Rapid COVID-19 Antigen Tests using Nasal or Nasopharyngeal Swabs

Detection of the Nucleocapsid Protein of SARS-CoV2 using a lateral flow immunoassay device.

This document describes a test of the analytical performance of the Rapid Test Ag 2019-nCov for the detection of the Nucleocapsid protein (NP) in nasal or nasopharyngeal swab specimens.

What is the scientific principle of this test?

In this test, antibodies specific to the NP are coated on the test line region of the nitrocellulose membrane.

During testing, antigens of SARS-CoV-2 in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate one colored line in the test region.

PRODUCT

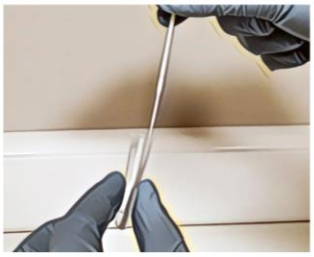
Rapid Test Ag 2019-nCoV (V1310/30)

	Time	Sample	Shelf life	Storage	Format
Rapid Test for the detection of SARS-CoV-2 Nucleocapsid Protein Antigen (NP) in nasal or nasopharyngeal specimens	15 mins	Nasal or nasopharyngeal swab specimens	12 months	4-30 °C	10/30 tests

NASAL PROCEDURE

Add Running Buffer till the second line of the extraction tube

Insert the swab less than one inch (about 2 cm) into the patient's nostril
Rotate the swab five times against the nasal wall & then repeat the collection procedure with the second nostril

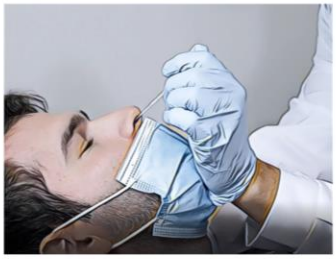


Place the swab into the tube for 60 seconds
Then remove the swab & carefully squeeze the sides of the tube for better extraction

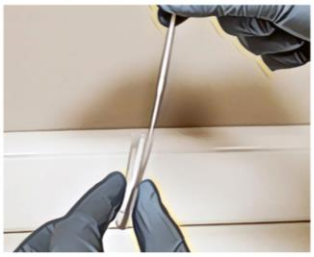
Place the test strip into the tube with the "arrows" pointing down
Wait for 15 mins

NASOLPHARYNGEAL

Add Running Buffer till the second line of the extraction tube



Place the swab into one of nostrils
Rotate 3 to 5 times over the posterior nasopharynx and then remove it slowly while rotating it



Place the swab into the tube for 60 seconds
Then remove the swab and carefully squeeze the sides of the tube for better extraction

Place the test strip into the tube with the "arrows" pointing down
Wait for 15 mins

RESULTS

RESULT INTERPRETATION TABLE

Control line & Test line	Positive result for SARS-CoV-2
Control Line & Weak Test line	Positive result for SARS-CoV-2
Control line only	Negative result for SARS-CoV-2
Test line only	Indicates an invalid result
No lines	Indicates an invalid result

The presence of this colored line indicates a positive result. To serve as a procedural control, a colored line will always appear in the control region if the test has been properly performed.

The procedure for testing and analysis is exemplified on the diagram below.

Performance Results

Cross-Reactivity

In order to determine the cross reactivity of Rapid Test Ag, an evaluation was performed; no cross reactivity against organism, pathogens that could cause infections. Rapid Test Ag 2019-nCov could have some cross reaction with SARS and very low with MERS.

Pathogens	
Adenovirus	Human Transferrin
Astrovirus	Influenza A virus
Alpha coronavirus 229E	Influenza B virus
Alpha coronavirus NL63	Listeria monocytogenes
Beta coronavirus OC43	Salmonella enteritidis
Beta coronavirus HKU1	Streptococcus pneumococcal
Escherichia Coli O157	Streptococcus pyogenes

Table 1. Cross-reactivity evaluation of the Rapid Test Ag 2019-nCov

Determination of the Limit of Detection (LOD)

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, we checked the concentration of heat inactivated SARS-CoV-2 isolate USA-WA1/2020 in Rapid Test Ag 2019-nCov. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates of 6 dilutions with heat inactivated virus are shown at the table below.

LOD: 358.75 $TCID_{50}$/mL

Concentration ($TCID_{50}$/ml)	Positive Replicates	Visual Interpretation of results
1.15×10^7	20 / 20	Strong positive
1.15×10^6	20 / 20	Strong positive
1.15×10^5	20 / 20	Strong positive
1.15×10^4	20 / 20	Strong positive
5.75×10^3	20 / 20	Positive
2.87×10^3	20 / 20	Positive
1.435×10^3	20 / 20	Positive
717.5	20 / 20	Positive
358.75	20 / 20	Positive
179	3 / 20	Negative

Table 2. LOD of the Rapid Test Ag 2019-nCov

High Dose Hook Effect

No high dose hook effect was observed up to 1.15×10^7 $TCID_{50}$/mL of inactivated SARS-CoV-2 or with the Rapid Test Ag 2019-nCoV.

Analytical performance comparisons with RT-PCR

Nasal Specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 386 negative and 142 positive nasal specimens confirmed with RT-PCR assay SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qiagen were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	140	1	141
Negative	2	385	387
Total	142	386	528

Table 3. Summary of performance for the Rapid Test Ag 2019-nCov compared to RT-PCR for nasopharyngeal swabs

	Mean Value	95% Confidence Interval
Sensitivity	98.59%	95.00% to 99.83%
Specificity	99.74%	98.57% to 99.99%
PPV	99.29%	95.18% to 99.90%
NPV	98.86%	97.54% to 99.58%

Table 4. Hypothetical Positive and Negative Predictive Values for the Rapid Test Ag 2019-nCov compared to RT-PCR

- PPV: Positive Predictive Value = True Positives / True Positive + False Positive.
- NPV: Negative Predictive Value = True Negatives / True Negative + False Negative

CT cycles	RT-PCR positives	Rapid Test Ag 2019-nCov positives	Positive Agreement (95% CI)
15-20	53	53	100% (92.28% to 100%)
21-25	44	44	100% (91.96% to 99.99%)
26-30	27	27	100% (87.23% to 100%)
31-35	18	16	88.89% (65.29% to 98.62%)

Table 5. Positive samples comparison results between Rapid Test Ag 2019-nCov and RT-PCR

Nasopharyngeal Specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 478 negative and 135 positive nasopharyngeal specimens confirmed with RT-PCR assay SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qiagen) were tested. The results are presented at the table below.

Rapid Test Ag 2019-nCoV	Real-time RT PCR		
	Positive	*Negative*	*Total*
Positive	129	2	131
Negative	6	476	482
Total	135	478	613

	Mean Value	95% confidence interval
Sensitivity	95.56%	90.58% to 98.35%
Specificity	99.58%	98.50% to 99.95%
PPV	98.47%	94.18% to 99.61%
NPV	98.76%	97.32% to 99.43%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	48	48	100% (92.60% to 100.00%)
21-25	43	43	100% (91.78% to 100.00%)
26-30	23	23	100% (85.18% to 100.00%)
31-35	21	15	71.43% (47.82% to 88.72%)

No	Interfering Substances	Final Test Concentration
1	Azithromycin	84 mg/ml
2	Amoxicillin	54 mg/L
3	Albuterol	0.05 mg/L
4	Acarbose	0.3 mg/L
5	Chlorpheniramine	0.8 mg/L
6	Chlorothiazide	27 mg/L
7	Rheumatoid factor	200 IU/ml
8	Triglycerides	1.5 mg/L
9	Hemoglobin	100 mg/L
10	Human Chorionic Gonadotropin Hormone (pregnancy)	10-fold dilution
11	Ibuprofen	219 mg/L
12	Xylometazoline (Otriven)	10%
13	Acetylsalicylic Acid	3 mg/ml
14	Mucin	0.5%

Table 6. Interference Data

References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
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4. Wu F, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-269.
5. https://www.who.int/health-topics/coronavirus#tab=tab_1
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For In Vitro Diagnostic Use. Product(s) not available in all countries.

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