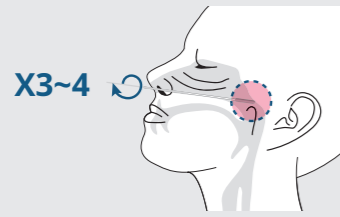


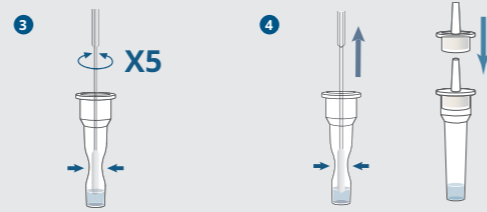
SPECIMEN PREPARATION

[Nasopharyngeal swab specimen] specimen preparation for immediate testing

- 1 Insert a sterile nasopharyngeal swab into the nostril of the patient.
- 2 Rotate the swab over the posterior nasopharynx surface 3-4 times.



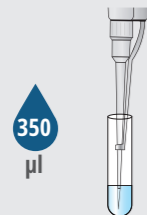
- 3 Insert the swab into an extraction buffer tube. While squeezing the tube, stir the swab more than 5 times.
- 4 Remove the swab while squeezing the sides of the tube. Press the nozzle cap tightly onto the tube.



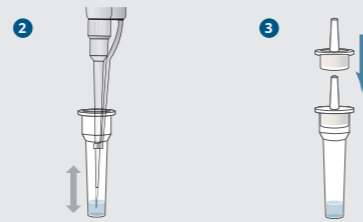
[Specimen in VTM] alternative procedure for the specimen without immediate testing

* If the specimen cannot be tested immediately, 1 ml VTM could be used instead of the extraction buffer for storage (~8 hr at 20 °C/~12 hr at 5 °C).

- 1 Using a micropipette, collect the 350 µl of specimen from the VTM.

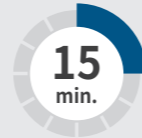
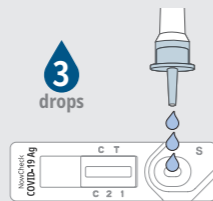
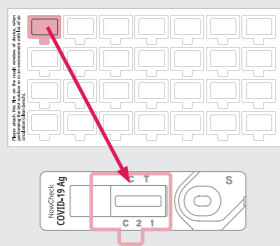


- 2 Add the specimen into an extraction buffer tube (VTM specimen solution : extraction buffer = 1:1) and mix well.
- 3 Press the nozzle cap tightly onto the tube.



TEST PROCEDURE

- 1 Attach the provided film on to the test device.
- 2 Apply 3 drops of the extracted specimen to the specimen hole of the test device.
- 3 Read the test result after 15~30 minutes. The test can be read up to 30 minutes.



**** Do not read test results after 30 min. It may give false results.**

TEST RESULT

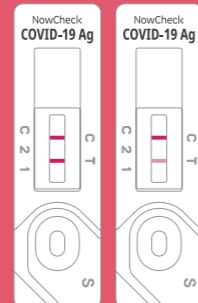
- Negative

One colored band ("C" Control line) within the result window indicates a negative result.



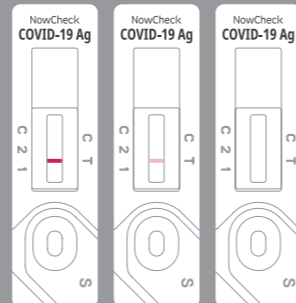
+ Positive

Two colored bands ("C" Control line and "T" Test line) within the result window indicate SARS-CoV-2 positive.



✗ Invalid

If the control band ("C" Control line) is not visible within the result window, the result is considered invalid.



Doc. No.: I1901-10E

NowCheck COVID-19 Ag Test

For *in vitro* diagnostics use only

PRINCIPLE

NowCheck COVID-19 Ag Test is a rapid chromatographic immunoassay for the qualitative detection of specific SARS-CoV-2 antigens present in human nasopharynx. This test is for administration by healthcare workers and labs only, as an aid to early diagnosis of COVID-19 in patients that are suspected to have a SARS-CoV-2 infection. It provides only an initial screening test result. More specific alternative diagnosis methods should be performed in order to obtain the confirmation of SARS-CoV-2 infection.

NowCheck COVID-19 Ag Test has two pre-coated lines, "C" Control line, "T" Test line on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Mouse monoclonal anti-SARS-CoV-2 antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. Mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles is used as detectors for SARS-CoV-2 antigen. During the test, SARS-CoV-2 antigen in the specimen interacts with mouse monoclonal anti-SARS-CoV-2 antibody conjugated with color particles, making antigen-antibody color particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen. The intensity of colored test line will vary depending upon the amount of SARS-CoV-2 antigen present in the specimen. If SARS-CoV-2 antigens are not present in the specimen, then no color appears in the test line. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working.

MATERIALS PROVIDED

Reagent	25 Tests/Kit
① Test device	25
② Extraction buffer tube	25
③ Nozzle cap	25
④ * Nasopharyngeal swab (Cat. No.: RG1901DG)	25
⑤ Paper stand	1
⑥ Film	1
⑦ Instructions for use	1

* Manufacturer and Authorized representative information for sterile swab is as below.

Noble Biosciences, Inc.
13-50, Sinbaek-gil, Jeongnam-myeon, Hwaseong-si, Gyeonggi-do, Korea

Authorized Representative
S.B. PHARMA GMBH
Bunsenstr, 14 53121 Bonn, Germany

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Timer
2. VTM
3. Micropipette

STORAGE AND STABILITY

1. Store the kit at room temperature (2~30°C / 36~86°F).
2. Store the kit out of direct sunlight.
3. Do not freeze the kit.
4. Shelf life is 24 months. Kit materials are stable until the expiration date printed on the outer box.

PRECAUTIONS

1. Do not reuse the test kit.
2. Do not use the test kit if the pouch is damaged or the seal is broken.
3. Do not use the buffer of another lot.
4. Use the test device immediately once taken out of the foil pouch.
5. Do not smoke, drink, or eat while handling the specimen or kit reagents.
6. Handle all specimens with caution as if they contain infectious agents.
7. Wear personal protective equipment, such as gloves and lab coats, when handling the specimen and kit reagents. Wash hands thoroughly after the tests are done.
8. Clean up spills thoroughly using an appropriate disinfectant.
9. The used test and all specimens should be discarded as biohazard waste and must be handled according to local regulations.

10. Observe established precautions against microbiological hazards throughout testing procedures.
11. Do not use the kit if the test result with positive/negative control is abnormal.

COLLECTION AND PREPARATION OF SPECIMEN

1. Specimen collection using a nasopharyngeal swab
 - ① Insert a nasopharyngeal swab into the nostril of the patient, reaching the surface of the posterior nasopharynx.
 - ② Using gentle rotation, push the swab until resistance is met at the level of the turinate.
 - ③ Rotate the swab 3-4 times against the nasopharyngeal wall.
 - ④ Remove the swab from the nostril carefully.
 2. Specimen should be tested as soon as possible after collection.
 3. Use the collected specimen and extraction buffer immediately. Be careful of contamination.
 4. If the specimen cannot be tested immediately after collection, viral transport medium (VTM)* could be used instead of extraction buffer.
- * As the sensitivity of this test can be affected by excessive dilution, it is recommended to use 1 ml VTM.

Specimen Storage Condition	5±3°C	20±5°C
Extraction buffer	4 hours	1 hour
Nasopharyngeal swab inoculated in VTM	12 hours	8 hours

INTERPRETATION OF THE RESULT

1. A colored band will appear in the top section of the result window to show that the test is working properly. This band is a control line (C).
 2. A colored band will appear in the lower section of the result window. This band is a test line of SARS-CoV-2 antigen (T).
 3. Even if the control line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as positive.
- * The presence of any line no matter how faint the result is considered positive.
- * Positive results should be considered in conjunction with the clinical history and other data available.

SPECIMEN COLLECTION AND TRANSPORT

Commercially available transport medium

Virus Transport Medium (VTM)	Recommended Storage Condition	
	5±3°C	20±5°C
UTM™ (COPAN Diagnostics Inc.)	12 hours	8 hours
Universal Viral Transport (BD™)	12 hours	8 hours
FA TRANSPORT MEDIUM (FA Inc.)	12 hours	8 hours



Allow the VTM containing the specimen to reach room temperature (15-30°C) prior to testing. Refrigerated specimen may fail to move through the device, causing erroneous or invalid results.

PERFORMANCE CHARACTERISTIC

1. Clinical evaluation
Performance characteristics for the NowCheck COVID-19 Ag Test for rapid detection of SARS-CoV-2 antigen was established in a prospective study at a Community Testing Clinic in Brazil during the 2020 SARS-CoV-2 pandemic. A total of 400 prospective specimens were tested using the NowCheck COVID-19 Ag test. These specimens consisted of nasopharyngeal swabs from symptomatic patients. The performance of the NowCheck COVID-19 Ag Test was compared to a Reference RT-PCR method (Lab-developed assay based on the US CDC protocol, which targets two regions (N1 and N2) of the nucleocapsid (N) gene of SARS-CoV-2, using nasopharyngeal swabs). This clinical evaluation was conducted by FIND (Geneva, Switzerland) and its partners, over the period of July - August 2020. The NowCheck COVID-19 Ag Test showed 89.2% of sensitivity (92.2% sensitivity when the days from symptom onset were ≤ 7 days) and 97.3% of specificity, compared to diagnostic RT-PCR assay.

Specimens from symptomatic patients (N=400)	Real-time PCR			
	Positive	Negative	Total	
NowCheck COVID-19 Ag Test	Positive	91	8	99
	Negative	11	290	301
	Total	102	298	400
Clinical Sensitivity = 89.2% (95%CI, 81.7% - 93.9%) 92.2% (95%CI, 84.8% - 96.2%) (Days ≤ 7) 91.4% (95%CI, 83.9% - 95.6%) (Ct ≤ 33)				
Clinical Specificity = 97.3% (95%CI, 94.8% - 98.6%)				

ANALYTICAL PERFORMANCE

- Limit of Detection (LoD): The study used "SARS-CoV-2 (2019-nCoV) NCCP 43326/2020 /Korea" strain. The titer of the cultured virus was confirmed by PCR. The inactivated virus was spiked into the negative nasopharyngeal swab. The LoD is $3.12 \times 10^{2.2}$ TCID₅₀/ml.

SARS-CoV-2 strain tested	Virus stock titer	Specimen type	LoD (Spiking titer)	Final working titer	Call rates of 20 replicates near cut-off
NCCP 43326/2020 /Korea	$1 \times 10^{6.2}$ TCID ₅₀ /ml	Direct nasopharyngeal swab	$3.12 \times 10^{2.2}$ TCID ₅₀ /ml	$6.24 \times 10^{1.2}$ TCID ₅₀ /ml	100% (20/20)

- Cross-Reactivity: SARS-CoV showed cross-reactivity, while the others did not show any cross-reactivity at high concentration.

Microbial Organisms	Test titer/value	Result
MERS-Coronavirus	4×10^4 TCID ₅₀ /ml	No cross-reactivity
Human Coronavirus 229E	$1 \times 10^{6.5}$ TCID ₅₀ /ml	No cross-reactivity
Human Coronavirus OC43	1×10^5 TCID ₅₀ /ml	No cross-reactivity
Human Coronavirus NL63	1×10^4 TCID ₅₀ /ml	No cross-reactivity
Influenza A	3×10^5 TCID ₅₀ /ml	No cross-reactivity
Influenza B	3×10^5 TCID ₅₀ /ml	No cross-reactivity
RSV A	3×10^5 TCID ₅₀ /ml	No cross-reactivity
RSV B	3×10^5 TCID ₅₀ /ml	No cross-reactivity
Human Metapneumovirus (hMPV)	1×10^5 TCID ₅₀ /ml	No cross-reactivity
Parainfluenza virus	1×10^5 TCID ₅₀ /ml	No cross-reactivity
Rhinovirus	1×10^5 TCID ₅₀ /ml	No cross-reactivity
Enterovirus	1×10^4 TCID ₅₀ /ml	No cross-reactivity
Adenovirus	3×10^5 TCID ₅₀ /ml	No cross-reactivity
Human immunodeficiency virus lysate	10 µg/ml	No cross-reactivity
Mycobacterium tuberculosis	5×10^4 cells/ml	No cross-reactivity
Haemophilus influenzae	5×10^4 cells/ml	No cross-reactivity
Mycoplasma pneumoniae	5×10^4 cells/ml	No cross-reactivity
Streptococcus pneumoniae	5×10^4 cells/ml	No cross-reactivity
Streptococcus pyogenes	5×10^4 cells/ml	No cross-reactivity
Legionella pneumophila	5×10^4 cells/ml	No cross-reactivity
Candida albicans	5×10^4 cells/ml	No cross-reactivity
Bordetella pertussis	5×10^4 cells/ml	No cross-reactivity
Moraxella catarrhalis	5×10^4 cells/ml	No cross-reactivity
Pseudomonas aeruginosa	5×10^4 cells/ml	No cross-reactivity
Staphylococcus epidermidis	5×10^4 cells/ml	No cross-reactivity
Streptococcus salivarius	5×10^4 cells/ml	No cross-reactivity
Chlamydia pneumoniae	1×10^5 cells/ml	No cross-reactivity
Pooled human nasal wash	N/A	No cross-reactivity

*Human coronavirus HKU1 has not been tested. The % identity of the nucleocapsid protein sequence between HKU1 and SARS-CoV-2 is below 35%.

- Endogenous/Exogenous Interference Substances Studies: The substances having potential interference are listed in the table below. There was no any interfering activity at high concentration.

Category	Interfering Substances	Test Concentration
Relevant medicines	Zanamivir (Influenza)	5 mg/ml
	Oseltamivir (Influenza)	0.039 mg/dL
	Artemether-lumefantrine (Malaria)	50 µM
	Doxycycline hyclate (Malaria)	70 µM
	Quinine (Malaria)	150 µM
	Lamivudine (Retroviral medication)	1.05 mg/dL
	Ribavirin (HCV)	1 mg/ml
Anti-inflammatory medication	Daclatasvir (HCV)	1 mg/ml
	Acetaminophen	1030 µM
	Acetylsalicylic acid	167 µM
Antibiotics	Ibuprofen	1060 µM
	Mupirocin	3 µM
	Tobramycin	70.6 µM
	Erythromycin (antibiotic)	188 µM
	Ciprofloxacin (antibiotic)	36.2 µM
Nasal sprays or drops	Neo-Synephrine (Phenylephrine)	10% (v/v)
	Afrin Nasal Spray (Oxymetazoline)	10% (v/v)
	Saline Nasal Spray	10% (v/v)
	Rhinocort (Nasal corticosteroids - Budesonide)	10% (v/v)

Homeopathic allergy relief medicine	Homeopathic Zicam Allergy Relief Nasal Gel	5% (v/v)
	Sodium Cromoglycate	20 mg/ml
	Olopatadine Hydrochloride	10 mg/ml
Oral anaesthetic	Anbesol (Benzocaine 20%)	5% (v/v)
	Strepsils (flurbiprofen 8.75 mg)	5% (w/v, 50 mg/ml)
Throat lozenges	Throat candy (mint)	5% (w/v, 50 mg/ml)
	Mucin: bovine submaxillary gland, type I-S	100 µg/ml
Others	Biotin	14.3 µM
	Human anti-mouse antibody	802 ng/ml
Autoimmune disease	Rheumatoid factor	3,480 IU/mL
	Whole Blood (human), EDTA anticoagulated	10% (w/w)
Serum protein	Human serum albumin	60 mg/ml

- High-dose Hook Effect: The highest concentration of heat and chemical inactivated SARS-CoV-2 stock available (TCID₅₀ of $1 \times 10^{6.2}$ per ml) was tested. There was no hook effect detected.
- SARS-CoV-2 was inactivated (non-CPE) by the extraction buffer of NowCheck COVID-19 Ag Test in 2 minutes.

Type	Virus Spiking	Cytopathic Effect	Interpretation
Extraction buffer	0	No CPE	Virus inactivated
Cell culture media		CPE	Positive control

- Matrix Equivalency: The matrix and VTM does not affect the detection of COVID-19 Ag in contrived specimen between direct nasopharyngeal swab, nasopharyngeal swab in VTM, direct nasal swab, nasal swab in VTM. (comparator : direct nasopharyngeal swab sample)

LIMITATIONS OF THE TEST

- The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
- The test should be used for the detection of SARS-CoV-2 antigen in human nasopharyngeal swab.
- Neither the quantitative value nor the rate of SARS-CoV-2 antigen concentration can be determined by this qualitative test.
- Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
- For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
- The test result must always be evaluated with other data available to the physician.
- A negative result may occur if the concentration of antigen in a specimen is below the detection limit of the test or if the specimen is collected or transported improperly. Therefore, a negative test result does not eliminate the possibility of SARS-CoV-2 infection, and should be confirmed by viral culture, a molecular assay, or ELISA.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results are not intended to rule in other coronavirus infection except the SARS-CoV-1.
- Children tend to shed virus for longer periods than adults, which may result in differences in sensitivity between adults and children.
- When using VTM, sensitivity can be reduced due to excessive dilution.

EXTERNAL QUALITY CONTROL

- Positive and negative controls are optional contents (NowCheck COVID-19 Ag Control(Cat No. **RG1901CD**)) and these controls can be provided as a means on additional quality control to demonstrate a positive or negative reaction.
- Quality controls should be treated and tested the same as patient specimens.
- It is recommended that positive and negative controls be run:
 - once for each new lot.
 - once for each untrained operator.
 - as required by test procedures in this instructions and in accordance with local, state, and federal regulations or accreditation requirements.

BIBLIOGRAPHY OF SUGGESTED READING

- Clinical management of severe acute respiratory infection when novel coronavirus(nCoV) infection is suspected. Interim guidance. WHO.2020
- Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR.2020
- Diagnosis and treatment of pneumonia caused by new coronavirus (trial version 4) National Health Commission. 2020

SYMBOL

Symbol	Description
	Manufacturer
	Consult instructions for use
	Reference number
	Date of manufacture To indicate the date of analyzer manufacture
	Note
	Contains sufficient for <n> tests
	Do not re-use
	Use by
	Caution! Indicates a situation, which if not avoided could result
	Indicates that you should keep the product dry
	Indicates that the product is fragile and to handle it with care
	Batch code To indicate the lot number
	Indicates to discard it separately from other household waste
	Keep away from sunlight
	Do not use if package is damaged
	Fulfill the requirements of Directive 98/79/EC on <i>in vitro</i> diagnostic medical devices
	Indicates the Authorized Representative in the European Community

Doc. No.: I1901-10E
Issued date : Nov. 17, 2020



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