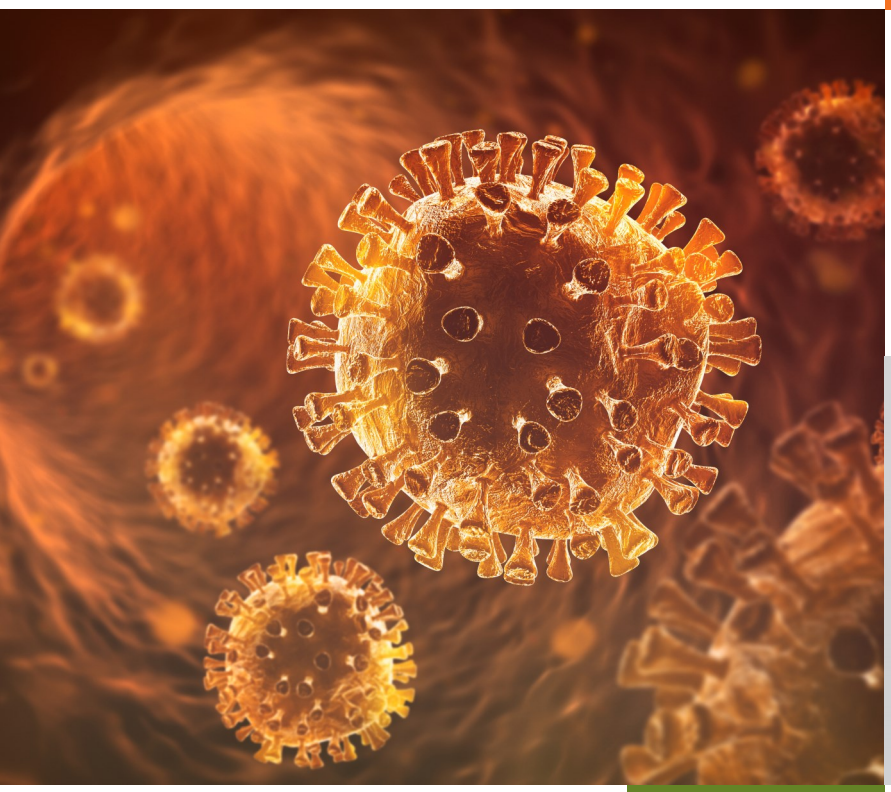
VALIDATION REPORT
Rapid Test Ag 2019-nCoV



PROGNOSIS
BIOTECH

Rapid Test Ag 2019-nCoV Test kit

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1. Introduction

A novel coronavirus (identified as 2019-nCoV) emerged in the Chinese province of Hubei (Wuhan) in December 2019, which has resulted in hundreds of thousands of confirmed human infections worldwide. Cases of severe illness and deaths have been reported. On February 11, 2020 the International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2. The median incubation time is estimated to be approximately 5 days with symptoms estimated to be present within 12 days of infection. The most common symptoms of COVID-19 (according to WHO), are similar to other viral respiratory diseases and include fever, dry cough and tiredness. The virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes. Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses with a nucleocapsid of helical symmetry and are composed of several proteins including the Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) proteins (Figure 1). Molecular and antigen testing are the only techniques capable of detecting the SARS-CoV-2 virus. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

1.1 Principle of the method

The Rapid Test Ag 2019-nCov is a qualitative, lateral flow immunoassay for the detection of Nucleocapsid protein (NP) in nasal or nasopharyngeal specimens. In this test, antibodies specific to the NP are coated on the test line region of the test card. During testing, the specimen reacts with the antibodies to NP that are coated onto particles. The mixture migrates up the membrane to react with the antibodies to NP on the membrane and generate one colored line in the test region. The presence of this colored line in the test region 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 performed properly.

1.2 Kit Characteristics

Refer to the instruction manual V13XX VERSION 2021-04-12/rev.12.
Kit V1301/1310/1320/1330/1340.

1.3 Specimen collection

1.3.1 Nasal specimen collection

- Add Running Buffer till the line to the extraction tube from the dropper bottle (300ul).
- Tilt the patient's head back 70 degrees.
- Remove a sterile swab from the pouch and place it into one of the patient's nostrils while rotating (insert the swab less than one inch-2cm). Rotate the swab five times against the nasal wall then slowly remove from the nostril. (Figure 2)
- Using the same swab repeat the collection procedure with the second nostril.

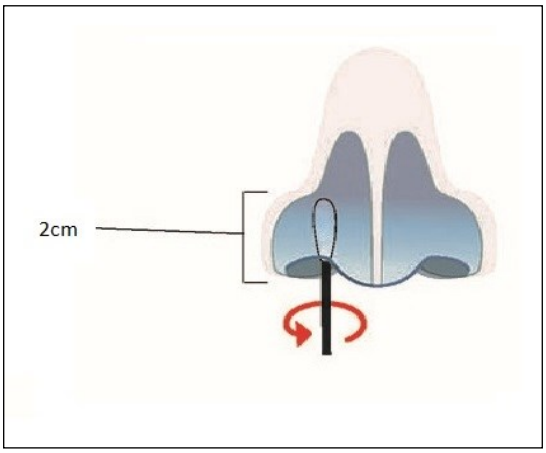


Figure 2. Nasal swab procedure

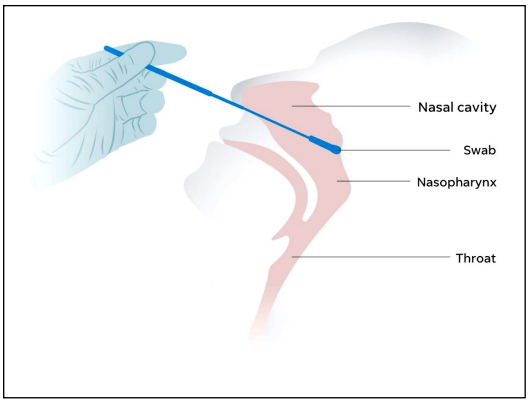


Figure 3. Nasopharyngeal swab procedure

1.3.2 Nasopharyngeal specimen collection

- Add Running Buffer till the line to the extraction tube from the dropper bottle (300ul).
- Tilt the patient's head back 70 degrees.
- Remove a sterile swab from the pouch and place it into one of the patient's nostrils. When it reaches the posterior nasopharynx rotate three to five times and then remove it slowly. (Figure 3)

1.4 Method procedure

1. Calculate the number of swabbing sticks and tubes needed, according to the number of samples to collect.
2. Mark the extraction tubes according to the specimens you intend to collect and add Running Buffer till the line to each one of them from the dropper bottle (300µl).

ATTENTION: In V1301 the extraction tubes are pre-filled.

3. After the specimen collection (see Chapter 1.3), place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the specimen is vigorously extracted in the solution.
4. Remove the swab, squeezing the sides of the tube to extract as much liquid as possible.
5. Discard the swab.
6. The stick format: Immerse the test stick following the direction shown by the arrows, so the uncovered area of the sticks gets soaked.

Note: In case the test stick gets inserted in the wrong direction (arrows pointing up) and gets wet at the top label area, it becomes useless and has to be replaced with a new test stick.

7. The cassette format: close the extraction tube with the dropper cup. Add 3 drops in the circular window of the cassette.
8. After 15 minutes, the test stick can be visually read and interpreted according to the corresponding figure.

POSITIVE CONTROL:

The stick format: Immerse the stick directly into the positive control tube.

The cassette format: Insert the swab into the positive control tube. Afterwards place it in the extraction tube and follow the described procedure.

NEGATIVE CONTROL:

The stick format: Immerse the stick directly into the negative control tube.

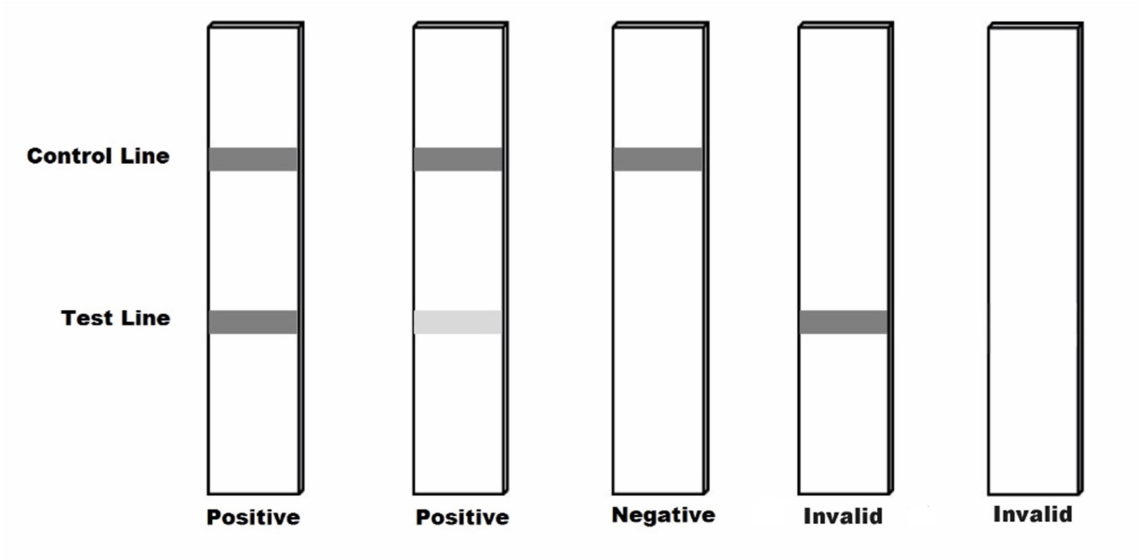
The cassette format: Insert the swab into the negative control tube. Afterwards place it in the extraction tube and follow the described procedure.

1.5 Interpretation of results

Positive: Two visible colored bands appear at both Test (T) and Control (C) line. It indicates a positive result for the SARS-CoV-2 Nucleocapsid Protein in the specimen.

Negative: One visible colored band appears at Control line. It indicates that the concentration of the SARS-CoV-2 NP is zero or below the detection limit of the test.

Invalid: No colored band appears at Control line no matter whether it appears at Test line or not.



2. Immunoassay Specifications

2.1 General Specifications

The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) for two hours. In this case use the cap to close the extraction tube without discarding the droplet.
The test should be used for the detection of SARS-CoV-2 antigen ONLY in nasal or nasopharyngeal swab specimens.
Failure to follow the guidelines for proper specimen collection, test procedure and interpretation of test results may adversely affect test performance and/or produce invalid result.
USE ONLY the sterile swabs that are provided in the kit for the specimen collection.
During specimen collection avoid contact with bleeding areas and excess of mucus of the nasopharynx as both of them may give a false positive result due to interference with the test performance.
Positive results indicate the presence of SARS-CoV-2 antigens but a diagnosis of an infection should only be made by a physician evaluating all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.
Positive test results do not rule out co-infection with other pathogens.
The Rapid Test Ag will indicate the presence of SARS-CoV-2 NP in the specimen from both viable and non-viable virus.

2.2 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.

Microorganism	Concentration	Result
Adenovirus	$1x10^6$ PFU/ml	negative
Astrovirus	$1x10^5$ PFU/ml	negative
Alpha coronavirus 229E	$1x10^5$ PFU/ml	negative
Alpha coronavirus NL63	$1x10^6$ PFU/ml	negative
Beta coronavirus OC43	$1x10^5$ PFU/ml	negative
Beta coronavirus HKU1	$1x10^6$ PFU/ml	negative
Escherichia Coli O157	$6.4x10^6$ CFU/ml	negative
Influenza A virus	$1x10^6$ PFU/ml	negative
Influenza B virus	$1x10^5$ PFU/ml	negative
Listeria monocytogenes	$2.5x10^6$ CFU/ml	negative
Salmonella enteritidis	$3.6x10^6$ CFU/ml	negative
Streptococcus pneumococcal	$4.2x10^6$ CFU/ml	negative
Streptococcus pyogenes	$3.6x10^6$ CFU/ml	negative

2.3 Interference Data

The following substances showed no significant interference on the test results of Rapid Test Ag 2019n-CoV.

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%

3. Validation

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 check 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 inactivate virus are shown at the table below.

LOD : 358.75 $TCID_{50}$/mL

3.1 Determination of the Limit of Detection LOD in the methodology

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 1 . LOD of Heat Inactivated Virus in liquid

3.2 High Dose Effect

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

3.3 Clinical performance characteristics

3.3.1 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

	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%

CT cycles	RT-PCR positive	Rapid Test Ag positive	Positive Agreement (95% CI)
15-20	53	53	100% (92.28% to 100.00%)
21-25	44	44	100% (91.96% to 99.99%)
26-30	27	27	100% (87.23% to 100.00%)
31-35	18	16	88.89% (65.29% to 98.62%)

Clinical Diagnostic Specificity: 99.74%

Clinical Diagnostic Sensitivity: 98.59%

3.3.2 Nasopharyngeal specimens

In order to determine the clinical performance of the Rapid Test Ag 2019-nCoV, 478 negative and 135 positive NASO-PHARYNGEAL specimens confirmed with RT-PCR assay SARS-COV-2 R-GENE® Biomerieux, RNeasy Mini Kit Qi-agen) 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%)

Clinical Diagnostic Specificity: 99.58%

Clinical Diagnostic Sensitivity: 95.56%

4. Results and discussion

During the COVID-19 pandemic, the development of highly sensitive and rapid diagnostic devices has become increasingly important. Currently there are two types of diagnostic tests– molecular tests, such as RT-PCR tests, that detect the virus's genetic material, and antigen tests that detect specific proteins from the virus.

In contrast with the gold standard for COVID-19 diagnosis (RT-PCR), antigen tests can be used immediately, thus enabling the rapid detection of new infected individuals, their isolation and the implementation of confinement measures.

We developed a Rapid Test for the detection of the nucleocapsid protein of SARS-CoV-2 virus from nasal or nasopharyngeal swabs that provides simple, rapid (15 minutes) and highly responsive detection of SARS-CoV-2 virus with excellent sensitivity and specificity.

The diagnostic value of the Rapid Test Ag-2019-nCov was determined in comparison to RT-PCR in 528 nasal swabs and 548 nasopharyngeal swabs collected from individual patients who were suspected of COVID-19. The Rapid Test Ag –2019-nCov showed 98.59% sensitivity, 99.74% specificity in nasal swabs and 95.56% of sensitivity, 99.56% of specificity in nasopharyngeal swabs.

Considering short turnaround times, user friendliness, low costs and opportunities for decentralized testing, this test can improve our efforts to control transmission of SARS-CoV-2.

- https://www.fda.gov/consumers/consumer-updates/coronavirus-disease-2019-testing-basics
- https://www.nature.com/articles/s41579-020-00461-z
- https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm
- Wu F, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265-269

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