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Evaluation of Coris COVID-19 antigen respi-strip

Evaluation Report

Final

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Main findings

- The sensitivity of the COVID-19 Ag respi-strip for cultured SARS-CoV-2 is: ~ $2*10^4$ $TCID_{50}$ and a Ct ~23 (E-gene PCR).
- The sensitivity was not influenced by GLY transport medium and minimally by VTM medium.
- The test did not detect other common respiratory viruses.
- The COVID-19 Ag respi-strip detected clinical samples with a Ct value below 20 (E-gene PCR).
- The COVID-19 Ag respi-strip failed to consistently detect samples at a Ct value of 20 or higher (E-gene PCR).
- Applying this test during routine testing in the SARS-CoV-2 outbreak would result in false negative incidence rate of 57-75%.
- A limitation of this study is that we did not have access to enough fresh clinical specimens and that we therefore had to perform the validation on frozen clinical material. The impact of freezing on the integrity of the antigen and thus on the sensitivity of the Coris Ag test is unknown to us.

Introduction

Rapid SARS-CoV-2 antigen test may be helpful in the diagnosis of infection with SARS-CoV-2. They can be used as a Point of Care test with a fast turn around time (~15 minutes) and are easy to use. A prerequisite is that these test need to be sensitive enough. The immunochromatographic assays are based on the detection of a specific SARS-CoV-2 antigen by a monoclonal antibody conjugated to colloidal gold nanoparticles. This complex diffuses across a nitrocellulose membrane and is captured by another SARS-CoV-2 monoclonal antibody fixed to the membrane resulting in a red line. We here evaluated the Coris COVID-19 antigen respi-strip base on a monoclonal antibody directed against the nucleoprotein of SARS-CoV and SARS-CoV-2.

Materials and Methods

The virus culture dilution range was prepared from SARS-CoV-2 virus stock COR20200415-F with titer of $3.2*10^7$/ml as determined by $TCID_{50}$ on Vero-E6 cells (ATCC CRL-1586) in virus culture medium (DMEM, 1% FCS and Pen/Strep) or in GLY or VTM when indicated. This stock is a 3rd passage from isolate hCoV-19/Netherlands/Zuid_Holland_0133R/2020. GLY and VTM transport medium were obtained from Mediaproducts B.V., Groningen, Netherlands and HiMedia laboratories Pvt Lfd, Mumbai, India. ,, respectively. The RT-qPCR was run according to the in-house PCR procedure based on the Corman protocol (Corman et al., 2020). Ct values for the 2-fold dilution were calculated by linear regression analysis of the 10 fold dilution range. Combined nasopharyngeal and oropharyngeal swab samples (Copan in GLY medium) obtained from the different public health services throughout the Netherlands stored in the repository of the Centre for Infectious Disease Research, Diagnostics and Laboratory Surveillance (IDS) were used for evaluation. The Coris COVID-19 antigen respi-strip test was performed in strict adherence to the prescription provided by the manufacturer. When the lower band, indicating a positive, sample was clearly visible this was scored by a '+' and when this was difficult to see this was indicated by a '+/-'. Both indications are considered positive, also according to the manufacturers prescription.

Results

Sensitivity of the COVID-19 Ag test based on cultured virus

To test the sensitivity of the COVID-19 Ag respi-strip, a 10-fold dilution range was prepared from cultured virus MEM medium . Viral content was determined by $TCID_{50}$ on Vero E6 cells and by the in-house 'Corman' RT-qPCR. From the dilution at which virus was clearly detected ($3.2*10^5$, $TCID_{50}$) a 2-fold serial dilution in GLY was prepared to finetune the limit of detection.

Table 1: Sensitivity test on a 10-fold dilution series of SARS-CoV-2 cultured virus.

Dilution (10log)	-1	-2	-3	-4	-5	-6	-7	-8
Virus titer ($TCID_{50}$)	$3.2*10^6$	$3.2*10^5$	$3.2*10^4$	$3.2*10^3$	$3.2*10^2$	$3.2*10^1$	$3.2*10^0$	$3.2*10^{-1}$
Ct (E-Gene PCR)	15.6	19.1	22.5	25.8	29.3	31.3	33.7	Inconcl.
Coris Ag test	+	+	+/-	-	-	-	-	-

Inconcl. = inconclusive because no RNA was detected in one of the duplo's

Table 2: Sensitivity test on a 2-fold dilution series of SARS-CoV-2 cultured virus.

Dilution (2log)	-1	-2	-3	-4	-5
Virus titer ($TCID_{50}$)	$1.6*10^5$	$7.9*10^4$	$4.0*10^4$	$2.0*10^4$	$1.0*10^4$
Coris Ag test 1	+	+	+	+/-	-
Coris Ag test 2	+	+	+	+/-	-

n.d.: not determined

The Limit of detection (LOD) of the COVID-19 Ag respi-strip for cultured virus was determined at approx. $2*10^4$ $TCID_{50}$ and at a Ct value of approx. 23 (E-Gene PCR - calculated).

Effect of transport medium

To test the possible inhibitory effects of transport medium the cultured virus was 2 fold serially diluted in two different frequently used virus transport media. Preparation of the dilution range was started with a 1 in 10 dilution in each of the transport media of cultured virus at $3.2*10^6$ $TCID_{50}$

Table 3: Sensitivity test on a 2 fold dilution series of SARS-CoV-2 cultured virus in different transport media.

Dilution (2log)	0	-1	-2	-3	-4	-5
Virus titer ($TCID_{50}$)	$3.2*10^5$	$1.6*10^5$	$7.9*10^4$	$4.0*10^4$	$2.0*10^4$	$1.0*10^4$
GLY-medium:						
Coris Ag test 1	+	+	+	+	+/-	-
Coris Ag test 2	+	+	+	+	+/-	-
VTM-medium:						
Coris Ag test 1	+	+	+	+	-	-
Coris Ag test 2	+	+	+	+	-	-

There was no negative effect of GLY on the outcome of the test when compared to virus culture medium and a minimal effect of VTM medium compared to GLY and virus culture medium.

Performance on clinical samples

To test the performance of the COVID-19 Ag respi-strip on clinical samples, samples with a range of Ct (E-Gene PCR) values were selected.

Table 4: Evaluation of COVID-19 Ag respi-strip test on SARS-CoV-2 RT-qPCR (E-gene) confirmed positive samples.

Sample	Ct (E-Gene PCR)	Coris Ag test
1	15.5	**+**
2	15.5	**+**
3	17.0	**+**
4	17.3	**+**
5	17.6	**+/-**
6	17.6	**+/-**
7	19.7	**+/-**
8	20.2	**-**
9	20.6	**-**
10	20.6	**-**
11	21.4	**+/-**
12	21.7	**-**
13	23.4	**-**
14	23.6	**-**
15	23.6	**-**
16	24.4	**-**
17	24.6	**-**
18	25.9	**-**
19	26.1	**-**
20	27.4	**-**
21	27.8	**-**
22	28.5	**-**
23	28.9	**-**
24	30.5	**-**
25	31.4	**-**
26	32.5	**-**
27	32.7	**-**
28	33.4	**-**

Figure 1 (Appendix) shows representative Ag strip tests, illustrating the visibility of the indicator lines that appear in a SARS-CoV-2 positive sample and the single control line that appears in a negative sample. All samples with a Ct value below 20 were detected. At Ct 20 and above, results were negative, with just one exception at Ct 21.4. In total 8 out of 28 clinical PCR-confirmed positive samples tested positive in the COVID-19 Ag respi strip test.

Specificity

To test the specificity of the COVID-19 Ag respi-strip, clinical samples containing other PCR-confirmed common respiratory viruses were used.

Table 5: clinical samples containing RT-qPCR-confirmed other respiratory viruses

Virus	Sample	Ct	Coris Ag test
RSV-A	1	24.3	-
RSV-B	2	20	-
Influenza A (H1)	3	22.5	-
Influenza A (H3)	4	21.1	-
Influenza A (H3)	5	18.9	-
Influenza B (Victoria)	6	23	-
Rhinovirus	7	15	-
Enterovirus (CV-A6)	8	24.6	-
Enterovirus (CV-A4)	9	16.6	-
Negative	10	n.a.	-

n.a.: not applicable

The COVID-19 Ag respi-strip was specific as no other respiratory viruses were detected. However, viral loads for some might have been to low to indicate cross-reactivity of the monoclonal antibodies.

Prediction range of false negative outcome

In this study we performed an evaluation with a limited number of clinical samples and cultured virus dilution repeats. Therefore we investigated the false negative incidence of a wide range of Ct values in which the limit of detection of the COVID-19 Ag respi-strip would fall based on the current evaluation. This allows for a best and worst case scenario prediction of false negative outcome. We therefore used the database containing the outcome of the 'in-house' SARS-CoV-2 PCR assay on clinical samples obtained from the different public health services during the COVID-19 outbreak in the Netherlands.

Table 6: Determination of the range of the false negative incidence during routine screening

E-gene Ct value	# below indicated Ct	% of total SARS-CoV-2 POS	False negative incidence (%)
<20	160	24.6	75.4
<21	207	31.8	68.2
<22	248	38.2	61.8
<23	281	43.2	56.8
total # clinical samples	2388		
# SARS-CoV-2 Pos samples	650		

Clinical samples were obtained from different public health services during the COVID-19 outbreak in the Netherlands. For each indicated Ct value (E-gene), the number of SARS-CoV-2 positive samples is displayed and the percentage of the total amount of positive samples is calculated. Percentage false negative was calculated as the percentage of PCR-positive specimens not detected by the COVID-19 Ag respi-strip taking the indicated Ct value as cutoff.

Based on the calculations in Table 6, the false negative incidence for the Coris COVID-19 Ag respi-strips would fall within in the range of 57-75% during routine screening in the Netherlands considering LOD of Ct 23 (Table 2) and clinical cutoff at Ct 20 (Table 4).

Conclusion

The sensitivity of the COVID-19 Ag respi-strip for cultured virus was determined at a LOD of approx. $2*10^4$ $TCID_{50}$ and a calculated Ct value of approx. 23 (E-Gene PCR). The sensitivity was not influenced by the widely used GLY transport medium and only minimally by the VMT buffer as compared to virus culture medium. The test was specific for SARS-CoV-2 and did not detect other common respiratory viruses, although some of the Ct values were above 20, at which SARS-CoV-2 is also not consistently detected in clinical samples. The sensitivity to clinical samples appeared to be less than for cultured virus as the COVID-19 Ag respi-strip failed to consistently detect samples at a Ct value of 20 or higher. In this evaluation, a limited number of clinical samples and test repeats on the dilution range of cultured virus were performed. Therefore only a wide range of Ct values could be provided in which the limit of detection of the Ag test would fall. From this range it was calculated that 57-75% of the SARS-CoV-2 PCR-confirmed samples would result in a false negative outcome by the COVID-19 Ag respi-strip test during routine screening in the Netherlands.

Appendix

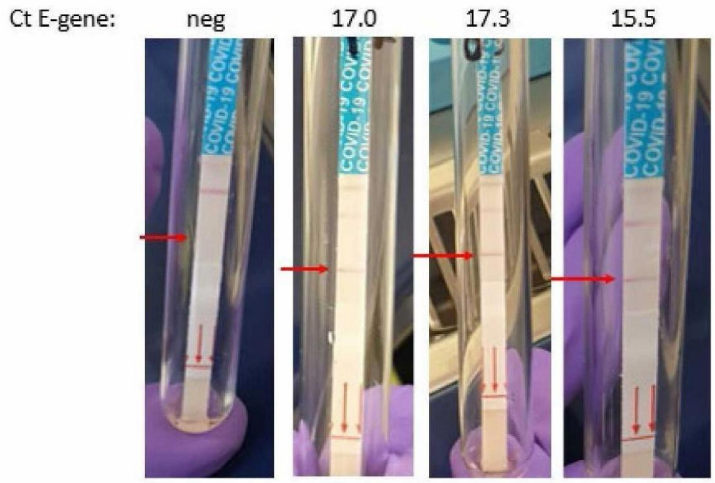

Figure 1. Typical examples of indicator lines appearing on the Coris Ag strips after incubation with clinical specimens for 15 minutes.
In the SARS-CoV-2 negative specimen only the control line appears and in positive samples a second line appears as indicated at the height of the red arrow. Values above each strip represent Ct values measured in the clinical specimens by the 'in house' SARS-CoV-2 RT-qPCR