COVID-19 pandemic:

Evaluation of Abbot's Panbio COVID-19 rapid antigen test in Norway

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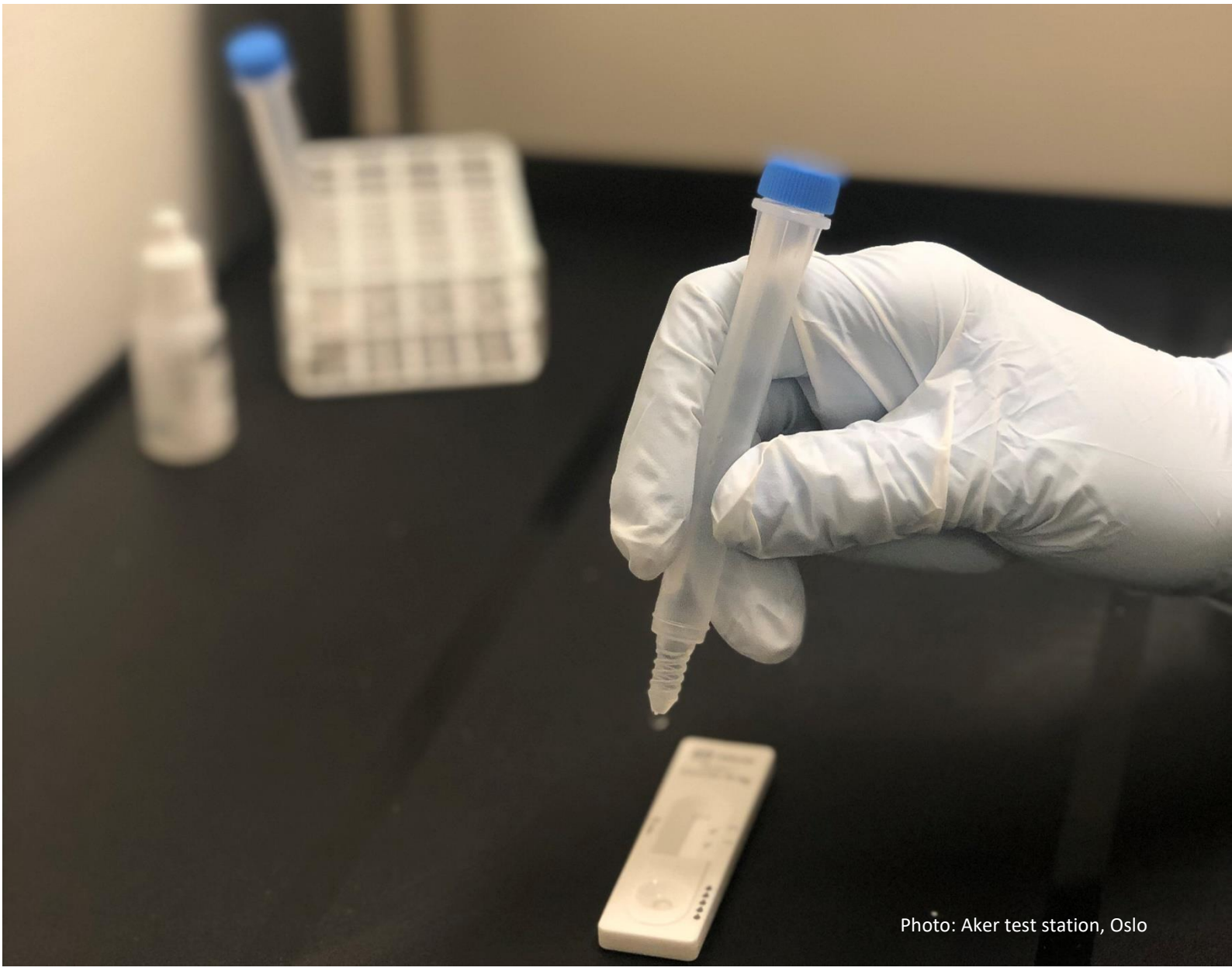

Photo: Aker test station, Oslo

Evaluation of Abbot's Panbio COVID-19 rapid antigen test in Norway

This report was written on behalf of the Norwegian Directorate of Health

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Summary

The Abbott Panbio™ COVID-19 Ag Rapid Test Device (Panbio rapid antigen test (RAT)) was compared to in-house SARS-CoV-2 PCR in an evaluation performed on 3991 samples from a test station in Oslo and 866 samples from outbreaks in Norway in the period October 30th to November 25th 2020. 250 samples (6.3 %) were positive at Aker test station and 60 (6.9 %) at the outbreaks all together. At Aker test station the over-all sensitivity was 74.4 % and the specificity was 99.9 %, compared to the PCR results from Oslo University Hospital. Positive and negative predictive values were 0.984 and 0.983, respectively, showing high accuracy of both positive and negative test results at the given prevalence (6,3%) at the time. In the outbreak material, the sensitivity was 70 %, and the specificity was 100 %. Due to the lower sensitivity of the Panbio RAT, we conclude that for diagnosing serious illness and hospitalized patients, PCR remains the preferred method. However, RATs have been launched for other purposes: Epidemiological surveillance and contact tracing. A lower sensitivity can be tolerated in this context and compensated for by higher availability, higher turnaround time and repeated testing. The Panbio RAT had a lower detection limit corresponding approximately to 1.4 million copies/mL. Studies have shown that clinical samples with less than 1 million copies/mL are unlikely to cultivate in the laboratory and thus also less likely to be infectious from one person to another. In the Aker test station material, the sensitivity of the Panbio RAT was 83.8 % for samples with a viral load above this limit, indicating that the majority of infectious individuals will be detected with the test. For patients symptomatic for less than five days, the sensitivity was 79.8 %. These sensitivity figures are within WHO's guidelines for RATs used in surveillance. For subjects without symptoms, the sensitivity was only 55.3 %, showing that the Panbio RAT is best suited for use in symptomatic patients, and that PCR will be a necessary backup method if RATs are used in this group. Due to the lower sensitivity of the Panbio RAT, guidelines for correct use are important. Correct use of RATs will for example be highly influenced by the actual disease prevalence. RATs may prove to be valuable tools in controlling the COVID-19 pandemic.

Summary in Norwegian

Et norsk prosjekt for evaluering av Abbots Panbio™ COVID-19 Ag Rapid Test Device (Panbio antigentest) ble gjennomført i perioden 30. oktober til 25. november 2020. Panbio antigentest ble sammenlignet med veletablert genteknologisk diagnostikk (SARS-CoV-2 PCR ved Oslo Universitetssykehus). 3991 prøver ble inkludert fra Aker teststasjon i Oslo. I tillegg ble 866 prøver fra utbrudd i hele landet inkludert. 250 prøver (6,3 %) var positive ved Aker teststasjon og 60 (6,9 %) fra utbruddene samlet. Sensitiviteten beregnet for materialet fra Aker teststasjon var 74,4 %, og spesifisiteten var på hele 99,9 %. Positiv og negativ prediktiv verdi var på henholdsvis 0,984 og 0,983. Dette betyr at både et positivt og et negativt testresultat er svært nøyaktig ved den aktuelle sykdomsforekomsten (6,3 %). Sensitiviteten var noe lavere for utbruddsmaterialet (70 %), men spesifisiteten var like høy. Med en såpass lav sensitivitet for antigentesten konkluderer vi med at PCR-tester forblir foretrukket metode i diagnostikken av alvorlig syke og innlagte pasienter. Antigentester har dog blitt lansert med et annet mål for øye: Epidemiologisk overvåking og smitteoppsporing. Kravene til sensitivitet vil da ikke være like høye og kan kompenseres for ved større tilgjengelighet, raskere svar og større muligheter for repetert testing. Her har antigentester en klar fordel. I laboratorieforsøk utført som del av evalueringsprosjektet fant vi at Panbio hurtigtests deteksjonsgrense var rundt 1,4 millioner kopier/ml. Flere studier har vist at ved viruskonsentrasjoner under 1 million kopier/ml luftveismateriale er det svært sjelden man påviser levende eller såkalt replikerende virus, noe som indikerer lav smittsomhet. I materialet fra Aker teststasjon fant vi en sensitivitet på 83,8 % for prøver med viruskonsentrasjoner over denne grensen, noe som viser at majoriteten av smittsomme pasienter detekteres. For pasienter med sykehistorier kortere enn fem dager var sensitiviteten 79,8 %. Slike sensitivitetstall er innenfor det WHO har anbefalt for antigentester til overvåkningsformål. For personer uten symptomer var sensitiviteten kun 55,3 %. Dette viser at testen er best egnet til overvåking av symptomatiske pasienter, og at ved bruk på ikke-symptomatiske bør man supplere med PCR. Ettersom antigentestene har sine begrensninger er det viktig å etablere gode retningslinjer for deres bruk. Bruken av antigentester vil blant annet være sterkt avhengig den gjeldende sykdomsforekomst. Brukt riktig kan disse testene bli et verdifullt verktøy i bekjempelsen av covid-19-pandemien.

Introduction

In September 2020, the Norwegian health authorities decided to purchase several million SARS CoV-2 rapid antigen tests (RATs) in order to secure testing capacity following the second wave of infections in Norway. Although the laboratory testing capacity in Norway has increased more than fivefold since early spring, the global exhaustive demand for the same reagents, making the testing capacity vulnerable to supply discontinuity. In addition, long distance to testing laboratories in rural areas mean that the response time may be too long for infection tracking to start quickly enough. The Norwegian strategy to fight the spread of virus is highly dependent on rapid identification of infected persons, swift isolation and infection tracing and quarantine (TISK). In order to supplement the laboratory testing with the Abbott's Panbio™ COVID-19 Ag Rapid Test Device (Panbio RAT), it was decided to initiate a field-evaluation of the test in a low to medium prevalence setting at a COVID-19 test station in Oslo. Additional study arms were included from outbreak settings where people more frequently have been exposed but often have less symptoms. Independent and setting-specific validations of RATs before their implementation is also in line with the ECDC recommendations (1). Analytical sensitivity and specificity measures given by the manufacturers do not necessarily reflect the actual sensitivity and specificity of the test, and evaluation studies from other countries might not reflect the Norwegian outbreak setting. Thus, a field or clinical evaluation of the most desirable areas of application for the tests are needed as this is the first time RATs are considered used for COVID-19 testing in Norway.

The RATs are based on lateral flow immunochromatography using antibodies to target the SARS-CoV-2 nucleoprotein in nasopharyngheal specimens for diagnosis of COVID-19 in symptomatic patients. The method is very different from PCR, which detects and amplifies RNA to millions of copies in order to give a signal for detection. PCR is the most sensitive method at hand with the highest analytical sensitivity and specificity and is considered the gold standard that other tests or assays are compared with. Due to its high sensitivity, PCR is able to detect not only infectious viral particles, but non-replicative RNA after an infection. The cycle threshold (ct) value from the PCR may give some indication of the amount of viral RNA present in a sample, but the PCR test results are reported as positive or negative and usually not interpreted in regard to infectivity. Compared to PCR, the sensitivity of RATs is lower. A key question is whether a positive RAT result may correlate with infectiousness and correctly identify infectious persons in specific settings. In that case, the immediate test result on-site could facilitate initiation of targeted contact tracing and isolation at an early time point.

The evaluation project for the Panbio RAT was conducted from October 30th to November 25th, 2020. The aim of the evaluation was to study the RAT's performance on a sample material collected in a routine setting 1) at a COVID-19 test station in Oslo, and 2) in outbreaks happening in Norway during the study period. The pilot was a clinical comparison of test results obtained with antigen tests and the gold standard test (SARS-CoV-2 real-time RT-PCR). Additionally, laboratory studies on analytic performance were performed.

Part 1: Aker test station, Oslo

Study design

People who signed up for a COVID-19 test at Aker test station during the study period, were asked to take part in the project. Two nasopharyngeal swabs were obtained from each person. The first swab was sent to Unit for Large Scale PCR Diagnostics for SARS CoV-2 at Department of Microbiology, Oslo University Hospital (OUH) for testing with an in-house SARS-CoV-2 PCR, and the second swab was used for Abbott's Panbio™ COVID-19 Ag Rapid Test Device (Panbio RAT) at the test site, and was performed according to the manufacturer's instructions. Participants were asked questions about known exposure and symptom duration. All participants gave an informed consent to take part in the project.

Clinical data as well as results from the RATs were compiled and delivered to the National Institute of Public Health (NIPH) on a weekly basis. Data from Aker test station was subsequently merged/cross-referenced with the Norwegian Laboratory database (Meldingssystem for smittsomme sykdommer (MSIS)) at NIPH, using a

personal identifier, in order to obtain results on the corresponding PCR results. PCR positive samples were identified, and ct values were provided by the analyzing laboratory at OUH. All data analyses were performed in an access-controlled folder in NIPH secure zone. All personal identifiers were removed before further data analysis, and only anonymized data was shared in the evaluation group.

Positive RAT results not confirmed by a positive PCR result were repeated at OUH. Furthermore, the samples were sent to the department's virology facilities at OUH Ullevål, where another SARS-CoV-2 PCR was conducted with the cobas 6800 system (Roche Diagnostics).

Data analysis was performed using Stata version 16 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC). Data were summarized with descriptive statistics mean, median, and standard deviation for numerical variables, frequencies and percentages for categorical variables. Sensitivity and specificity with 95 % confidence intervals, as well as positive and negative predictive value were computed using the PCR as a gold standard. Agresti-Coull confidence intervals are shown. Bivariate associations between independent categorical variables and RAT results were calculated using Chi-Square tests. For independent numerical variables Mann Whitney U tests were used to compare medians in two groups. For bar graphs showing the distribution of ct values by RAT results, rounded ct values are shown.

Of the 4025 samples collected at Aker test station, 3998 were successfully matched to their corresponding PCR results in the database. Out of the 3998 samples, one PCR result was inconclusive, whereas six antigen tests were either inconclusive or defective. A total of seven samples were thus omitted from further analysis.

The sample size was calculated based on the formula by Malhotra et al (2). A sample size of 4000 was deemed acceptable.

Results

A total of 3991 cases were successfully included in the study from Aker test station. A known exposure to the virus was reported by 35.7 % (n = 1423) of the cases, and of these 9.8 % (n = 139) were PCR positive (Table 1). The majority of the cases (62.0 %, n = 2475) reported symptoms of COVID-19, and of these 8.0 % (n = 199) obtained a positive PCR, compared to 3.3 % (n = 47) of those who reported no symptoms. Of those with symptoms, 86.6 % (n = 2143) reported a symptom duration of ≤ 5 days.

Table 1. Descriptive characteristics of persons tested at Aker test station during the study period.

	Total	PCR negative (row %)	PCR positive (row %)
n	3991	3741 (93.7)	250 (6.3)
Exposed			
No	2234	2143 (95.9)	91 (4.1)
Yes	1423	1284 (90.2)	139 (9.8)
Unknown	325	305 (93.9)	20 (6.2)
Missing	9	9 (100)	0
Symptoms			
No	1408	1361 (96.7)	47 (3.3)
Yes	2475	2276 (92.0)	199 (8.0)
Unknown	101	97 (96.0)	4 (4.0)
Missing	7	7 (100)	0
Symptom duration			
≤ 5 days	2143	1965 (91.7)	178 (8.3)
> 5 days	327	306 (93.6)	21 (6.4)
Unknown	5	5 (100)	0

The comparison of the PCR positive, RAT negative and RAT positive cases (Table 2) showed that the presence of COVID-19 symptoms was significantly associated with a positive RAT result ($p < 0.001$), - the percentage of RAT positive was 78.9 (n = 157) among those reporting symptoms and 55.3 (n = 26) among those reporting no symptoms. The duration of symptoms (≤ 5 days vs. > 5 days), however, was not significantly associated with the RAT result ($p = 0.375$). The mean and median ct values from the PCR were significantly lower in the RAT positive than the RAT negative cases ($p < 0.001$), indicating higher viral loads in the RAT positive, but the range from the lowest to the highest ct value was not significantly different between the two groups (see also Figure 1). A known exposure to the virus did not have significant impact on the RAT result among the PCR positive cases ($p = 0.469$).

Table 2. Characteristics of the PCR positive samples.

	Total	RAT negative (row %)	RAT positive (row %)	p-value*
n	250	64 (25.6)	186 (74.4)	
Exposed				
No	91	21 (23.1)	70 (76.9)	0.469
Yes	139	38 (27.3)	101 (72.7)	
Unknown	20	5 (25.0)	15 (75)	
Symptom				
No	47	21 (44.7)	26 (55.3)	< 0.001
Yes	199	42 (21.1)	157 (78.9)	
Unknown	4	1 (25.0)	3 (75.0)	
Symptom duration				0.375
≤ 5 days	178	36 (20.2)	142 (79.8)	
> 5 days	21	6 (28.6)	15 (71.4)	
ct values				
Mean (SD)	25.8 (4.7)	29.9 (4.7)	24.4 (3.9)	< 0.001
Median	25.3	29.8	23.8	
Min - Max	16.16 - 38.99	17.5 - 38.27	16.16 - 38.99	

*Categories such as "Unknown" were not included in chi square test

As illustrated in Figure 1, a significantly larger share of the RAT positive cases had ct values in the mid and lower range (higher viral load), while the highest ct values (lower viral load) were more often obtained by the RAT negative cases. However, there was a considerable overlap between the two groups.

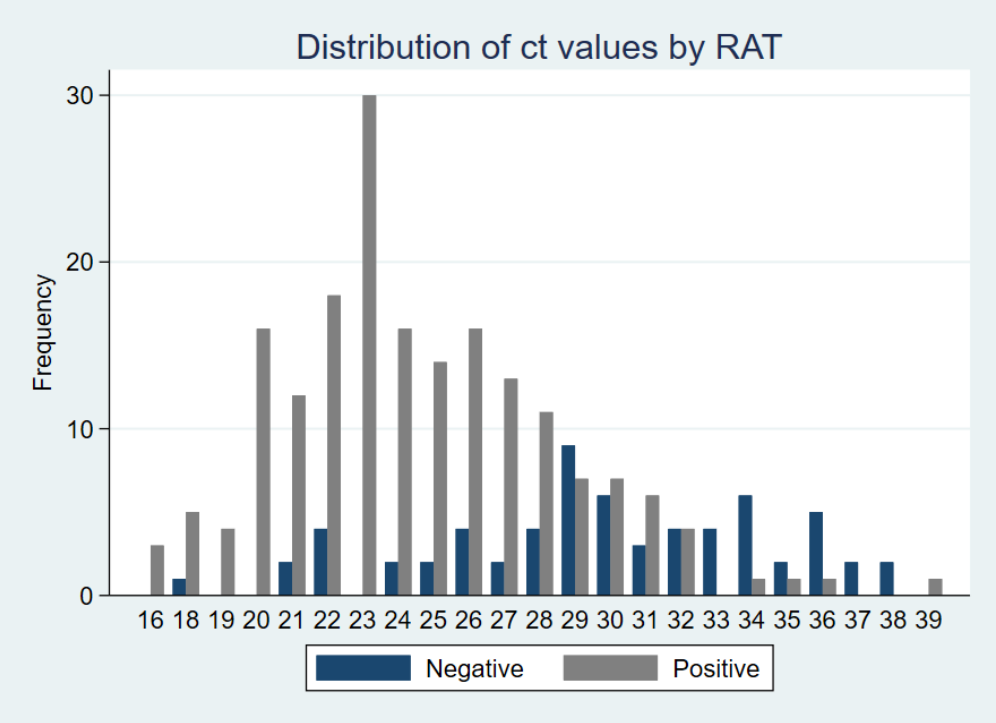

Figure 1. Distribution of ct values by RAT result.

As shown in Figure 2, the distribution of reported days of symptoms was not markedly different between the PCR positive cases that are RAT positive and negative (see also Table 2). Furthermore, both the RAT positive and negative cases had ct values, which covered a large range of the overall obtained ct values. This was also seen for asymptomatic cases, and cases with shorter and longer symptom duration.

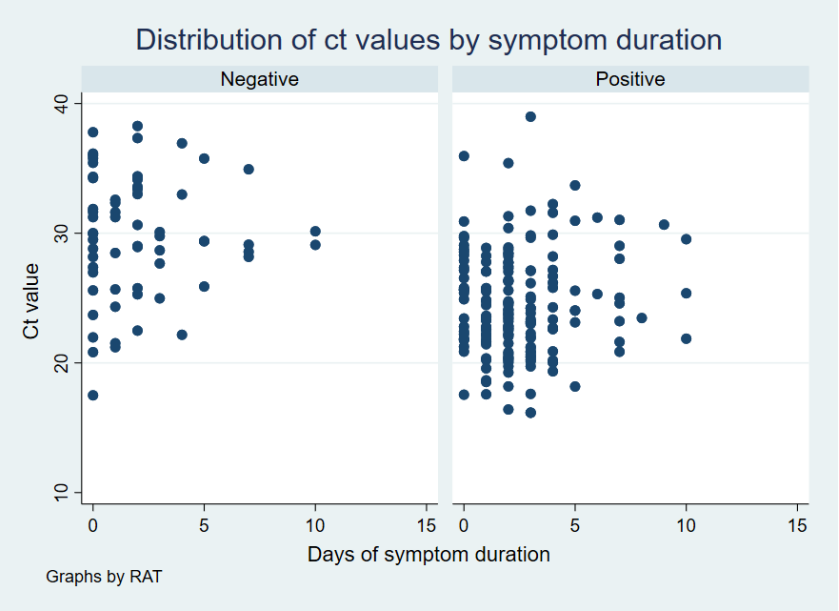

Figure 2. Distribution of ct values by symptom duration by antigen test result. Asymptomatic cases were categorised as zero days of symptoms.

Of the 250 PCR positive cases 186 were RAT positive, giving an overall sensitivity of the RAT of 74.4 % (Table 3). Thus, 64 cases were false negatives (1.7 % of the total number of samples). The median ct value for the RAT false negatives was 29.8 (Table 2), Only 3 of the 3741 PCR negative cases were RAT positive (false positives 1.2%), giving a specificity of 99.9 % (CI 95 %: 99.7 - 99.9). For the 3 false positive cases the PCR was repeated and also conducted on a different platform at OUH, with the results confirming that they were truly SARS-CoV-2 PCR negatives and thus false positive RAT results. In two of these samples rhinovirus RNA was detected. The positive predictive value (PPV) was 0.984, while the negative predictive value (NPV) was 0.983.

When only the cases with ct values below 30 were considered, the sensitivity increased to 83.8 %. Including only the cases reporting symptoms of COVID-19 in the analyses resulted in a sensitivity of 78.9 %, and it was further slightly higher (79.8 %) when only those reporting a symptom duration ≤ 5 days were included. In the group of PCR positive cases who reported no symptoms, the sensitivity of the RAT was 55.3 %. The sensitivity of the RAT was 72.7 % among the PCR positive cases who had known exposure to SARS-CoV-2.

Table 3. Test performance (sensitivity) of the Panbio RAT compared to PCR, overall and when using different ct and clinical cut-offs.

	RAT neg (n)	RAT pos (n)	Total (n)	Sensitivity (%)	CI 95 % (%)
PCR positive	64	186	250	74.4	69 - 79
PCR negative	3738	3	3741		
ct < 30	33	171	204	83.8	78 - 88
ct ≥30/neg	3769	18	3787		
PCR positive symptomatic	42	157	199	78.9	73 - 84
PCR positive symptom duration ≤5 days	36	142	178	79.8	73 - 85
PCR positive symptom duration > 5 days	6	15	21	71.4	50 - 86
Asymptomatic PCR positive	21	26	47	55.3	41 - 69
Exposed PCR positive	38	101	139	72.7	65 - 79

Figure 3 shows how the positive predictive value (PPV) and the negative predictive value (NPV) of the RAT results are affected by variations in prevalence rates of SARS-CoV-2 given a sensitivity of 74.4 % and a specificity of 99.9 %. A sharp decrease in PPV at prevalence rates below 1 % is demonstrated. The exact numbers for PPV, NPV at different prevalence's are given in Supplementary Table 3 (Appendix).

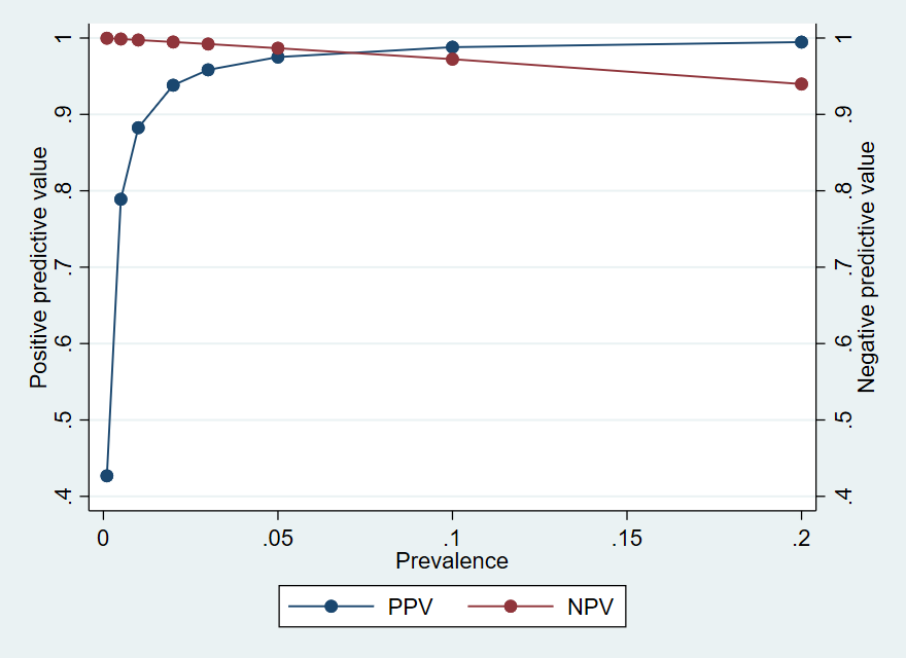

Figure 3. Positive predictive value (PPV) and negative predictive value (NPV) at different prevalence rates of SARS-CoV-2, given a sensitivity of 74.4 % and a specificity of 99.9 %.

Part 2: Outbreaks

Study design

Municipality physicians in charge of infection control were asked, countrywide, to participate if they were experiencing COVID-19 outbreaks during the evaluation of the Panbio RAT. The Norwegian Organization for Quality Improvement of Laboratory Examinations (Noklus) offered support in training and planning of logistics. Two nasopharyngeal swabs were obtained from each person enrolled in the evaluation. One swab was used for the RAT at the test site. The second swab was sent to the local microbiology laboratory for routine SARS-CoV-2 PCR.

Results

During the study period, six Norwegian municipalities were included. The largest outbreak was in Farsund municipality in Agder County, where RATs were introduced at an early stage in the outbreak investigation. In the municipalities of Våler/Åsnes and Lindesnes, RATs were only used late in the outbreak-related testing and after the first rounds of close contacts had been tested. In Rana, Lurøy and Vindafjord there were no major outbreaks or mass testing, and RATs were used targeted and mainly because of long response time for PCR results.

A total of 866 RATs were performed in the six municipalities, 304 in Farsund (28 PCR positive), 75 in Rana (18 PCR positive), 404 in Våler/Åsnes (9 PCR positive), 54 in Lindesnes (1 PCR positive), 21 in Vindafjord (3 PCR positive), and 8 in Lurøy (1 PCR positive) (Supplementary Table 1, Appendix). Among the 806 participants with a negative PCR result, no false positive RAT was recorded in any municipality, yielding an overall specificity of 100 % (95 CI 99.5-100). Among the 60 participants with a positive PCR result, 42 tested positive with the RAT, yielding an overall sensitivity of 70 % (57-81).

In Farsund, the only outbreak where RATs were introduced at an early stage and mass-testing performed, sensitivity of the RAT was estimated to 71 % (95 % CI 53 - 85) (Supplementary Table 2, Appendix). For individuals presenting with symptoms, the sensitivity was 81 % (95 % CI 59-93). We did not pool the data for subgroup analyses since the clinical situations varied, and ct values were obtained from different laboratories.

Part 3: Laboratory analyses

Study design

A ten-fold dilution series with known concentrations of SARS-CoV-2 RNA was performed both at the NIPH and at OUH in order to determine the detection limit of the PCR methods in use and to generate a standard curve of copies pr. mL.

The RAT was tested in serial 10-fold dilutions, in duplicate, of a clinical sample with known concentration of virus in order to estimate the limit of detection. The last dilution giving a positive RAT result was read as as approximately detection limit. The RATs were further tested on a blinded panel of 40 samples, comprising, rhinovirus, influenza A virus and respiratory virus negative clinical samples in order to get an impression of cross-reactivity. Furthermore, the RATs were challenged with SARS-CoC-2 samples of high viral load to investigate what effect such clinical samples would have on the readout of the analysis result. The tests were also read at different time points after test result was achieved in order to see whether the result would fade out over time.

Results

Both PCR methods could detect SARS-CoV-2 RNA concentrations down to 1,000 RNA copies/mL, but the highest dilution consistently detecting RNA in all parallels was 10,000 copies/mL. The limit of detection of the RAT was approximately 1.4 million copies/mL. This was the last tenfold dilution with a positive RAT result. The

actual limit of detection is therefore expected to be between the last dilution giving a positive result (1.4 million copies/mL) and the first dilution giving a negative result (650 000 copies/mL).

The blinded panel of clinical samples consisted of dilution series (in duplicate) of SARS-CoV-2, one rhino virus positive clinical sample, four influenza A virus positive clinical samples and 5 respiratory virus negative clinical samples in addition to ten negative controls of virus transport medium. All non-SARS-CoV-2 samples were RAT negative. In order to investigate if the RATs would perform equally well on clinical samples with a very high viral load, five clinical samples with average viral load of $2{,}1x10^9$ copies/mL were tested and the high viral load did not affect the test results negatively.

The tests were read at different time points before and after the 15 minutes recommendation from the manufacturer. Test results were appearing already after a couple of minutes. The test results appeared to be stable over several hours and even days. Still, it is highly recommended to follow the instructions from the manufacturer.

Discussion

Molecular amplification tests, mainly PCR tests, are widely used in clinical virology laboratories today, and they are regarded as the gold standard in SARS-CoV-2 diagnostics. These tests are extremely accurate, but resource intensive. Reagents, machines, disposable equipment, transport- and personnel resources are in high demand during larger SARS-CoV-2 outbreaks, or when infection rates are high in the general population. Some, or even all, of the above factors may prove to be scarce in periods with high test activity. This may lead to unacceptable delays in test processing. For this reason, antigen tests, using old and well-known immunological technology, have gained renewed attention. These tests are far less demanding when it comes to resource use. Laboratory personnel with less training than a laboratory technician can perform them outside laboratories, and after 10-15 minutes, a result can be obtained. Conversely, it is well known that RATs are less sensitive than PCRs, and in traditional clinical virology they have been abandoned for this reason. However, a new idea was launched in summer 2020: It was proposed that RATs could be used for infection surveillance and contact tracing rather than clinical diagnostics. In other words, they could be used for testing infectiousness instead of clinical disease (3). Modelling studies have shown that in this context, sensitivity is less important than test frequency and turn-around time (3,4). Here, the important premise is that patients with low viral loads in nasal secretions are less contagious.

Based on similar arguments, WHO has recommended that antigen tests used in infection surveillance should have sensitivity and specificity of at least 80 % and 97 %, respectively, compared to PCR (5). Further, WHO and ECDC have recommended local evaluations of antigen tests to account for local differences in prevalence, test technology, test availability and infrastructure (1). A few clinical evaluations of the PanBio RAT and other similar tests have been performed elsewhere (6-8) and are ongoing, and results indicate that the test accuracy may vary significantly depending on the population tested.

Viral loads around one million RNA copies per mL (or per swab) of respiratory secretions have been proposed as a reasonable cut off for evaluating infectiousness (9-13). Replicating virus is rarely detected in samples with viral loads below this limit. Furthermore, it has been shown that the patients more than a week into their disease course are very little infectious (12). At that time, viral loads in upper respiratory tract samples usually fall below one million copies/swab (9). Therefore, it is an interesting question whether the detection limit of the Panbio RAT is below or above this threshold. Due to differences in PCR technology across laboratories, it is hard to establish an accurate threshold. To account for this uncertainty, we estimate that the threshold will be in an area between 10^5 and 10^7 copies per mL. Most patients reach far higher levels in the acute phase of the disease (7, 14, 15).

Overall results

As expected, we found the overall sensitivity of the Panbio RAT to be about 75 % when compared to PCR. Of the 250 samples with a positive PCR, a total of 186 (74.4 %) were RAT positive. Based on the ct values of the positive samples, RAT positive samples had significantly higher viral loads than the PCR positive samples that were not detected by the RAT. In our laboratory, a ct-value of 30 roughly corresponds to 10^6 copies/mL, which was considered the approximate threshold for infectiousness as described above. For samples with a copy number above this limit, we found test sensitivity to be 83.8 %. This means that the majority of infectious cases can be correctly identified with RAT. Nevertheless, more than 15 % of the potentially infectious individuals that were tested with RAT received negative test results, underscoring that negative test results should be interpreted with caution. The risk of inaccurate results differed within different subgroups as discussed below, supporting a pre-test risk stratification for selecting patient groups eligible for RAT. Our results are in line with other evaluations of the Panbio RAT (summarised in annex 1 in the ECDC guidelines Options for the use of RATs for COVID-19 in the EU/EEA and the UK (1)). The evaluation in Oslo is to our knowledge the largest conducted on the PanBio RAT to date. However, the prevalence in Oslo at the time was low to medium and therefore a larger number of test-persons was needed in order to get reliable results.

Evaluation of testing in a low-prevalence setting

The use of RATs is generally recommended as an alternative to PCR when availability of laboratory testing is limited, especially in individuals with COVID-19 compatible symptoms in areas where the proportion of test positivity is high or very high. e.g. >10% (1). Our results support this, as we find a lower sensitivity in the low prevalence settings (outbreaks), and our analyses illustrate how the PPV falls drastically when the prevalence is low.

Evaluation for testing of symptomatic patients

For COVID-19 patients symptomatic for less than five days, the sensitivity of the RAT was about 80 %. Indeed, the sensitivity was 87.6 % for patients with less than five days of symptoms and a high viral load above the suggested infectivity threshold of 10^6 copies/mL. This is in line with findings in other evaluations, and with WHO's recommendations, and support the use of RAT for rapid infectivity testing among patients with mild symptoms. However, the risk of false negative results needs to be considered also in this group. RAT positivity rate was slightly higher among cases with brief duration of symptoms (≤ 5 days) compared to those with symptoms lasting > 5 days, but the numbers were small as the majority of the symptomatic cases in the study (90 %) had had symptoms lasting for less than five days.

Evaluation for testing of non-symptomatic patients

For asymptomatic individuals, sensitivity of the Panbio RAT was low (55.3 %), indicating that it is best suited for symptomatic patients. The lower viral loads found among asymptomatic subjects may explain the lower sensitivity. These individuals could still be in the incubation period (pre-symptomatic) or in a late phase of their infection. Both phases are usually characerised by viral loads below the detection limit of the RAT. In the late phase, the individual can be regarded as non-infectious, although they are important in an infection tracing perspective. If tested in the incubation period, the person may become infectious after a day or two. Repeated testing may compensate for this to some extent, but it is important to consider the risk of false negative RAT results in pre-symptomatic persons. It is likely that in most of the pre-symptomatic false negative cases viral load will increase over the next few days and therefore could be detected if RAT was repeated after 24-48 hours. However, this could not be verified in the current study as repeated testing was not included in the study design.

Evaluation of the test based on viral load

The Panbio RAT had a lower detection limit corresponding approximately to 1.4 million copies/mL. Laboratory data from mid-August to mid-November 2020 was collected from the large-scale PCR diagnostics unit for SARS-CoV-2 at OUH. Ct-values of all positive PCRs were examined to get an impression of the regular viral load in

samples from several other test stations in the Oslo area, including more than 3600 positive results from more than 100 000 PCR tests performed. The average viral load in samples with positive PCR OUH in this period was estimated to be approximately 10 million copies/mL (range < 1 copy/mL - 2.3 x 10^{10} copies/mL, median ct value of 25.5). The majority of the samples (75.7 %) had ct values below 30 (approximately 1 million copies/mL), thus within the detection limit of the RATs, and 89.5 % of samples were below ct 33 (85 000 copies/mL) (personal communication). Considering the estimated sensitivity of 83 % for Panbio RAT on samples with ct < 30, these numbers suggest that Panbio RAT would have correctly identified a high proportion of samples from infectious patients received at OUH in this period.

Although the human infectious dose is unknown, studies have shown that clinical samples with less than 1 million copies/mL or RAT negative, but PCR positive samples, are unlikely to cultivate in the laboratory (and thus also less likely to be infectious from one person to another) (9, 16). The Panbio RAT evaluated will with high certainty detect most COVID-19 infectious patients that are more likely to infect others based on their amount of virus in the upper airways.

Risk of false negatives and false positives

The over-all specificity of the antigen test was very high (99.9 %). This means that false positive results are extremely rare. However, in situations with low disease prevalence (<1 %), the proportion of false positives still becomes noticeable. If disease prevalence numbers are below 1 % in the population being tested, positive results with the antigen test should be confirmed with a PCR test.

The main concern with RAT is the risk of false negative results. In our data, there were a total of 64 cases with positive PCR and negative antigen-tests. Although viral load was lower among cases with negative RAT, the mean ct-value equaled the threshold for infectivity, and more than half of the individuals with false negative results had symptoms lasting less than five days. This should be considered when negative RAT results are interpreted and emphasizes the importance of continued follow-up in spite of negative test results, and to consider repeated testing.

Experiences with the test device and sampling logistics

Experience from the study was that performing the RAT analyses in parallel to sampling for laboratory PCR test, reporting results, and dissemination of test results to the patient was time consuming and stressful in an already busy test-station. Performing the analyses correctly is imperative to optimize the test performance, and the staff in the pilot study expressed the need for trained personnel dedicated to these tasks. Dissemination of test results to the patient was not straight forward, as individual test results may be difficult to interpret and patients in several cases presented complex dilemmas. These factors need to be considered before implementing RAT in a test station. Personnel dedicated to perform RATs and reporting results, should be trained and recruited in addition to the regular staff in a test station, and implementation of RAT will require adequate facilities for performing the analyses in order to minimize the risk of pre-analytical, analytical, and post-analytical errors.

In conclusion, the test results are in line with WHO's recommendations and previous field evaluations of the RAT, and the Panbio RAT can be utilized in specified situations. However, it is important to be aware of its limitations, and to keep in mind that that sensitivity is lower compared to the well-established PCR tests. For this reason, PCR tests remain the preferred option, especially for patients with serious respiratory symptoms. Furthermore, the accuracy of the test is particularly low in asymptomatic individuals. Thus, the use in persons without symptoms should be limited, and the results must be interpreted with caution. The lower sensitivity of the antigen test can be compensated for by repeated and frequent RAT use, or by use of PCR as additional test in selected cases. In the coming weeks and months, it will be of utter importance to focus on correct use of antigen tests. Otherwise, false negative results may jeopardize infection control. However, used correctly antigen tests may be a valuable tool in controlling the COVID-19 pandemic.

Recommendations for the use of Panbio RAT will be made based on the results of this evaluations pilot in combination with evaluation results from others.

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Appendix

Table S1. Descriptive characteristics of persons tested in outbreak municipalities.

	Total (n)	PCR negative (n)	PCR positive (n)
FARSUND	304	276	28
Exposed			
No	82	80	2
Yes	159	134	25
Unknown	62	61	1
Missing	1	1	0
Symptoms			
No	143	138	5
Yes	139	118	21
Unknown	21	19	2
Missing	1	1	0
RANA	75	57	18
Exposed			
No	3	3	0
Yes	56	41	15
Unknown	16	13	3
Missing	0	0	0
Symptoms			
No	10	7	3
Yes	48	37	11
Unknown	17	13	4
Missing	0	0	0
VÅLER/ÅSNES	404	395	9
Exposed			
No	170	170	0
Yes	194	184	9
Unknown	41	41	0
Missing	1	0	0
Symptoms			
No	216	212	3
Yes	175	172	3
Unknown	14	11	3
Missing	1	0	0
LINDESNES	54	53	1
Exposed			
No	2	2	0
Yes	47	46	1
Unknown	5	5	0

Missing	0	0	0
Symptoms			
No	39	39	0
Yes	15	14	1
Unknown	0	0	0
Missing	0	0	0
VINDAFJORD	21	18	3
Exposed			
No	0	0	0
Yes	20	17	3
Unknown	1	1	0
Missing	0	0	0
Symptoms			
No	15	14	1
Yes	6	4	2
Unknown	0	0	0
Missing	0	0	0
LURØY	8	7	1
Exposed			
No	1	1	0
Yes	7	6	1
Unknown	0	0	0
Missing	0	0	0
Symptoms			
No	4	4	0
Yes	3	2	1
Unknown	1	1	0
Missing	0	0	0

Table S2 Test performance of Abbotts' PanBioTM COVID-19 Ag Rapid Test (RAT) compared to PCR in municipalities with outbreaks.

	RAT neg (n)	RAT pos (n)	Total (n)	Sensitivity (%)	CI 95% (%)	Specificity (%)	CI 95% (%)
FARSUND							
PCR pos	8	20	28	71.4	53-85		
PCR neg	276	0	276			100.00	98-100
PCR pos symptomatic	4	17	21	81.0	59-93		
PCR pos asymptomatic	3	2	5	40.0	5-85		
PCR pos exposed	8	17	25	68.0	48-83		
RANA							
PCR pos	6	12	18	66.7	44-84		
PCR neg	57	0	57			100.00	92-100
PCR pos symptomatic	2	9	11	81.8	51-96		
PCR pos asymptomatic	1	2	3	66.7	20-94		
PCR pos exposed	3	12	15	80.0	54-94		
VÅLER/ÅSNES							
PCR pos	3	6	9	66.7	35-88		
PCR neg	395	0	395			100.00	99-100
PCR pos symptomatic	0	3	3	100.0	38-100		
PCR pos asymptomatic	1	2	3	66.7	20-94		
PCR pos exposed	3	6	9	66.7	35-88		
LINDESNES							
PCR pos	0	1	1	100.0	17-100		
PCR neg	53	0	53			100.00	92-100
VINDAFJORD							
PCR pos	1	2	3	66.7	20-94		
PCR neg	18	0	18			100.00	79-100
LURØY							
PCR pos	0	1	1	100.0	17-100		
PCR neg	7	0	7			100.00	60-100

Table S3 PPV and NPV at the different prevalence's, as shown in figure 3.

Prevalence	PPV	NPV
0.001	0.427	1.000
0.005	0.789	0.999
0.010	0.883	0.997
0.020	0.938	0.995
0.030	0.958	0.992
0.050	0.975	0.987
0.100	0.988	0.972
0.200	0.995	0.940

Table S4 Detection limit of antigen rapid test:

Clinical sample	**RAT test 1**	**RAT test 2**	**Average ct-values in RAT buffer (NIPH)**	**Estimated copies/mL**
Undiluted	Pos	Pos	19,3	$1.4x10^7$
10^{-1}	Pos	Pos	22,1	$3.1x10^6$
10^{-2}	Pos (weak)	Pos (weak)	23,52	$1,4x10^6$
10^{-3}	Neg	Neg	24,97	$6.5x10^5$

Sample size calculation Aker test station

The sample size was calculated based on the formula by Malhotra et al., 2010 (2).

$$\text{Sample size } (n) \text{ based on sensitivity} = \frac{Z^2_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times Prevalence}, \text{ and}$$

$$\text{sample size } (n) \text{ based on specificity} = \frac{Z^2_{1-\alpha/2} \times S_P \times (1-S_P)}{L^2 \times (1-Prevalence)},$$

n= sample size, α = 0.05, Z_(1-α)/2 = 1.96, and L = absolute precision desired on either side (half-width of the confidence interval) of sensitivity or specificity. L was set to 0.03, 0.05, 0.07 and 0.1 for an estimated sensitivity of 60% and 80% and prevalence of 4%. A sample size of 4000 was deemed acceptable.

Experiences reported by the test performers at Aker test station

Between 180 and 250 persons visited Aker test station daily during the project period. A total of 5412 persons had their samples tested with PCR. Out of these, 4025 people accepted to include the RAT, giving a participation rate at 74.4%. When participants attended Aker test station, they were given an information leaflet obtaining regulate information and an informed consent form. This was distributed while participants were waiting in line prior to the testing procedure.

Working conditions

The test station experienced good workflow and testers cooperated well, so that samples for both PCR and RAT could be taken from the same patient. During the project period, Aker test station expanded their working staff so that one dedicated person took the samples to be analyzed with PCR, and another performed the RAT. The RAT kit was found to be somewhat difficult to use because the test stick was more rigid and less manageable than the PCR test stick. Concerns about contamination risk was reported in this context. Another challenge associated with the RAT kit was that the lysis buffer was sometimes difficult to pipette. At times the test pin blocked the pipette opening, and the tester had to turn the test tube to get the sample material out. This also resulted in difficulties in controlling the number of drops applied to the RAT kit, which was supposed to be exactly 5 drops. There were surplus amounts of buffer solution for each of the test kits.

Analysis routines

The analysis logistics were established in the start-up phase. In order to keep the analysis time within the recommended 15 minutes from sampling to reading the results, a timer was used. This made it possible to analyze several samples simultaneously. The employees reported well-functioning routines for handling samples. Some workers reported stress related to a poor working position when performing the tests. An iPad was used as registration tool. The test station found this challenging as the system was at times perceived as unstable. Therefore, the test station had to establish routines for double-checking when registering test results.

Reporting of test results

The communication of test results proved to be a challenge because many workers lacked the necessary medical knowledge to answer questions. More in-depth training and use of regular callers to ensure continuity was suggested. Telephone operation was also challenging due to the high number of calls, and sometimes due to the complexity of the problems presented.