Diagnostic accuracy of rapid antigen tests in asymptomatic and presymptomatic close contacts of individuals with confirmed SARS-CoV-2 infection: cross sectional study

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ABSTRACT

OBJECTIVE
To assess the diagnostic test accuracy of two rapid antigen tests in asymptomatic and presymptomatic close contacts of people with SARS-CoV-2 infection on day 5 after exposure.

DESIGN
Prospective cross sectional study.

SETTING
Four public health service covid-19 test sites in the Netherlands.

PARTICIPANTS
4274 consecutively included close contacts (identified through test-and-trace programme or contact tracing app) aged 16 years or older and asymptomatic for covid-19 when requesting a test.

MAIN OUTCOME MEASURES
Sensitivity, specificity, and positive and negative predictive values of Veritor System (Beckton Dickinson) and Biosensor (Roche Diagnostics) rapid antigen tests, with reverse-transcriptase polymerase chain reaction (RT-PCR) testing as reference standard. The viral load cut-off above which 95% of people with a positive RT-PCR test result were virus culture positive was used as a proxy of infectiousness.

RESULTS
Of 2678 participants tested with Veritor, 233 (8.7%) had a RT-PCR confirmed SARS-CoV-2 infection of whom 149 were also detected by the rapid antigen test (sensitivity 63.9%, 95% confidence interval 57.4% to 70.1%). Of 1596 participants tested with Biosensor, 132 (8.3%) had a RT-PCR confirmed SARS-CoV-2 infection of whom 83 were detected by the rapid antigen test (sensitivity 62.9%, 54.0% to 71.1%). In those who were still asymptomatic at the time of sampling, sensitivity was 58.7% (51.1% to 66.0%) for Veritor (n=2317) and 59.4% (49.2% to 69.1%) for Biosensor (n=1414), and in those who developed symptoms were 84.2% (68.7% to 94.0%; n=219) for Veritor and 73.3% (54.1% to 87.7%; n=158) for Biosensor. When a viral load cut-off was applied for infectiousness (≥5.2 log10 SARS-CoV-2 E gene copies/mL), the overall sensitivity was 90.1% (84.2% to 94.4%) for Veritor and 86.8% (78.1% to 93.0%) for Biosensor, and 88.1% (80.5% to 93.5%) for Veritor and 85.1% (74.3% to 92.6%) for Biosensor, among those who remained asymptomatic throughout. Specificities were ›99%, and positive and negative predictive values were ›90% and ›95%, for both rapid antigen tests in all analyses.

CONCLUSIONS
The sensitivities of both rapid antigen tests in asymptomatic and presymptomatic close contacts tested on day 5 onwards after close contact with an index case were more than 60%, increasing to more than 85% after a viral load cut-off was applied as a proxy for infectiousness.

WHAT IS ALREADY KNOWN ON THIS TOPIC

At the end of 2020, rapid antigen tests had been evaluated and considered sufficient to be used in the Dutch public health service test sites in people with mild to moderate symptoms of covid-19 without the need for retesting with reverse-transcriptase polymerase chain reaction

The few evaluations of rapid antigen tests in asymptomatic people at that time were of small sample size, largely did not account for whether the tested individual had been exposed to an index case, and did not perform virus culture

WHAT THIS STUDY ADDS

The sensitivity of both the Veritor (Beckton Dickinson) and the Biosensor (Roche Diagnostics) for detecting SARS-CoV-2 in this population was more than 60%, increasing to more than 85% after a viral load cut-off was applied as a proxy of infectiousness

The results suggest that close contacts of people with confirmed SARS-CoV-2 infection can accurately be tested for SARS-CoV-2 using either rapid antigen test from day 5 onwards, even when they have not (yet) developed symptoms

The tests should not be used when the consequences of missed infections will be severe

Introduction

The cornerstone of control during the covid-19 pandemic has been the implementation of generic infection control measures (hand hygiene, physical distancing, and staying at home when symptoms develop) combined with test-and-trace programmes. Mathematical modelling studies have shown that test-and-trace programmes in combination with generic infection control measures can successfully control SARS-CoV-2 epidemics, even when assuming that at least 40% of transmissions might result arise from asymptomatic people or those whose symptoms have not yet developed.[1 2] Such measures can only reduce the reproductive number below 1.0, however, when delays in test and trace are minimised.[3 4] In test-

and-trace programmes, contacts of infected people are actively traced and offered testing, initially only when symptoms develop, but increasingly also when asymptomatic or before symptoms develop.[5]

In the first phase of the pandemic, only people with symptoms had access to (free-of-charge) testing at Dutch public health service test sites, and testing was performed using reverse-transcriptase polymerase chain reaction (RT-PCR) of combined oral and nasal or nasopharyngeal swabs. The sensitivities of these tests increase as the upper respiratory tract viral load increases, which is known to reach a high plateau on day 5 after infection.[6-9] From 1 December 2020 onwards, it was possible to test asymptomatic close contacts of index cases on day 5 after exposure.

Although RT-PCR is considered the reference test for SARS-CoV-2, it also has disadvantages. RT-PCR testing platforms are typically only available in centralised laboratories and require sample batching, thereby introducing delays in testing. Point-of-care SARS-CoV-2 tests soon became available and, of these, rapid lateral flow antigen diagnostic tests are promising.[10] These tests require no or minimal equipment, provide a result within minutes, and can be performed in a range of settings with relatively little training.

Multiple studies have now compared rapid antigen tests with RT-PCR testing.[11] Based on these studies, the Dutch Ministry of Health concluded at the end of 2020 that the performance of rapid antigen tests was sufficient to be used in the Dutch public health service test sites in those with mild to moderate symptoms without the need for retesting with RT-PCR.[12] The ministry recommended to continue RT-PCR testing in those with severe symptoms, at risk medical groups, people working in high risk settings such as hospitals, and asymptomatic or presymptomatic close contacts.[13] The reason being that the diagnostic accuracies of rapid antigen tests were expected to be lower in asymptomatic people and in samples containing lower SARS-CoV-2 viral loads.[11 14] This is not necessarily problematic if lower viral load translates into lower subsequent expected infectiousness.[15] A recently published Cochrane review showed that 12 evaluations of rapid antigen tests in asymptomatic people had been performed up to 30 September 2020, of which only four took into account exposure to an index case.[16] The identified studies were small and did not perform virus culture. Evaluations published since 1 October 2020 had the same limitations.[11 17-19] In the current study we quantified the accuracy of two rapid antigen tests for detecting SARS-CoV-2 infection with RT-PCR testing as the reference standard in asymptomatic and presymptomatic close contacts of index cases.

Methods
Study design and population
This prospective cross sectional diagnostic test accuracy study was embedded within the Dutch routine testing infrastructure. In the Netherlands, asymptomatic and presymptomatic close contacts can be identified by either the Dutch public health service test-and-trace programme, the Dutch contact tracing mobile phone application (the CoronaMelder app), an individual with confirmed SARS-CoV-2 infection (index case), or a combination of these. Between 1 December 2020 and the end of the study period, Dutch testing policy stipulated that asymptomatic and presymptomatic close contacts should schedule an RT-PCR test from the fifth day onwards after the last exposure. As individuals are generally not tested on the day of their test request, some might develop symptoms by the time of sampling. Close contacts who were still asymptomatic at the time of sampling and had a negative RT-PCR test result ≥5 days after exposure were encouraged to get retested if they developed symptoms and to avoid close contact with vulnerable people.[20]

Participants were recruited consecutively at four Dutch public health service test sites, located in the West-Brabant region (Raamsdonksveer and Roosendaal) and in the city of Rotterdam (Rotterdam Ahoy and Rotterdam The Hague Airport; travellers were excluded). Close contacts, presenting at these test sites, were considered eligible if they were aged 16 years or older, scheduled for a test ≥5 days after exposure, asymptomatic at the time of the test request, and willing and able to sign an informed consent in Dutch.

Inclusion procedure
Participants arrived at the test sites by car (West-Brabant) or on foot (Rotterdam). Test site staff verbally verified study eligibility. Eligible individuals received a study flyer and a participant information letter. After signing the informed consent form, a short questionnaire on presence, type, and onset of symptoms (see supplementary material 1) was self-completed by participants (West-Brabant) or by test site staff (Rotterdam) while participants waited for sampling. Two people independently extracted questionnaire data in duplicate.

Specimen collection and testing and virus culture procedures
Supplementary material 2 provides a detailed description of how the specimens were collected and tested, including culturing. A trained staff member took two combined oropharyngeal-nasal (West-Brabant) or oronasopharyngeal (Rotterdam) swabs from the study participant: one for RT-PCR testing and the other for rapid antigen testing. Swabs were transported to relevant offsite and onsite laboratories, respectively.

During the study period, all study sites were using Roche cobas 6800/8800 platforms for RT-PCR testing (supplementary material 2). The sites in West-Brabant were using the BD Veritor System (Becton Dickinson, Franklin Lakes, NJ) and the Rotterdam sites the Biosensor test (Roche Diagnostics, Basel, Switzerland). Both tests were applied according to the manufacturer's instructions. The results of Veritor were determined visually instead of using a Veritor Plus Analyzer. Interpretation of the rapid antigen test results

was always done before (thus staff were blinded) the RT-PCR result. Similarly, the results of the rapid antigen tests were not available to those assessing the RT-PCR results. Participants received the RT-PCR test result, but not the rapid antigen test result, to direct further management (such as quarantine advice).

At the Erasmus Medical Center Viroscience diagnostic laboratory, samples from participants in Rotterdam with a positive RT-PCR test result were cultured for seven days. Once cytopathic effects were visible, the presence of SARS-CoV-2 was confirmed with immunofluorescent detection of SARS-CoV-2 nucleocapsid protein (Rabbit polyclonal antibody; Sino Biological, Eschborn, Germany; supplementary material 2). Samples from participants in West-Brabant were not cultured.

Outcomes and statistical analyses

The primary outcome was the diagnostic accuracy (sensitivity, specificity, and positive and negative predictive values with corresponding 95% confidence intervals) of each rapid antigen test, with RT-PCR as reference standard. The Roche cobas platforms for RT-PCR testing were used according to the manufacturer's instructions; amplification curves and cycle threshold values were interpreted using the manufacturer's interpretation algorithms, which complied with the European in-vitro diagnostic devices directive. As the number of individuals without RT-PCR or rapid antigen test results was low (n=21 (0.5%); fig 1), we performed a complete case analysis.

Secondary outcomes included the diagnostic accuracy variables stratified for presence of covid-19-like symptoms between the test request and time of sampling (yes or no), number of days between last contact and date of sampling (<5, 5, and >5 days), and different viral load cut-offs and viral load cut-off above which 95% of people with a positive RT-PCR test result had a positive culture as a proxy of infectiousness. Cycle threshold values were first converted into viral loads (SARS-CoV-2 E gene copies/mL) using a standard curve (supplementary material 2). The infectiousness cut-off was defined as the viral load above which 95% of people with a RT-PCR test result showed in vitro infectivity in cell culture.

Finally, to capture any missed infections by the day 5 RT-PCR test, we prespecified the use of the SARS-CoV-2 test result databases of participating public health services to determine through pseudonymised linkage whether study participants with a negative day 5 RT-PCR test result had tested positive in the subsequent 10 days by either RT-PCR test or a rapid antigen test.

Sample size calculation

Previous performance studies of rapid antigen tests in people with covid-19 symptoms found sensitivities of around 85%.[11 15 21 22] We based our sample size calculation on an expected sensitivity of 80%, with a margin of error of 7%, type I error of 5%, and power of 90%. We therefore aimed for 140 positive RT-PCR test results for each rapid antigen test compared with RT-PCR test. In our target population, we anticipated a SARS-CoV-2 prevalence (based on RT-PCR testing) of 10%, and closely monitored RT-PCR test positivity proportion over time to prolong recruitment if needed.

Patient and public involvement

Patients and the public were indirectly involved in this research. Strong signals and requests were made by the public via news outlets and social media to determine whether rapid antigen tests, which provide a test result quicker than RT-PCR testing, can also be used to test close contacts of individuals already infected with SARS-CoV-2 at day 5 since the contact with the index case. Because the pandemic was at its height in the Netherlands, the urgency of the study, and the short time from conception to conduct of the study, we did not reach out actively to individuals outside our large, multidisciplinary study group.

Results

Between 14 December 2020 and 6 February 2021, 5190 people were considered eligible for participation of whom 4295 participated (fig 1). Results for both RT-PCR and rapid antigen tests were available for 2678 (99.5%) in the Veritor group and 1596 (99.6%) in the Biosensor group. The Veritor and Biosensor groups were similar: the mean ages were 45.9 (SD 17.6) years and 40.7 (SD 16.4) years, respectively, 1370 (51.3%) and 751 (47.3%) were female participants, and 219 (8.6%) and 158 (10.1%) had developed symptoms at the time of sampling (supplementary table S1).

In the Veritor group, 233 (8.7%) participants had an RT-PCR confirmed SARS-CoV-2 infection; of these, 149 were also detected by Veritor resulting in an overall sensitivity of 63.9% (95% confidence interval 57.4% to 70.1%; table 1). Specificity and positive and negative predictive values were 99.6% (99.3% to 99.8%), 94.3% (89.5% to 97.4%), and 96.7% (95.9% to 97.3%), respectively. In the Biosensor group, 132 (8.3%) participants had an RT-PCR confirmed SARS-CoV-2 infection; of these, 83 were also detected by Biosensor resulting in an overall sensitivity of 62.9% (54.0% to 71.1%). Specificity and positive and negative predictive values were 99.5% (98.9% to 99.8%), 91.2% (83.4% to 96.1%), and 96.7% (95.7% to 97.6%), respectively.

In participants who developed symptoms between the test request and time of sampling, sensitivity was 84.2% (68.7% to 94.0%) for Veritor (n=219; prevalence 17.4%) and 73.3% (54.1% to 87.7%) for Biosensor (n=158; prevalence 19.0%). Specificity and positive and negative predictive values were 99.4% (97.0% to 100%), 97.0% (84.2% to 99.9%), and 96.8% (93.1% to 98.8%) for Veritor, and 98.4% (94.5% to 99.8%), 91.7% (73.0% to 99.0%), and 94.0% (88.6% to 97.4%) for Biosensor, respectively.

In participants who remained asymptomatic up to the time of sampling, sensitivity was 58.7% (51.1% to 66.0%) for Veritor (n=2317; prevalence 7.7%) and 59.4% (49.2% to 69.1%) for Biosensor (n=1414; prevalence 7.1%). Specificity and positive and negative
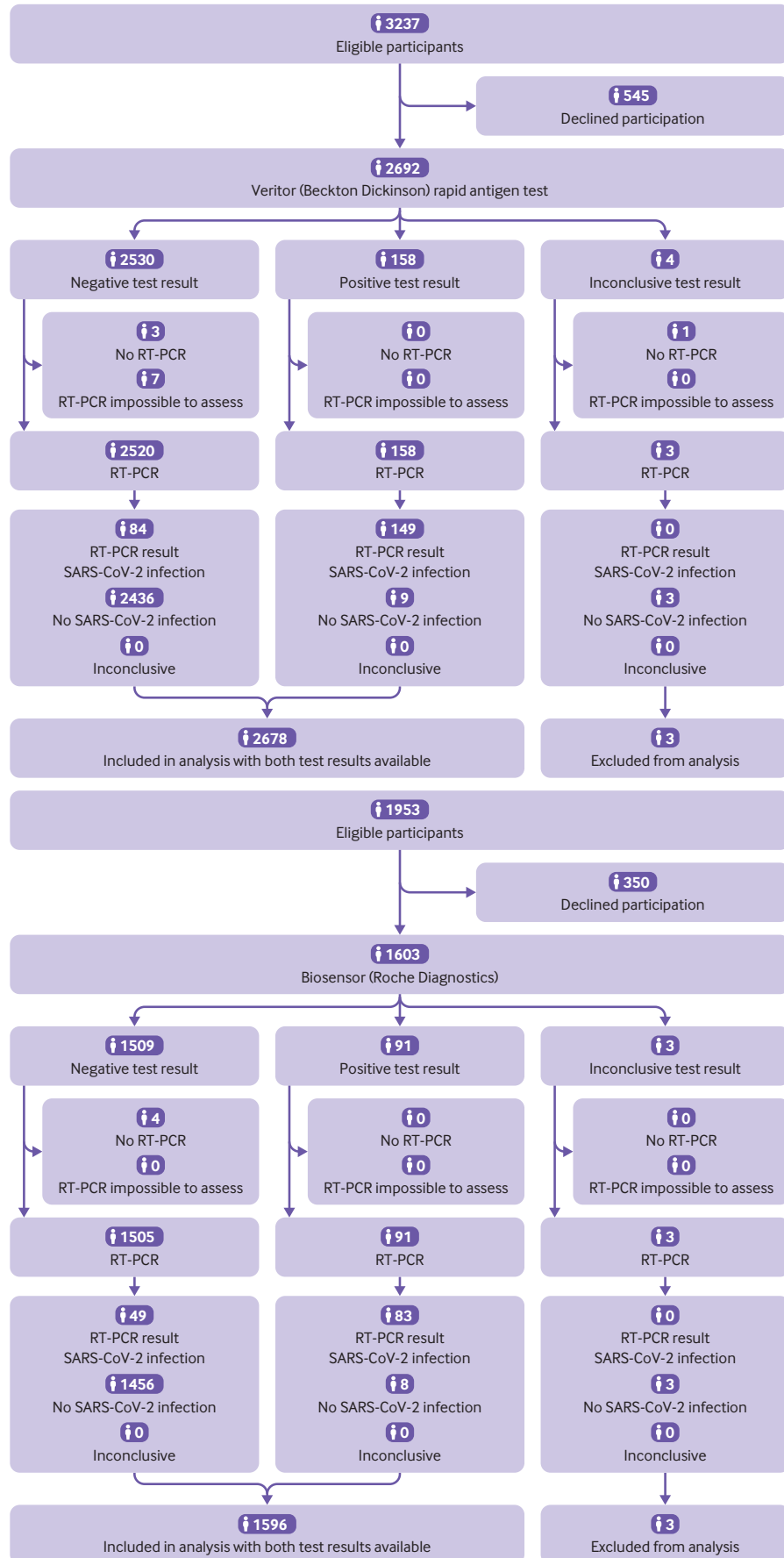
Fig 1 | Flow of study participants. RT-PCR=reverse-transcriptase polymerase chain reaction

Table 1 | Diagnostic accuracy variables of two rapid antigen tests. Values are percentages (95% confidence interval) unless stated otherwise

Analysis	No	Prevalence* (%)	Sensitivity	Specificity	PPV	NPV
Veritor System (Beckton Dickinson)						
Primary analysis	2678	8.7	63.9 (57.4 to 70.1)	99.6 (99.3 to 99.8)	94.3 (89.5 to 97.4)	96.7 (95.9 to 97.3)
Secondary (stratified) analysis:						
Infectiousness viral load cut-off†	2677‡	5.7	90.1 (84.2 to 94.4)	99.2 (98.8 to 99.5)	87.3 (81.0 to 92.0)	99.4 (99.0 to 99.7)
Symptoms present at sampling§:						
Yes	219	17.4	84.2 (68.7 to 94.0)	99.4 (97.0 to 100)	97.0 (84.2 to 99.9)	96.8 (93.1 to 98.8)
No	2317	7.7	58.7 (51.1 to 66.0)	99.6 (99.3 to 99.8)	92.9 (86.5 to 96.9)	96.6 (95.8 to 97.4)
Interval (days) between sampling and last contact with index case¶:						
‹5	379	14.8	69.6 (55.9 to 81.2)	99.7 (98.3 to 100)	97.5 (86.8 to 99.9)	95.0 (92.1 to 97.1)
5	1303	6.5	62.4 (51.2 to 72.6)	99.9 (99.5 to 100)	98.1 (90.1 to 100)	97.4 (96.4 to 98.2)
›5	511	9.0	56.5 (41.1 to 71.1)	99.1 (97.8 to 99.8)	86.7 (69.3 to 96.2)	95.8 (93.7 to 97.4)
Biosensor (Roche Diagnostics)						
Primary analysis	1596	8.3	62.9 (54.0 to 71.1)	99.5 (98.9 to 99.8)	91.2 (83.4 to 96.1)	96.7 (95.7 to 97.6)
Secondary (stratified) analysis:						
Infectiousness viral load cut-off†	1596	5.7	86.8 (78.1 to 93.0)	99.2 (98.6 to 99.6)	86.8 (78.1 to 93.0)	99.2 (98.6 to 99.6)
Symptoms present at sampling§:						
Yes	158	19.0	73.3 (54.1 to 87.7)	98.4 (94.5 to 99.8)	91.7 (73.0 to 99.0)	94.0 (88.6 to 97.4)
No	1414	7.1	59.4 (49.2 to 69.1)	99.5 (99.0 to 99.8)	90.9 (81.3 to 96.6)	97.0 (95.9 to 97.8)
Interval (days) between sampling and last contact with index case¶:						
‹5	153	13.1	75.0 (50.9 to 91.3)	99.2 (95.9 to 100)	93.8 (69.8 to 99.8)	96.4 (91.7 to 98.8)
5	1095	7.8	61.2 (50.0 to 71.6)	99.5 (98.9 to 99.8)	91.2 (80.7 to 97.1)	96.8 (95.6 to 97.8)
›5	205	6.3	69.2 (38.6 to 90.9)	99.5 (97.1 to 100)	90.0 (55.5 to 99.7)	97.9 (94.8 to 99.4)

PPV=positive predictive value; NPV=negative predictive value.
*SARS-CoV-2 infection based on reverse-transcriptase polymerase chain reaction (RT-PCR) test result.
†Viral load cut-off for infectiousness, defined as viral load above which 95% of people with a positive RT-PCR test result had a positive viral culture, was 5.2 log10 SARS-CoV-2 E gene copies/mL.
‡Viral load unavailable for one participant in Veritor group with a positive RT-PCR test result.
§Symptoms not available for 142 participants in Veritor group and 24 in Biosensor group.
¶Interval between moment of sampling and last contact with an infected individual was not available for 488 participants in Veritor group and 143 in Biosensor group, mainly because this question was added to the questionnaire later in study. Initially, a three item questionnaire was used. Questions 1 and 2 in the five item questionnaire (see supplementary material 1) were added after the first week of the study. The time interval between the last contact and time of sampling is not the same as the time between the test request and time of sampling.
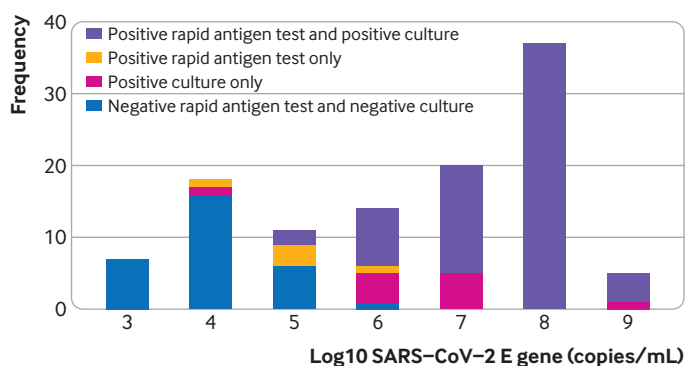
predictive values were 99.6% (99.3% to 99.8%), 92.9% (86.5% to 96.9%), and 96.6% (95.8% to 97.4%) for Veritor, and 99.5% (99.0% to 99.8%), 90.9% (81.3% to 96.6%), and 97.0% (95.9% to 97.8%) for Biosensor, respectively.

Table 1 shows the results of additional secondary analyses. Supplementary tables S2 and S3 show the results for 2×2 tables of all primary and secondary analyses.

Figure 2 shows the distribution of viral loads in participants with a positive RT-PCR test result, stratified by a combination of the Biosensor test result and the ability to culture SARS-CoV-2. When viral load was ≥5.2 log10 SARS-CoV-2 E gene copies/mL, the specimens of 95% of participants with positive RT-PCR test results could be cultured. The percentage of

Fig 2 | Distribution of viral loads in participants with a positive reverse-transcriptase polymerase chain reaction (RT-PCR) test result, stratified by Biosensor rapid antigen test result (negative or positive) and ability to culture, or not culture, SARS-CoV-2

participants with a viral load ≥5.2 log10 SARS-CoV-2 E gene copies/mL was 5.7% in both groups (152/2677 for Veritor and 91/1596 for Biosensor), and among those with a positive RT-PCR test result, 65.2% (152/233) in the Veritor group and 68.9% (91/132) in the Biosensor group. Using that viral load cut-off as a proxy for infectiousness, sensitivity was 90.1% (84.2% to 94.4%) for Veritor and 86.8% (78.1% to 93.0%) for Biosensor. Specificity and positive and negative predictive values were 99.2% (98.8% to 99.5%), 87.3% (81.0% to 92.0%), and 99.4% (99.0% to 99.7%) for Veritor and 99.2% (98.6% to 99.6%), 86.8% (78.1% to 93.0%), and 99.2% (98.6% to 99.6%) for Biosensor, respectively. Figure 3 shows diagnostic accuracy variables stratified by different viral load cut-offs. The sensitivity of both rapid antigen tests at the infectious viral load cut-off in participants without symptoms at the time of sampling was 88.1% (80.5% to 93.5%) for Veritor (n=2317; prevalence 4.7%) and 85.1% (74.3% to 92.6%) for Biosensor (n=1414; prevalence 4.7%). Specificity and positive and negative predictive values were 99.2% (98.8% to 99.6%), 85.0% (77.0% to 91.0%), and 99.4% (99.0% to 99.7%) for Veritor and 99.3% (98.7% to 99.7%), 86.4% (75.7% to 93.6%), and 99.3% (98.6% to 99.6%) for Biosensor, respectively. Supplementary figure 1 shows the diagnostic accuracy variables for this group at varying viral load cut-offs.

After linkage with test result databases of participating public health services, only 57 (1.6%) participants were found to have a positive SARS-CoV-2 test result within 10 days after their negative day 5 test
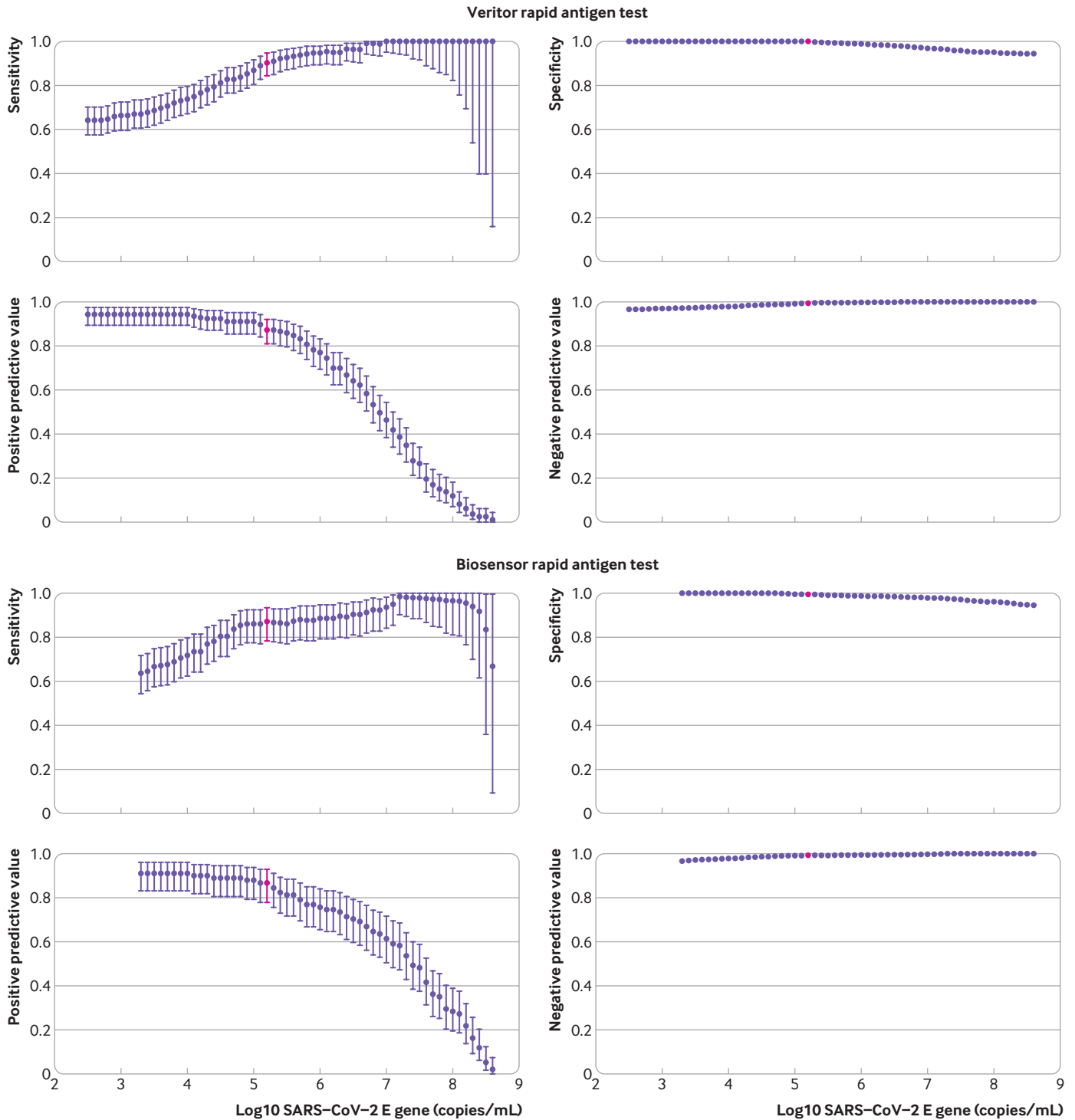
Fig 3 | Diagnostic accuracy variables of both rapid antigen tests for different definitions of reverse-transcriptase polymerase chain reaction (RT-PCR) test positivity based on viral load cut-offs, where a positive RT-PCR test result with a viral load below the viral load cut-off threshold is considered a negative result. Points highlighted in red indicate a viral load cut-off of 5.2 log10 SARS-CoV-2 E gene copies/mL, which was considered the viral load cut-off for infectiousness as determined by viral culture. PPV=positive predictive value; NPV=negative predictive value

result by RT-PCR. None of them had a positive day 5 test result by rapid antigen test.

Discussion

The Veritor and Biosensor lateral flow rapid antigen tests are among the most used diagnostic rapid tests for SARS-CoV-2 in the Netherlands but have only been evaluated in people with symptoms of covid-19. We determined the performance of these tests in asymptomatic and presymptomatic close contacts on the fifth day after exposure to an index case. At the time of the study, the prevalence of SARS-CoV-2 in our study population was around 8.5% and in the Dutch testers population as a whole (people with symptoms and

asymptomatic or presymptomatic testers combined) around 18%.[23] Both tests had a sensitivity of about 63% compared with RT-PCR testing. The sensitivity increased to 87-90% when a viral load cut-off of ≥5.2 log10 SARS-CoV-2 E gene copies/mL was used as a proxy for infectiousness. Specificities and positive and negative predictive values were high in analyses for both tests.

Comparison with other studies

The sensitivities of the tests were expectedly lower than those generally reported for people with symptoms and higher than those for asymptomatic people who are tested at random.[11] Our study population consisted of participants who were asymptomatic at test request and developed symptoms between test request and actual testing (about 10%) and those who did not. The sensitivities of the rapid antigen tests were close to 90% in the former group, which is comparable to previous studies of people with symptoms.[11 16] The sensitivities were close to 60% in the latter group, which is higher than those reported in previous studies of asymptomatic people.[11 16] However, those studies generally focused on those who were tested at random and not because they had been exposed to an index case (close contacts). At the time of our study, the prevalence of SARS-CoV-2 in the Dutch population as a whole was about 2%,[17] which is more than four times lower than the prevalence in our study population of those with known exposures to index cases.

RT-PCR as reference standard

We used RT-PCR on Roche cobas platforms as the reference test. RT-PCR tests are considered the preferred reference tests,[24] but with one important caveat: previous studies have shown that, on average, viral load and RT-PCR test sensitivity gradually increase in the 5-7 days after infection, reach a plateau that lasts for 1-2 weeks, and then decline.[7] By the time people develop symptoms, they generally have a sufficiently high viral load for RT-PCR test sensitivity to be considered optimal. However, the viral load in most people who do not (yet) have symptoms is lower, and RT-PCR test sensitivity might therefore be suboptimal. At the same time, many of these people are able to transmit SARS-CoV-2 to others. The Dutch Ministry of Health dealt with this delicate balance between infectiousness and RT-PCR test sensitivity by encouraging asymptomatic and presymptomatic close contracts of index cases to be tested from the fifth day after exposure to an index case. Our data suggest that this is appropriate because only 1.6% of those with a negative RT-PCR and rapid antigen test result on the fifth day after exposure had a positive test result within the subsequent 10 days.

Virus culture result as proxy for infectiousness

We used the viral load cut-off above which 95% of people with a positive RT-PCR test result had a positive virus culture as a proxy of infectiousness. Although this cut-off is not fully evidence based, it is a best guess

based on current knowledge and is less arbitrary than using RT-PCR cycle threshold value cut-offs of 25 or 30, as is often done.[21 25] Animal models have provided some evidence for an association between SARS-CoV-2 infectiousness and the ability to culture virus. For example, in a golden hamster model, infectiousness correlated with the detection of infectious virus in culture but not with detection of viral RNA.[26] Data from human studies are limited but mounting. Correlations between infectivity in culture and viral load, and between viral load and secondary attack rate, have been established, but variability between laboratories and studies was high.[27-30] Furthermore, the exact upper respiratory tract viral load cut-off below which no transmissions take place is still not known; some reports have suggested infectiousness at viral loads as low as 10 000 SARS-CoV-2 RNA copies/mL.[31]

The ability to culture virus is not only affected by the viral load of the sample but also by other factors related to the host, type of viral culture kits and methods used, and experience level of the laboratory team. An important host factor is the presence of SARS-CoV-2 neutralising antibodies.[25] To address concerns about high variability between laboratories, we performed all virus cultures in one experienced laboratory (at the Erasmus Medical Center Viroscience diagnostic laboratory in Rotterdam)[15 21 25] on fresh material (no freeze-thaw cycles). Therefore, only specimens collected by the Rotterdam study sites were cultured, and the infectiousness cut-off was extrapolated to specimens collected by the West-Brabant sites. Reassuringly, the two laboratories had similar RT-PCR test calibration curves, indicating that cycle threshold values corresponded to similar viral loads.

Strengths and limitations of this study

Strengths of our study include the well defined study population, large sample size, collection of samples for the reference and index test at the same time, and reference and index tests performed by trained staff who were blinded to the result of the other test. We also consider the use of virus culture results in our definition of infectiousness to be a strength, despite some of the limitations, because RT-PCR cycle values are even more influenced by laboratory workflow than the methods that we used.

Our study also has limitations. Firstly, we did not assess the type of the close contacts (eg, at home, at work, at school, or on public transport) or duration of the close contacts. This is especially problematic for household contacts, because exposure might take place over a prolonged period, which is associated with a high probability of testing positive (20% v 10%).[23] In our study, 12% of the participants reported that their last contact was within the past five days, and this group had a higher RT-PCR test positivity percentage than the study population as a whole. We suspect that they might have been household contacts with prolonged exposure but this cannot be verified. Secondly, we did not actively follow-up participants who had a negative RT-PCR test result

at day 5, but we explicitly designed per protocol to apply pseudonomysed linkage of these participants to the test result databases of the participating public health services. The 1.6% of infected close contacts who according to that database had a positive test result within 10 days after their negative day 5 RT-PCR test result in our study likely represent only those who developed symptoms and requested a new test for that reason at one of the participating public health services. Active follow-up, including repeat testing in all study participants, would have reduced the uncertainty around false negative RT-PCR test results completely, as was also recommended in a recent guidance paper.[24] Unfortunately, we could not implement this for ethical and logistic constraints, as our study was embedded in busy public health service test sites during the height of the second wave in the Netherlands.

Policy implications

As a result of this study, early in 2021 Dutch test sites implemented the two (and other nationally approved) rapid antigen tests for testing of close contacts, even when they have not (yet) developed symptoms. Close contacts regardless of symptoms are encouraged to get tested as soon as possible after known exposure to avoid delays in identifying people who are positive for SARS-CoV-2. However, if they are tested before the fifth day after exposure, they are retested on the fifth day and remain in quarantine until the fifth day test result is negative. Rapid antigen tests are, however, still not used in high risk situations, such as testing of vulnerable people in care facilities, severly ill patients, or healthcare workers.

The advantages of rapid antigen testing compared with RT-PCR testing include simplified logistics and reduced dependence on equipment (which in turn allow for testing in the community and for self-testing) and reduced delays. The extent to which these advantages outweigh the lower sensitivity compared with RT-PCR testing is currently unknown. With the increasing use of rapid antigen tests instead of RT-PCR testing, we expect the number of missed infections to increase. This underlines the importance of immediate self-quarantine and repeat testing when symptoms develop after a negative result by rapid antigen test or RT-PCR. Furthermore, false positivity of rapid antigen test results was rare in our study but might become a larger issue as the prevalence of SARS-CoV-2 declines. In that case, positive results with rapid antigen tests might have to be confirmed by RT-PCR.[32] We will continue to monitor the advantages and disadvantages of rapid antigen tests compared with RT-PCR testing utilising national test-and-trace databases and mathematical modelling.

Conclusions

The sensitivities of both rapid antigen tests compared with RT-PCR tests in asymptomatic and presymptomatic close contacts on the fifth day after exposure to the index case was more than 60%, increasing to more than 85% after a viral load cut-off was applied as a proxy of infectiousness. The Veritor and Biosensor rapid antigen tests can therefore reliably be used to test close contacts for infectiousness from the fifth day after infection, even when they have not (yet) developed symptoms, but the tests should not be used when the consequences of missed infections will be severe.

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Dissemination to participants and related patient and public communities: The Dutch Outbreak Management Team that provides guidance to the Ministry of Health, Welfare, and Sport on policy regarding covid-19, advised, based on the results of this study, that close contacts of people with a confirmed SARS-CoV-2 infection can be tested for SARS-CoV-2 using a rapid antigen test from day 5 onwards, even when they have not (yet) developed covid-19 symptoms. As such, the results of our study have been disseminated and are currently incorporated in a nationwide testing policy. At the time, this change in policy has been covered by different news outlets.

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Supplementary file: Tables S1-S3, figure S1, and supplementary material 1 and 2
Supplementary file: Excel file showing calculation of 2×2 tables based on diagnostic accuracy of both tests with differing prevalence or sample size