Evaluation of two rapid tests for detection of antibodies against SARS-CoV-2

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1. Summary

Background

SARS-CoV-2, the virus causing COVID-19, has emerged to cause a human pandemic during the last two years. Detection of SARS-CoV-2 in respiratory samples by using RT-PCR has been the standard diagnostic tool, later antigen rapid tests for detection have also emerged. Immunological tests detecting antibodies (immunoglobulins type G (IgG) and/or IgM or total antibodies) against SARS-CoV-2 are available, including many rapid tests (point-of-care-tests).

Objective

Our aim in this study was to evaluate the analytical accuracy of two rapid tests for detection of antibodies against SARS-CoV-2, and specifically their abilities to confirm past COVID-19, where detection of IgG antibodies is considered essential.

Methods

We evaluated the analytical sensitivity of the antibody detecting rapid tests using serum samples from 64 recovered RT-PCR confirmed COVID-19 patients, who had not required hospitalization. We evaluated the analytical specificities of the rapid tests using 132 serum samples collected pre-COVID-19. Performance was evaluated against criteria suggested by the ECDC, sensitivity of ≥90% and specificity of ≥98%. User-friendliness was evaluated by the biomedical laboratory scientists performing the tests.

Results

One of the tests was a lateral flow immunoassay read visually and the other used a sandwich immunodetection method for detection of antibodies against SARS-CoV-2 and an instrument for reading of results. Test 1 had sensitivity ≥90% and specificity <80%, this test required additional laboratory equipment. Test 2 likely had a sensitivity of 90% and specificity ≥98%, and the test was easy to perform.

Conclusion and recommendations

Test 1 met the ECDC recommendation for analytical sensitivity, while the recommendation for analytical specificity was not met. Test 2 likely met the recommendation for analytical sensitivity and analytical specificity. Based on the analytical performance, test 1 is well suited to rule out, but not rule in past infection. Test 2 targets the SP, which is affected by vaccination, can be used to rule in or out past infections if the patient is not vaccinated, or to rule in or out antibody response after vaccination. The user-friendliness was regarded acceptable for test 1 and good for test 2.

2. Background

The clinical presentation of Coronavirus disease 2019 (COVID-19), caused by coronavirus SARS-CoV-2, varies from asymptomatic disease, via mild upper respiratory infection to severe pneumonia with respiratory failure and death. As of March 6th 2022, over 433 million confirmed cases of COVID 19 has been reported, in addition to over 5.9 million deaths, globally (1). In late 2020, the first vaccines against SARS-CoV-2 became available. Most of the vaccines produced worldwide so far, and all of the vaccines available in Norway, target the spike protein (SP) of the SARS-CoV-2 virus (2).

Laboratory methods for diagnosing COVID-19

The most commonly used methods for detecting current SARS-CoV-2 infection, are nucleic acid amplification tests (typically reverse transcription polymerase chain reaction (RT-PCR)), and antigen rapid tests. These methods commonly use swab specimens collected from the upper airways. RT-PCR is highly sensitive, performed at medical microbiology laboratories, and requires advanced analytical instruments and trained personnel, while the antigen rapid tests are cheap and fast and less sensitive.

Detecting humoral immune response to the virus is a different analytical approach. Generally, immunoglobulin type M (IgM) is produced during the early stages of infection, usually followed by production of immunoglobulin type G (IgG). For infection with SARS-CoV-2, however, there is some evidence that IgG can be observed at the same time as IgM, or even earlier (3, 4). Since IgM can be a sign of an unspecific immune response, IgG detection is usually required to determine past infection.

Several enzyme immune assays (EIA-methods) detecting and quantifying antibodies against SARS-CoV-2, both commercial and in-house, are available in Norwegian hospital laboratories. Also, a substantial number of point-of-care rapid test kits have been commercialized. Most of these kits are for professional use, they usually make use of capillary or venous whole blood, plasma, or serum, and they are designed to qualitatively detect IgG and/or IgM antibodies against various antigens of SARS-CoV-2. The results are read, visually or by instrument, after 10-15 minutes.

ECDC recommends using tests with an analytical sensitivity of at least 90%, and an analytical specificity of at least 98% (5). ECDC further recommends to not use the tests for diagnostic purposes. Examples for recommended use could be to confirm antibody response after vaccination or to investigate antibody response after confirmed infection with a NAAT (6).

3. Objective

Our main objective in this study was to perform a limited evaluation of the analytical accuracy of two different rapid tests for detection of SARS-CoV-2 antibodies. In particular, we aimed to evaluate if these two tests could be used to confirm past infection. Furthermore, we wanted to evaluate the user-friendliness of the two rapid tests.

4. Methods

The evaluation was organized and performed by the Norwegian Organization for Quality Improvement of Laboratory Examinations (Noklus), based on previously performed evaluations (7, 8).

Rapid tests included

The two rapid tests in the evaluation were selected after Noklus sent an invitation to suppliers that previously had requested such an evaluation of their rapid test (Table 1). The rapid tests were provided by the suppliers and the evaluation was free of charge. In sending the tests, they consented to having the results published. All rapid tests were performed in accordance with manufactures´ instructions under optimal and standardized conditions, by biomedical laboratory scientists.

Test number 1 used a sandwich immunodetection method for detection of IgG against both the spike protein (SP) and the nucleocapsid protein (NP) of SARS-CoV-2, while test number 2 was a lateral flow immunoassay for detection of both IgG and IgM against the spike protein of SARS-CoV-2. (Table 1).

Table 1. Rapid Tests evaluated.

Test Number	Test name	Manufacturer	Antigen target
1	ichroma COVID-19 Ab + ichroma II instrument	Boditech Med Incorporated, Republic of Korea	SP IgG + NP IgG
2	SARS-CoV-2 IgG/IgM Rapid Test	ACON Biotech (Hangzhou) Co., Ltd.	SP IgG + SP IgM

Hospital methods

Three automated immunoassay systems were available at the time of testing (Table 2), but due to the limited volumes of particularly the pre-COVID-19 serum samples, they were not all analysed on all systems. All measurements on the automated systems were performed during the previous evaluations.

Table 2: Automated immunoassay used under the evaluation.

TEST NAME	MANUFACTURER	ANTIGEN TARGET
IFLASH 1800	SHENZHEN THLO BIOTECH CO., LETD., China	NP
DYNEX DS2	DYNEX Technologies, USA	NP
ALINITY I	Abbot, USA	NP

Study Design

We evaluated the performance of the rapid tests in two study arms:

1. 64 serum samples from recovered RT-PCR-confirmed COVID-19 outpatients who had not required hospitalization.
2. 132 serum samples collected pre-COVID-19, of which 74 were from Vejle Biobank (9) and 58 from Vestre Viken Hospital trust.

Samples used for the evaluation were left over samples from the earlier evaluations of rapid tests (8).

All antibody rapid test results were compared to the RT-PCR results, and to hospital laboratory antibody results, where available.

Statistical analysis

For test number 1, results from antibodies against SP and NP were evaluated both together and separately. For test number 2, only IgG results were evaluated. For the RT-PCR comparison, the sensitivity was calculated from study arm 1, defined as the proportion of recovered COVID-19 patients who had detectable antibodies. Specificity was calculated from study arm 2 (pre-COVID-19 sera), defined as the proportion of SARS-CoV-2 antibody negative samples. We computed 95% confidence intervals (CI) for the sensitivities and specificities using the adjusted Wald method (10). When calculating the sensitivity and specificity for test 1, a positive result for either SP or NP measurements was regarded as a positive result. Borderline results (n=5) were omitted. For test 2, detection of IgG was regarded as a positive result.

Evaluation of user friendliness

User friendliness was considered “not acceptable” if the test was complicated to perform/required many steps or it was difficult to read the result, “acceptable” if the test required slightly more advanced laboratory equipment (such as an automated pipette), and otherwise “good”.

Ethical considerations

The project was considered a method evaluation study and therefore exempt from ethical board approval in Norway. Recovered COVID-19 patients gave written informed consent to participate. In Denmark, use of rest material as separated plasma/serum from anonymous healthy persons for technical quality control is not restricted. The project was approved by the Data protection officers in Kristiansand Municipality, at Vestre Viken Hospital Trust, and at Noklus.

5. Results

Test 1 had a sensitivity of 100% (97,0-100), and a specificity of 78,7% (70,8-85,0), and test 2 had a sensitivity of 89,1% (78,8-94,9) and a specificity of 98,5% (94,3-99,9) (Table 3).

Table 3. Analytical sensitivity and specificity, user-friendliness and overall evaluation for the evaluated tests. Results compared to RT-PCR results.

Rapid Test	Sensitivity (95% CI)	Specificity (95% CI)	User-friendliness
1[1]	100 (97,0-100)	78,7 (70,8-85,0)	Acceptable
2	89,1 (78,8-94,9)	98,5 (94,3-99,9)	Good

1. Borderline results omitted.

Table 4 shows the results of antibodies against SP and NP from test 1 compared with the RT-PCR results. Stratification on antigen target decreased the sensitivities and increased the specificities correspondingly.

Table 4. The separate results for SP and NP for test 1. Sensitivity and specificity compared to RT-PCR results.

Antigen target	Sensitivity (95% CI)	Specificity (95% CI)
NP	93,8 (84,6-98,0)	86,7 (79,7-91,6)
SP	95,3 (86,6-98,9)	90,8 (84,4-94,8)

Figures 1-3 show all available results from the hospital methods and antibody rapid tests for detecting IgG antibodies.

Most of the false negative samples on test 2 were either false negatives or showed borderline results on the hospital laboratory methods (Figure 1). For the false positives for test 1, however, we could not see any correlation between the hospital laboratory results and the rapid antibody results (Figures 2 and 3).

Figure 1. Samples from study arm 1 (confirmed RT-PCR positives).

ID	iFlash	DS2	Alinity	Test 1	Test 2
1001					
1002					
1003					
1004					
1005					
1007					
1008					
1009					
1010					
1011					
1012					
1013					
1014					
1015					
1016					
1017					
1018					
1020					
1021					
1022					
1023					
2001					
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2020					
2021					
2022					
2023					
2024					
3001					
3002					
3003					
3004					
3005					
3006					
3007					
3008					
3009					
3011					
3012					
3013					
3014					
3015					
3016					
3017					
3018					
3019					
3020					

	Positive
	Borderline
	Negative
	Not analysed

Figure 2. Samples from study arm 2 (pre-COVID-19, Vejle Biobank).

ID	iFlash	Test 1	Test 2
5001			
5002			
5003			
5006			
5007			
5008			
5009			
5010			
5011			
5012			
5013			
5014			
5015			
5016			
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5018			
5020			
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5081			
5082			
5084			
5085			
5086			
5088			
5089			
5091			
5094			
5098			
5100			

	Positive
	Borderline
	Negative

Figure 3. Samples from study arm 2 (pre-COVID-19, Vestre Viken Hospital trust)

ID	DS2	Test 1	Test 2
3			
4			
5			
6			
9			
10			
11			
14			
15			
16			
17			
18			
20			
21			
22			
23			
24			
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29			
30			
31			
32			
33			
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36			
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43			
44			
45			
49			
53			
54			
55			
60			
63			
64			
65			
66			
76			
77			
86			
100			
108			
109			
110			
111			
112			
113			
117			
118			
119			
120			
121			

	Positive
	Borderline
	Negative

User-friendliness

Test 1 required pre-analytical mixing of blood and buffer, an automated pipette for analyses and an instrument for reading the result. Test 2 was a simpler lateral flow test, easy to perform and to read.

6. Discussion

Test 1 had a sensitivity of 100% (97,0-100) and a specificity of 78,7% (70,8-85,0), while test 2 had a sensitivity of 89,1% (78,8-94,9) and a specificity of 98,5% (94,3-99,9). User-friendliness was considered acceptable for test 1 and good for test 2.

The methodology/test-principle of the two tests differed a lot. Test 1 measured both SP and NP IgG antibodies, while test 2 detected SP IgG and IgM antibodies. Additionally, test 1 was an immunodetection test and test 2 was a lateral flow test.

When evaluating the specificity of test 1, we used the combined result of antibodies against SP and/or NP. Since the specificity was low using the combined result, the specificities for the individual antigen targets were also calculated. This increased the specificities somewhat, especially for the SP result. However, the sensitivities decreased correspondingly. Test 1 met the ECDC recommendation for analytical sensitivity, however it did not meet the recommendation for analytical specificity.

A clinical area in which such a test, like test 1, could potentially be useful, would be in examination of whether an antibody response was due to vaccination and not infection. In this situation, a positive result for antibodies targeting SP while at the same time a negative result for antibodies targeting NP, could indicate this. However, this is not likely to be a very frequent nor very important clinical indication.

Test 2 allowed detection of both IgG and IgM against SP. Past infection is diagnosed with IgG alone, and an isolated positive IgM result may be due to an unspecific immune response or cross-reactivity with other antibodies. It may therefore be considered a disadvantage that these tests come with both IgG and IgM on the same cassette, as an isolated positive IgM result could potentially cause confusion. When considering only the IgG results, test 2 likely demonstrated analytical sensitivity and specificity as recommended by ECDC.

In general, if a person with previously RT-PCR-confirmed COVID-19 has no detectable antibodies against SARS-CoV-2, the stage of infection could be too early for antibodies to have been formed. In our populations this was not the case, as median seroconversion time has been reported at around 13-14 days after onset of symptoms (3, 11). However, not all COVID-19 patients seem to form detectable antibodies (3, 12), and we would therefore not expect to find 100% sensitivity for the antibody tests when using RT-PCR as the reference method. Furthermore, a false negative rapid test result, or a false positive RT-PCR result, could be possible explanations for no detectable antibodies.

On the other hand, if there were detectable antibodies in sera collected before the virus was in circulation, this could be due to cross-reactivity with other antibodies (i.e., other types of the coronavirus), a faulty test, or technical errors when performing the test.

A number of antibody tests target antibodies against SP, thus, the intended use of the rapid tests may be more relevant to check the antibody response after injections of SP targeting vaccines. Rapid tests that target the nucleocapsid NP on the other hand, are well suited to detect antibodies from previously COVID-19 infections, even after vaccination. However, studies have shown that the antibody response, from both vaccination and infection, vary from person to person. Nevertheless, a low antibody response does not mean low protection against possible future infection of SARS-CoV-2 (13-15).

The user-friendliness criteria were adapted for health care professionals without much laboratory training. This means that if a test did not get classified as having “good” user-friendliness, the test may still be well suited for a laboratory facility of moderate complexity.

Strengths and limitations

Strengths of our evaluations include the reasonably large number of samples from recovered COVID-19 outpatients, where all had had enough time to develop IgG antibodies against SARS-CoV-2. Furthermore, having access to a substantial number of pre-COVID-19 sera allowed us to evaluate the analytical specificities of the tests.

One weakness of this study is that we did not have access to sera with known antibodies to further challenge the tests for cross-reactivity. No accredited reference method for measurement of SARS-CoV-2 antibodies was available at the time of collection of the samples, so we had to use the confirmed RT-PCR as reference. The automated immunoassay systems used for the evaluation were in early stages of development and probably had a poorer analytical precision compared to current assays available. Also, although all manufacturers stated that serum, plasma or whole blood could be used for their tests, we only had access to serum samples which may not be the most commonly used material in primary care. Additionally, the serum samples used for the evaluation of analytical specificity had been kept in -80 degrees for >1 year and have been frozen and thawed more than once before analysing. Finally, since testing was performed under optimal conditions by laboratory biomedical scientists and not by intended users, both pre-analytical and analytical errors were minimized, and performance could be poorer in real life.

Conclusion

We examined analytical sensitivity and specificity of two antibody detecting rapid tests, as well as their user-friendliness. The analytical performance of the tests was compared against ECDC performance recommendations for rapid tests Test 1 met the performance recommendation for analytical sensitivity (≥90%), however it did not meet performance recommendation for analytical specificity (≥98%). Test 2 most likely met the performance recommendation for analytical sensitivity, and it also met the performance recommendation for analytical specificity. Based on the analytical performance of the tests, test 1 is well suited to rule out, but not to rule in past infection (and possibly vaccination). As test 2 targets the SP, which is affected by vaccination, it can be used to rule in or out past infections if the patient is not vaccinated, or to rule in or out antibody response after vaccination. The user-friendliness was acceptable for test 1 and good for test 2.

7. References

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