem.

Patients who are not suspected for COVID-19 infection on admission are hospitalized at their specific hospital ward, in a single or double room. Notwithstanding the recommendation to wear a mask and to keep the curtain closed between the beds awaiting the rRT-PCR result, this involves a risk of SARS-CoV-2 transmission in case of highly infectious patients (pre/asymptomatic or atypical). For the HCW, COVID-19 should be excluded even in case of mild complaints. Using the in-house rRT-PCR test, this takes at least five hours.

Implementation of a RAD assay could help overcome some of the current issues, by allowing quick identification of (highly) contagious patients/HCW.

The first aim of this study was to evaluate the analytical performance of five new commercially available SARS-CoV-2 RAD assays, in comparison to rRT-PCR. Secondly, we describe the performance and effect of the implementation of a RAD assay as a first line screening test for patients at our emergency department who need to be hospitalized. We hypothesize that the RAD test has potential as a fast diagnostic tool for decision making concerning isolation measures and targeted admission for patients admitted to the hospital in an emergency setting.

Material and methods

Samples/patients

Part one: validation

Sensitivity was analyzed prospectively from 27th October to 1st November 2020 at the OLV Hospital Aalst, Belgium, on 60 SARS-CoV-2 positive nasopharyngeal swabs, confirmed by an in-house rRT-PCR (N1 gene) [2] (stored at room temperature for a maximum of 12 hours). Nasopharyngeal samples were collected in various transport media routinely used in our lab (ESwab™ Amies, COPAN; Sigma Transwab® liquid Amies, MWE; UTM®, COPAN; Sigma Virocult®, MWE; Viral stabilization tube, VACUETTE®; Virus Sampling Tube, Lingen®).

The selection of SARS-CoV-2 positive samples was based on their cycle threshold (Ct) value, regardless of the time since exposure or symptom onset. Samples from confirmed COVID-19 patients were grouped into four categories according to their Ct value: Ct<20 (n=16); 20≤Ct<26 (n=18); 26≤Ct<30 (n=18); 30≤Ct<36 (n=8).

Specificity was evaluated using 40 samples, 24 nasopharyngeal SARS-CoV-2 RNA negative swabs (collected between October 27 and November 1st 2020) and a cross-reactivity panel consisting of 16 samples (nasopharyngeal swabs and aspirates) positive for one or multiple other respiratory pathogens (obtained prior to the COVID-19 outbreak and stored at -80°C). These samples contained human metapneumovirus (n=3), human coronavirus HKU (n=3), human coronavirus OC43 (n=2), human coronavirus NL63 (n=2), human coronavirus 229E (n=2), influenza virus A/B (n=6), bocavirus (n=1), respiratory syncytial virus (n=4), rhinovirus (n=2), adenovirus (n=3), enterovirus (n=1), herpes simplex virus (n=1) and *Streptococcus pneumoniae* (n=7) (see Appendix Table II).

Part two: implementation

On the 5th November 2020 the SD Biosensor RAD assay was introduced at the emergency room as a screening method for SARS-CoV-2 on nasopharyngeal swabs, for all patients with an indication for hospital admission of whom there was no recent positive SARS-CoV-2 rRT-PCR result available (<8 weeks). All RAD tests were followed by an in-house rRT-PCR (from 07:00 to 22.00). Prevalence of COVID-19 was calculated based on these rRT-PCR results. Sensitivity, specificity and positive predictive value (PPV) of the RAD test were determined in comparison to the in-house method.

During the study period, variants of concern (VOCs) began to circulate in Belgium. From January 26th, 2021 on, variant analysis using targeted rRT-PCRs was performed on all SARS-CoV-2 RNA positive

samples. In this way, we were able to identify the 20I/501Y.V1 (British), 20H/501Y.V2 (South African) and 20J/501Y.V3 (Brazilian) variant. Since the mutations present in these variants don't target the nucleocapsid protein, it is unlikely that the analytical performance of the RAD test would be affected. This initially was confirmed by retrospectively analyzing some samples containing VOCs. We also determined the sensitivity of the RAD test in VOC containing samples.

SARS-CoV-2 testing

Part 1: validation

After a detailed marketing research, considering performance data, rapid and sustained availability and price, we selected five SARS-CoV-2 RAD tests for evaluation: COVID-19 ag BSS (Biosynex), SARS-CoV-2 Ag card (Biotical), Coronavirus AG Rapid test cassette (Orient Gene), Panbio™ COVID-19 Ag Rapid Test Device (Abbott) and SARS-CoV-2 Rapid Antigen test (SD Biosensor), all based on the detection of viral nucleocapsid protein SARS-CoV-2 antigen (Appendix Table III). The manufacturer's instructions recommend performing the tests directly on the nasopharyngeal swabs included in the test kits. We adapted the protocol, as we wanted to validate the antigen tests on the transport media used for rRT-PCR in routine practice, to avoid additional sampling (limiting discomfort, risk of complications and workload). To be able to compare all five RAD tests on the same sample, we adapted the sample and buffer volumes. For all assays, we used a one on one dilution of sample-extraction buffer. Depending on the test, results were manually read after 10-15 minutes. In the presence of a control line, any signal was interpreted as positive. Tests were read by two independent persons who were blinded for the rRT-PCR results.

Part two: implementation

On the 5th November 2020 the SARS-CoV-2 Rapid antigen test from SD Biosensor was implemented in our laboratory. The SD Biosensor RAD test was selected based on the good sensitivity and specificity in combination with the price and ease of use. The test was performed according to the manufacturer's instructions, with a sample volume of 350µl, but starting from Amies transport medium instead of the swabs included in the test kit. Every sample tested by RAD was confirmed by our in-house rRT-PCR. In case of discordant results, i.e. a RAD positive/rRT-PCR negative or RAD negative/rRT-PCR positive result with Ct value <26, the RAD test cassette was retrospectively checked by a second reader. The RAD test and the rRT-PCR were repeated on the same sample. If no explanation was found from these additional interventions, a new sample was requested to repeat both RAD and rRT-PCR.

Clinical data

To gain insight in the effect of implementation of the RAD test, clinical data were obtained retrospectively from RAD positive patients from 5th November 2020 to 14th March 2021.

RAD positive patients were divided in two groups based on their clinical presentation at the emergency ward, in accordance with the case definition of Sciensano, the national public health institute of Belgium [9]: 'COVID-19 suspected cases' who would have been admitted to the precohort ward and 'non-COVID-19 suspected cases', who would have been admitted to a regular ward.

Ethical approval

This study was approved by the local Ethics Committee OLV Hospital Aalst, study number 2020/123.

Statistical analysis

Sensitivity and specificity were calculated by means of Excel (version 16.0, Microsoft, Washington, USA). Sensitivity was evaluated for all samples, as well as for the four subgroups based on the Ct values. Positive predictive value (PPV) was calculated at the beginning and at the end of the study period, and a projection was made to other prevalences.

The statistical analysis of the comparison between the Ct values from RAD positive and RAD negative samples was performed in MEDCALC® Statistical Software version 17.1 (MedCalc Software Ltd, Ostend, Belgium). D'Agostino-Pearson test was used as normality test. In case of a normal distribution the unpaired Student's t-test was applied, otherwise the Mann-Whitney U test for independent samples. A p-value <0.05 was considered statistically significant.

Results

Part one: validation

60 SARS-CoV-2 rRT-PCR positive and 40 SARS-CoV-2 rRT-PCR negative samples were selected (demographics, see Appendix Table IV). The first two samples collected in the Virus Sampling Tube, Lingen® gave invalid results (no control line) for the SD Biosensor assay and were excluded for all assays.

The specificity of the RAD assays ranged from 46.2% to 100%. The overall sensitivity ranged from 67.2% to 89.7%. More important, in the subgroup analyses based on the Ct values, all five RAD assays showed an excellent sensitivity for samples with Ct-values <20, with sensitivity dropping to <50% for samples with Ct ≥ 30. Detailed results are presented in Table I.

A statistically significant (P<0.001) difference was observed in Ct values of the positive RAD test compared to the negative RAD test (Figure 1), resulting in a sensitivity of 100% for all studied RAD assays for samples with rRT-PCR CT values between 25 and 27.

Part two: implementation

From the 5th November to 14th March 2021 4195 SD Biosensor RAD tests were performed (demographics, see Appendix Table IV). The median rRT-PCR Ct value was 22.4 for the RAD positive samples and 33.9 for the RAD negative/rRT-PCR positive samples (p<0.0001) (Figure 1). 212/4195 (5.1%) RAD tests were positive, compared to 369/4195 (8.8%) rRT-PCR tests. In the observed time period, the SD Biosensor RAD showed an overall sensitivity of 54.2% and a specificity of 99.7% compared to rRT-PCR. In the Ct value range <20 and 20 - < 26, the test showed sensitivities of 100% and 92.6%, respectively (see Table I and Appendix Figure 1). There were 12 false positive results. Review of the test cassette, in combination with repeat RAD testing, revealed incorrect reading of three out of 12 false positive results. There were nine false positives which remained unexplained.

Of the 212 patients who tested positive using the antigen test, as mentioned above, 200 were true positive and 12 were false positive. 157/200 (78.5%) patients who tested true positive were admitted to the hospital. Of these, 47/157 (29.9%) patients had symptoms not typical of COVID-19. They were transferred immediately to the cohort instead of the regular ward. One hundred and ten patients (70.1%) were suspected of having COVID-19 disease and were transferred directly to the cohort instead of the pre-cohort ward (Figure 2).

Ten patients with a false positive result were admitted to the hospital and transferred to a cohort ward. Only one of these ten patients had symptoms suspected for COVID-19. Eight patients were transferred to the cohort ward in a single room. Two patients were hospitalized in a multiperson room. After the

negative rRT-PCR test was known, they were immediately transferred to a single room on a regular ward. One of the patients who stayed in the multiperson room was tested on day 4 and day 8 after his admission and remained SARS-CoV-2 negative. Unfortunately, the second patient hospitalized in a multiperson room tested positive on day 4 after his admission. He was transferred to the cohort ward. Two of the twelve patients with a false positive result were not admitted to the hospital. One of them had signs of fever and dyspnoea. After investigation he was discharged to home from the emergency department. He was readmitted after 48 hours and rRT-PCR was COVID-19 negative. Cardial investigation showed he suffered from infective endocarditis. The second patient suffered from epigastric pain, caused by a duodenal ulcer. This patient was not retested.

During the study period, 32 SD Biosensor RAD tests were performed on samples containing a VOC. There were 25 samples with 20I/501Y.V1, five samples with 20H/501Y.V2 and two samples with 20J/501Y.V3. For 20I/501Y.V1 containing samples, the SD Biosensor RAD showed an overall sensitivity of 76%, and a sensitivity of 100% and 87.5% for samples in the Ct value range of <20 and 20 - < 26 respectively. Except for one sample with a Ct value of 27 containing 20H/501Y.V2, all 20H/501Y.V2 and 20J/501Y.V3 containing samples tested positive using the RAD test.

At the beginning of the study period, the COVID-19 prevalence was 25%. At that time, the PPV for the RAD was 98.4%. During the study period, the prevalence declined to 4%, resulting in a marked decrease of the PPV to 88.3%. The impact of the prevalence of COVID-19 on the PPV is shown in Appendix Figure 2.

Discussion

In a hospital setting, with the ongoing spread of COVID-19 disease, rapid and easy to perform diagnostic tests are very important for fast diagnosis and optimization of infection control measures in order to limit further nosocomial spread.

In this study the analytical performance of five rapid SARS-CoV-2 antigen tests was determined. Besides, we describe the performance in routine practice of the SD Biosensor RAD and the added value of this RAD test for guiding isolation decisions and optimal patient care in a hospital setting.

The SD Biosensor RAD was the only assay which met the WHO performance criteria in the validation study, but not in the implementation study (overall sensitivity 54.2%) [4]. In the subgroups with a Ct value <26, all assays during the validation period and the SD Biosensor during the implementation period met the WHO sensitivity criterion. As the Biotical and Panbio assays also showed an excellent specificity, they seem to be appropriate for the use in settings similar to ours. This is in line with recently published literature on the SD Biosensor antigen test and the Panbio antigen rapid test [7,10-15]. In general, comparison of sensitivity between studies remains difficult as various Ct value categories are used. Moreover, Ct values between different rRT-PCR platforms can't be compared directly.

The specificity in our validation study was 100% for the Biotical, Panbio and SD Biosensor assays. In literature, specificities of the SD Biosensor and Panbio assay vary between 92% and 100%, and 95% and 100% respectively [7,10-15]. However, there was only one study which, like ours, included a cross-reactivity panel [10]. Orient Gene and Biosynex showed a specificity of 92.5% and 46.2% respectively. These suboptimal to bad specificity results are very different from the data provided in the package inserts. This can probably be attributed to the use of transport medium instead of the swab provided in the test kit. We observed that one transport medium was not compatible with the SD Biosensor assay. Additionally, Biotical has declared a partial incompatibility of his test with Amies media for high Ct samples. This highlights the importance for every laboratory to verify the performance of a RAD assay as it will be used in practice.

In the implementation period, we observed a lower specificity of the SD Biosensor RAD in comparison with the validation period (99.7% vs. 100%). This probably can be explained by the bigger data set included. Worth noticing, in three out of 12 samples the false positive results could not be confirmed by repeat RAD testing, emphasizing the risk of sample mix-up and subjective interpretation of the test result. The use of a reader would help to standardize interpretation of test results. Besides, 10 out of 12 false

positive results were reported in the first 6 weeks of the implementation period. Thus, we believe that specificity, partially, improves with the learning curve of the staff.

For all RAD tests (validation and implementation part) the rRT-PCR Ct values of RAD positive samples were significantly lower than the Ct values of the RAD negative samples. This confirms the lower sensitivity of RAD assays compared to rRT-PCR. It almost exclusively concerns samples of patients with relatively low viral loads (Ct values >26). The cut-offs mentioned in literature for infectivity are between >25 and >34 based on Ct values and between <100 000 and 6.63 log10 RNA copies/mL based on viral load, probably depending on the test characteristics and the type of samples used [7,16-20]. For our in-house rRT-PCR, a Ct value of 26 correlates with approximately 10^5 RNA copies/mL. This implies that the SD Biosensor RAD assay, with a sensitivity of 95.3% for samples with Ct values <26, can probably differentiate between contagious and non-contagious individuals [17].

Early identification of SARS-CoV-2 infected patients is essential to isolate confirmed cases on cohort wards to prevent nosocomial transmission of SARS-CoV-2 to other patients or HCW [21]. We implemented the SD Biosensor SARS-CoV-2 antigen test as a rapid screening test at the emergency department of our hospital. As the Biotical and Panbio RAD tests also had an acceptable analytical performance, our decision was guided by ease of use, price and rapid and sustained availability.

During the implementation period of approximately four months, more than half of the rRT-PCR positive patients presenting at the emergency department tested positive with the RAD test. Due to the rapid positive result of the antigen test, 47 ‘non-COVID-19’ suspected cases were immediately transferred to the COVID-19 area instead of a regular ward, limiting the risk of nosocomial transmission and outbreaks. In our institution the results from the RAD test was available approximately 30 minutes after the nasopharyngeal swab was send to the lab. Thanks to the user-friendly procedure, the test could be performed 24/7. In contrast the rRT-PCR test results were available at best five hours after the sample was send to the lab. For samples taken after 19:00, the result was not known until 12:00 on the next day, since no runs were performed at night. With reports of nosocomial transmission of SARS-CoV-2 in patients that are hospitalized in the same room as early as day one [22,23], the fast results of the RAD test hold the potential to reduce transmission in the hospital environment.

One hundred and ten ‘COVID-19 suspected’ cases were admitted immediately to the cohort area instead of the precohort area, diminishing the pressure on the single room hospital beds in the precohort area and limiting extra transfers of infectious patients between precohort and cohort ward. All but eight of the remaining 157 (RAD negative) rRT-PCR positive patients all had a Ct-value >26, corresponding to limited to no contagiousness. Unfortunately, ten COVID-19 negative patients were admitted to the cohort

area, due to a false positive RAD test, exposing them to the potential risk of nosocomial transmission of COVID-19. One patient became infected. This highlights the importance of the confirmation of each RAD test with rRT-PCR.

When evaluating the performance of an antigen test, it is crucial to perform the evaluation in the context the test will be used. We calculated that in a situation with a low COVID-19 prevalence in the target population, for example 0.5%, the PPV for the RAD is reduced to less than 50%. In this context, the disadvantages outweigh the benefits. We decided to stop using the RAD test as a screening tool when the prevalence of SARS-CoV-2 falls below 3% in the targeted population. This is in accordance with the recommendations of the WHO and earlier studies [4,24].

Meanwhile, the RAD test is also used in our hospital as a screening tool for hospital staff with mild symptoms when they start their shift. A RAD test is always followed by a rRT-PCR test, but with a negative RAD, the person can go to work while waiting for his/her rRT-PCR result. This implies an enormous advantage in terms of work planning and absenteeism. We currently don't use the RAD test as a pre-operative screening method, as endotracheal intubation is an aerosol-generating procedure, which has been linked to an increased risk of transmission [25,26]. We feel a negative RAD test on an upper respiratory sample is insufficient in this context, so we reserve a rapid molecular test for unplanned/urgent surgical procedures.

One of the strengths of this study is the head to head comparison of five RAD tests of which three assays aren't previously described in literature (Biosynex, Biotical, Orient Gene). Furthermore, to investigate the specificity, we included a cross-reactivity panel consisting of respiratory samples positive for other respiratory viruses. With the exception of Mak et al., none of the previous studies on the SD Biosensor or Panbio assay included such a panel [10]. All RAD assays and the rRT-PCR were performed in parallel on the same samples, so between-sample variations due to pre-analytical issues, such as the sample collection technique, are excluded. RAD assays were performed in the same time frame as rRT-PCR results without cooling or freezing.

One of the main limitations of our study, is that we did not perform the RAD tests directly on the nasopharyngeal swabs supplied in the kits as described by the manufacturer's instructions. A second limitation is that the subgroup analyses were performed on small sample cohorts. Furthermore, Ct values obtained by our in-house rRT-PCR are used as a proxy for viral load and infectivity, in the absence of viral culture. Clinical data used to study the effect of the implementation of the RAD assay were retrieved

retrospectively, and only for the RAD positive patients. Unfortunately, we did not record the incidence of nosocomial SARS-CoV-2 infections before and after the implementation of the RAD tests.

During the study period, VOCs were first identified and became present in Belgium. In the meantime, VOCs are present in approximately 77.2% [27] of the positive samples in Belgium. Since the mutations included in these variants don't target the nucleocapsid protein, it is unlikely that the analytical performance of the RAD test would be affected. Our limited data show an equal performance of the RAD test in VOC containing samples compared to all samples. The overall sensitivity of the RAD in 20I/501Y.V1 containing samples was 76%. The sensitivity met the WHO sensitivity criterion in samples with a Ct value <20 (100% for 20I/501Y.V1; no samples for 20H/501Y.V2; 100% for 20J/501Y.V3) and 20 - <26 (87.5% for 20I/501Y.V1; 100% for 20H/501Y.V2; 100% for 20J/501Y.V3). Similar findings have already been published for the British variant (20I/501Y.V1) [28].

We can conclude that the Biotical, Panbio and SD Biosensor SARS-CoV-2 antigen test had an acceptable performance in this study. All RAD assays are less sensitive than the current gold standard, rRT-PCR. However, from a clinical point of view, they seem to be able to detect most of the infectious patients. Exclusive use of an antigen test cannot replace rRT-PCR in a hospital setting, because a negative RAD test result can't rule out the diagnosis of COVID-19 disease. In the context of high prevalence of SARS-CoV-2 in the community, RAD tests can be used as a rapid screening tool complementary to rRT-PCR, to guide infection prevention measures and aid targeted admission.

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Conflict of interest statement

None declared.

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Reference

[1] Smithgall MC, Dowlatshahi M, Spitalnik SL, Hod EA, Rai AJ. Types of Assays for SARS-CoV-2 Testing: A Review. Lab Med 2020;51:e59–65. https://doi.org/10.1093/labmed/lmaa039.

[2] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

[3] Lippi G, Simundic A-M, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med 2020;58:1070–6. https://doi.org/10.1515/cclm-2020-0285.

[4] World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays 2020. https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays (accessed November 28, 2020).

[5] Mak GC, Cheng PK, Lau SS, Wong KK, Lau CS, Lam ET, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. J Clin Virol 2020;129:104500. https://doi.org/10.1016/j.jcv.2020.104500.

[6] Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. Cochrane Database Syst Rev 2020;8:CD013705. https://doi.org/10.1002/14651858.CD013705.

[7] Albert E, Torres I, Bueno F, Huntley D, Molla E, Fernández-Fuentes MÁ, et al. Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2020. https://doi.org/10.1016/j.cmi.2020.11.004.

[8] EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL. Infection prevention and control and preparedness for COVID-19 in healthcare settings. Fith update - 6 October 2020 2020. https://www.ecdc.europa.eu/sites/default/files/documents/Infection-prevention-and-control-in-healthcare-settings-COVID-19_5th_update.pdf (accessed November 21, 2020).

[9] SCIENSANO. GEVALSDEFINITIE, INDICATIES VOOR TESTEN EN VERPLICHTE MELDING VAN COVID-19 versie 23 november 2020 2020. https://covid-19.sciensano.be/sites/default/files/Covid19/COVID-19_Case definition_Testing_NL.pdf (accessed December 7, 2020).

[10] Mak GC, Lau SS, Wong KK, Chow NL, Lau CS, Lam ET, et al. Analytical sensitivity and clinical sensitivity of the three rapid antigen detection kits for detection of SARS-CoV-2 virus. J Clin Virol 2020;133:104684. https://doi.org/10.1016/j.jcv.2020.104684.

[11] Krüttgen A, Cornelissen CG, Dreher M, Hornef MW, Imöhl M, Kleines M. Comparison of the SARS-CoV-2 Rapid antigen test to the real star Sars-CoV-2 RT PCR kit. J Virol Methods 2020;288:114024. https://doi.org/10.1016/j.jviromet.2020.114024.

[12] Cerutti F, Burdino E, Milia MG, Allice T, Gregori G, Bruzzone B, et al. Urgent need of rapid tests for SARS CoV-2 antigen detection: Evaluation of the SD-Biosensor antigen test for SARS-CoV-2. J Clin Virol 2020;132:104654. https://doi.org/10.1016/j.jcv.2020.104654.

[13] Nalumansi A, Lutalo T, Kayiwa J, Watera C, Balinandi S, Kiconco J, et al. Field Evaluation of the Performance of a SARS-CoV-2 Antigen Rapid Diagnostic Test in Uganda using Nasopharyngeal Samples. Int J Infect Dis IJID Off Publ Int Soc Infect Dis 2020. https://doi.org/10.1016/j.ijid.2020.10.073.

[14] Fenollar F, Bouam A, Ballouche M, Fuster L, Prudent E, Colson P, et al. Evaluation of the Panbio Covid-19 rapid antigen detection test device for the screening of patients with Covid-19. J Clin Microbiol 2020. https://doi.org/10.1128/JCM.02589-20.

[15] Linares M, Pérez-Tanoira R, Carrero A, Romanyk J, Pérez-García F, Gómez-Herruz P, et al. Panbio antigen rapid test is reliable to diagnose SARS-CoV-2 infection in the first 7 days after the onset of symptoms. J Clin Virol 2020;133:104659. https://doi.org/10.1016/j.jcv.2020.104659.

[16] Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465–9. https://doi.org/10.1038/s41586-020-2196-x.

[17] Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020. https://doi.org/10.1093/cid/ciaa638.

[18] van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, et al. Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants. MedRxiv 2020:2020.06.08.20125310. https://doi.org/10.1101/2020.06.08.20125310.

[19] La Scola B, Le Bideau M, Andreani J, Hoang VT, Grimaldier C, Colson P, et al. Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards. Eur J Clin Microbiol Infect Dis 2020;39:1059–61. https://doi.org/10.1007/s10096-020-03913-9.

[20] Korean centers for Disease Control & prevention. FINDINGS FROM INVESTIGATION AND ANALYSIS OF RE-POSITIVE CASES. 2020 May 19. 2020. https://is.cdc.go.kr/upload_comm/syview/doc.html?fn=159118745823700.pdf&rs=/upload_comm/docu/0030/ (accessed December 7, 2020).

[21] Cheng VCC, Wong S-C, Chen JHK, Yip CCY, Chuang VWM, Tsang OTY, et al. Escalating

infection control response to the rapidly evolving epidemiology of the coronavirus disease 2019 (COVID-19) due to SARS-CoV-2 in Hong Kong. Infect Control Hosp Epidemiol 2020;41:493–8. https://doi.org/10.1017/ice.2020.58.

[22] Rickman HM, Rampling T, Shaw K, Martinez-Garcia G, Hail L, Coen P, et al. Nosocomial Transmission of Coronavirus Disease 2019: A Retrospective Study of 66 Hospital-acquired Cases in a London Teaching Hospital. Clin Infect Dis an Off Publ Infect Dis Soc Am 2021;72:690–3. https://doi.org/10.1093/cid/ciaa816.

[23] Vanhems P, Saadatian-Elahi M, Chuzeville M, Marion E, Favrelle L, Hilliquin D, et al. Rapid nosocomial spread of SARS-CoV-2 in a French geriatric unit. Infect Control Hosp Epidemiol 2020;41:866–7. https://doi.org/10.1017/ice.2020.99.

[24] Chaimayo C, Kaewnaphan B, Tanlieng N, Athipanyasilp N, Sirijatuphat R, Chayakulkeeree M, et al. Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. Virol J 2020;17:177. https://doi.org/10.1186/s12985-020-01452-5.

[25] World Health Organization. Infection prevention and control during health care when coronavirus disease (COVID-19) is suspected or confirmed: interim guidance, 29 June 2020 2020. https://apps.who.int/iris/bitstream/handle/10665/332879/WHO-2019-nCoV-IPC-2020.4-eng.pdf?sequence=1&isAllowed=y (accessed November 21, 2020).

[26] Tran K, Cimon K, Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. PLoS One 2012;7:e35797. https://doi.org/10.1371/journal.pone.0035797.

[27] National Reference Laboratory Belgium (UZ Leuven & KU Leuven). Genomic surveillance of SARS-CoV-2 in Belgium Report of the National Reference Laboratory (UZ Leuven & KU Leuven) Situation update – 16th of March 2021 (report 2021_18) 2021. https://www.uzleuven.be/nl/laboratoriumgeneeskunde/genomic-surveillance-sars-cov-2-belgium (accessed March 19, 2021).

[28] Public Health England. Guidance SARS-CoV-2 lateral flow antigen tests: evaluation of VUI-202012/01 2020. https://www.gov.uk/government/publications/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-vui-20201201/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-vui-20201201 (accessed March 7, 2021).

[29] SCIENSANO. COVID-19 WEKELIJKS EPIDEMIOLOGISCH BULLETIN 2020. https://covid-19.sciensano.be/nl/covid-19-epidemiologische-situatie (accessed March 19, 2021).

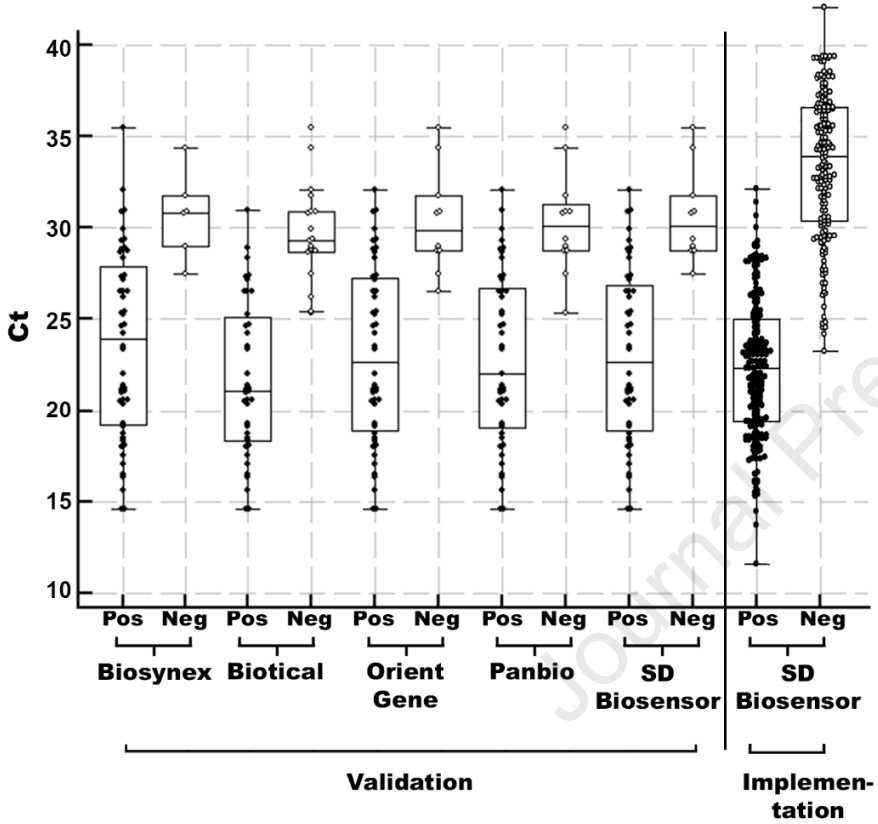
Table I: Sensitivity and specificity of the included RAD tests on validation and the SD Biosensor on implementation

				Biosynex	*Biotical*	*Orient Gene (Validation)*	*Panbio*	*SD Biosensor*			*SD Biosensor (implementation)*
Sensitivity											
	Ct-value	**Median Ct**	**Range**						**Median Ct**	**Range**	
	<20	17.7	14.6-19.2	14/14 (100%)	14/14 (100%)	14/14 (100%)	13/13* (100%)	14/14 (100%)	18.4	11.7-19.9	61/61 (100%)
	20 - <26	21.7	20.3-25.4	18/18 (100%)	16/18 (88.9%)	18/18 (100%)	17/18 (94.4%)	18/18 (100%)	23.1	20.0-25.9	100/108 (92.6%)
	26 - <30	28.5	26.2-29.9	16/18 (88.9%)	8/18 (44.4%)	13/18 (72.2%)	13/18 (72.2%)	13/18 (72.2%)	28.2	26.1-29.9	35/64 (54.7%)
	30 - <36	31.3	30.8-35.5	4/8 (50.0%)	1/8 (12.5%)	3/8 (37.5%)	2/8 (25.0%)	3/8 (37.5%)	33.2	30.0-35.9	4/84 (4.8%)
	>36								37.6	36.1-42.0	0/52 (0.0%)
	Overall			**52/58 (89.7%)**	**39/58 (67.2%)**	**48/58 (82.8%)**	**45/57 (78.9%)**	**48/58 (82.8%)**			**200/369 (54.2%)**
Specificity											
	Negative samples			7/23* (69.6%)	0/24 (100%)	2/24 (91.7%)	0/24 (100%)	0/24 (100%)			
	Cross-reactivity samples			14/16 (12.5%)	0/16 (100%)	1/16 (93.8%)	0/16 (100%)	0/16 (100%)			
	Overall			**21/39 (46.2%)**	**0/40 (100%)**	**3/40 (92.5%)**	**0/40 (100%)**	**0/40 (100%)**			**3814/3826 (99.7%)**

Abbreviations: Ct=cycle threshold; No.=number
*** One sample with insufficient volume to perform the test**

Figure 1: Real-time RT-PCR Ct values in positive versus negative test results per RAD assay: validation and implementation

The average Ct value was significantly lower in all positive RAD test compared to negative RAD test results ($p<0.001$). In the Box-and-whisker plot, the central box represents the interquartile range with the central line being the median. The whiskers represent the range, excluding outlier values.



Abbreviation: Pos= positive; Neg= negative, Ct= cycle treshold

Figure 2: Flowchart describing the impact of positive RAD test results for guiding isolation decisions

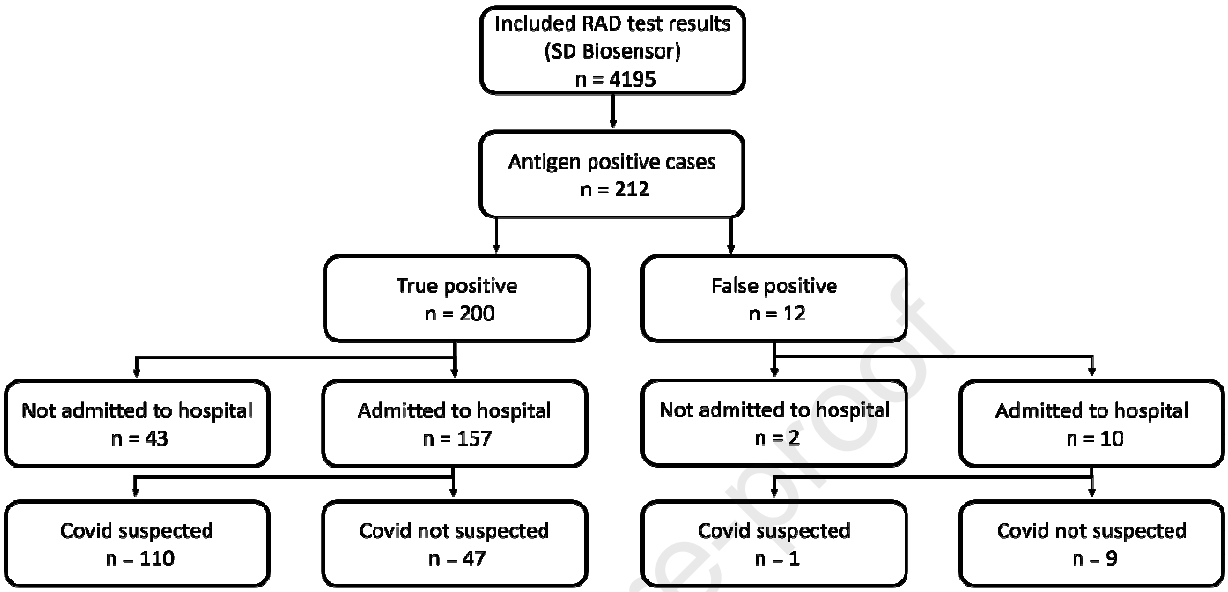


Figure legends

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