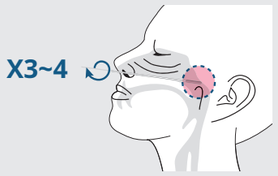
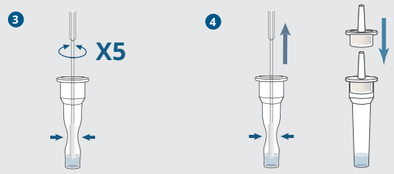
SPECIMEN PREPARATION

[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.



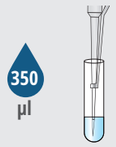
3. Insert the swab into an extraction buffer tube. While squeezing the tube, stir the swab more than 5 times.
4. Remove the swab while squeezing the sides of the tube. Press the nozzle cap tightly onto the tube.



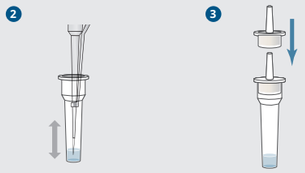
[Specimen in VTM] alternative procedure for the specimen without immediate testing

* 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.

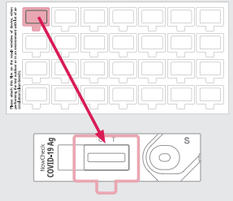


2. Add the specimen into an extraction buffer tube (VTM specimen solution : extraction buffer = 1:1) and mix well.
3. Press the nozzle cap tightly onto the tube.



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.

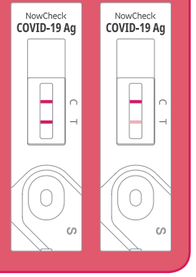
TEST RESULT

Negative

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

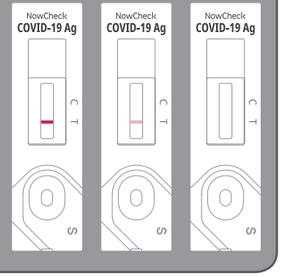
Positive

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.: I1901-13E
Cat. No.: RG1901DG

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	25
⑤	Paper stand	1
⑥	Film	1
⑦	Instructions for use	1

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 turbinate.
   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.**
5. The specimen storage condition is as follows.

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

CAUTION

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 (nasopharyngeal version and nasal version) were established in a prospective study at a community testing clinic in Brazil. This clinical evaluation was conducted by FIND (Geneva, Switzerland) and its partners, over the period of January – February 2021.
   A total of 218 nasopharyngeal swab and nasal swab specimens from symptomatic patients were tested. These specimens were determined to be positive or negative using a reference RT-PCR method (Lab-developed assay based on the US CDC protocol). The NowCheck COVID-19 Ag Test showed a sensitivity of 89.9% (95% CI: 81.3-94.8%) and a specificity of 98.6% (95% CI: 94.9-99.6%).

Specimens from symptomatic patients (N=218)		RT-PCR (Nasopharyngeal)		
		Positive	Negative	Total
NowCheck COVID-19 Ag (Nasopharyngeal)	Positive	71	2	73
	Negative	8	137	145
	Total	79	139	218

	NowCheck COVID-19 Ag (Nasopharyngeal)	NowCheck COVID-19 Ag (Nasal)
Clinical Sensitivity (95% CI)	89.9% (81.3, 94.8)	89.9% (81.3, 94.8)
Sensitivity days ≤ 7	92.5% (83.7, 96.8)	92.5% (83.7, 96.8)
Sensitivity Ct ≤ 33	97.2% (90.4, 99.2)	97.2% (90.4, 99.2)
Sensitivity Ct ≤ 25	100% (92.3, 100)	100% (92.3, 100)
Clinical Specificity (95% CI)	98.6% (94.9, 99.6)	98.6% (94.9, 99.6)
Positive percent agreement – nasal/NP (95% CI)	N/A	100% (95, 100)
Negative percent agreement – nasal/NP (95% CI)	N/A	100% (97.4, 100)

* NowCheck COVID-19 Ag Test can detect both the United Kingdom variant (B.1.1.7), South African variant (B.1.351), and Brazil variant (B.1.1.248). Refer to the No. 7 of the **[ANALYTICAL PERFORMANCE]** for more details.

ANALYTICAL PERFORMANCE

1. 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 X $10^{2.2}$ $TCID_{50}$/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 X $10^{6.2}$ $TCID_{50}$/ml	Direct nasopharyngeal swab	3.12 X $10^{2.2}$ $TCID_{50}$/ml	6.24 X $10^{1.2}$ $TCID_{50}$/ml	100% (20/20)

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

Name	Test Titer/value	Result
Human coronavirus 229E	1X$10^{5.5}$ $TCID_{50}$/mL	No cross-reactivity
Human coronavirus OC43	1X$10^{7.77}$ $TCID_{50}$/mL	
Human coronavirus NL63	1.70X10^{5} $TCID_{50}$/mL	
MERS-coronavirus	4.17X10^{5} $TCID_{50}$/ mL	
SARS-coronavirus	35 µg/ml	
Adenovirus Type1	2.57X10^{8} $TCID_{50}$/mL	
Adenovirus Type2	1.15X10^{7} $TCID_{50}$/mL	
Adenovirus Type5	1X$10^{7.53}$ $TCID_{50}$/mL	
Adenovirus Type6	1X$10^{7.29}$ $TCID_{50}$/mL	
Adenovirus Type7A	1X$10^{5.15}$ $TCID_{50}$/mL	
Adenovirus Type11	1X$10^{7.29}$ $TCID_{50}$/mL	
Adenovirus Type14	1X$10^{5.39}$ $TCID_{50}$/mL	
Adenovirus Type40	1X$10^{6.58}$ $TCID_{50}$/mL	
Human Metapneumovirus3 type B1	1X$10^{6.34}$ $TCID_{50}$/mL	
Human Metapneumovirus16 type A1	1X$10^{6.98}$ $TCID_{50}$/mL	
Parainfluenza virus 1	1X$10^{8.49}$ $TCID_{50}$/mL	
Parainfluenza virus 2	1X$10^{6.10}$ $TCID_{50}$/mL	
Parainfluenza virus 3	1X$10^{6.82}$ $TCID_{50}$/mL	
Parainfluenza virus 4A	1X$10^{6.58}$ $TCID_{50}$/mL	
Influenza A H1N1 pdm/Michigan/45/15	1X$10^{6.10}$ $TCID_{50}$/mL	
Influenza A H1N1 Brisbane/59/07	1X$10^{5.86}$ $TCID_{50}$/mL	
Influenza A H3N2 Singapore/ INFIMH-16-0019/16	4.68X10^{4} $TCID_{50}$/mL	
Influenza A H3N2 South Australia/55/14	1X$10^{5.07}$ $TCID_{50}$/mL	
Influenza A H3N2 Hong Kong/8/68	1X$10^{5.70}$ $TCID_{50}$/mL	
Influenza A H3N2 Victoria/361/11	1X$10^{5.15}$ $TCID_{50}$/mL	
Influenza B Massachusetts/2/12	1X$10^{5.39}$ $TCID_{50}$/mL	
Influenza B Malaysia/2506/04	4.17X10^{5} $TCID_{50}$/mL	
Influenza B Lee/40	1X$10^{5.39}$ $TCID_{50}$/mL	
Influenza B Yamagata/16/88	1X$10^{5.39}$ $TCID_{50}$/mL	
Influenza B Victoria/2/87	1.86X10^{4} $TCID_{50}$/mL	
Influenza B Texas6/11	1X$10^{6.58}$ $TCID_{50}$/mL	
Influenza B Colorado6/17	4.68X10^{4} $TCID_{50}$/mL	
Influenza B Florida/02/06	3.8X10^{6} $TCID_{50}$/mL	
Enterovirus type 68 09/2014 Isolate 4	3.55X10^{5} $TCID_{50}$/mL	
Respiratory syncytial virus A	1X$10^{6.58}$ $TCID_{50}$/mL	
Respiratory syncytial virus B	5.01X10^{5} $TCID_{50}$/mL	
Rhinovirus 1A	1X$10^{5.55}$ $TCID_{50}$/mL	
Rhinovirus A16	1X$10^{6.1}$ $TCID_{50}$/mL	
Rhinovirus B42	1.05X10^{6} $TCID_{50}$/mL	
Haemophilus influenzae (NCCP 13815)	2.54X10^{7} CFU/mL	
Haemophilus influenzae (NCCP 13819)	3.39X10^{7} CFU/mL	
Haemophilus influenzae (NCCP 14581)	4.10X10^{7} CFU/mL	
Haemophilus influenzae (NCCP 14582)	1.06X10^{7} CFU/mL	
Streptococcus pneumoniae type1	1.54X10^{6} CFU/mL	
Streptococcus pneumoniae type2	1.04X10^{7} CFU/mL	
Streptococcus pneumoniae type3	1.34X10^{7} CFU/mL	
Streptococcus pneumoniae type5	1.24X10^{7} CFU/mL	
Streptococcus pyogenes	3.22X10^{7} CFU/mL	
Candida albicans	1.78X10^{6} CFU/mL	
Bordetella pertussis	6.24X10^{7} CFU/mL	
Mycoplasma	2.48X10^{9} CFU/mL	
Chlamydia pneumoniae	9.1X10^{7} IFU/mL	
Legionella pneumophila	1.9X10^{8} CFU/mL	
Staphylococcus aureus	1.00X10^{9} CFU/mL	
Staphylococcus epidermidis	6.22X10^{8} CFU/mL	
Mycobacterium tuberculosis	58.6 µg/mL	
Pooled human nasal wash – representative of normal respiratory microbial flora	N/A	

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

3. 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
	Daclatasvir (HCV)	1 mg/ml
	Tamiflu (Oseltamivir Phosphate)	5 mg/ml
Anti-inflammatory medication	Acetaminophen	1030 µM
	Acetylsalicylic acid	167 µM
	Ibuprofen	1060 µM
Antibiotics	Mupirocin	10 mg/mL
	Tobramycin	4 µg/mL
	Erythromycin (antibiotic)	188 µM
	Ciprofloxacin (antibiotic)	36.2 µM
Nasal sprays or drops	Neo-Synephrine (Phenylephrine) ≒ CVS Nasal Drops	15% (v/v)
	Afrin Nasal Spray (Oxymetazoline)	10% (v/v)
	Afrin(Oxymetazoline)	15% (v/v)
	Saline Nasal Spray	10% (v/v)
	Rhinocort (Nasal corticosteroids - Budesonide)	10% (v/v)
	Naso GEL (NeilMed)	5% (v/v)
	CVS Nasal Spray (Cromolyn)	15% (v/v)
	Sore Throat Phenol Spray	15% (v/v)
	CVS Health Fluticasone Propionate	5% (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
	Zicam	5% (v/v)
	Homeopathic (Alkalol)	1:10 dilution
	Anbesol (Benzocaine 20%)	1.5 mg/ml
Throat lozenges	Strepsils (flurbiprofen 8.75 mg)	5% (w/v, 50 mg/ml)
	Thoat candy (mint)	5% (w/v, 50 mg/ml)
Others	Mucin: bovine submaxillary gland, type I-S	0.5%
	Biotin	14.3 µM
Autoimmune disease	Human anti-mouse antibody	802 ng/ml
	Rheumatoid factor	3,480 IU/mL
Serum protein	Whole Blood (human), EDTA anticoagulated	10% (w/w)
	Human serum albumin	60 mg/ml

4. High-dose Hook Effect: The highest concentration of heat and chemical inactivated SARS-CoV-2 stock available ($TCID_{50}$ of 1 X $10^{6.2}$ per ml) was tested. There was no hook effect detected.
5. 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

6. 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)
7. SARS-CoV-2 Variants Study: The performance of the NowCheck COVID-19 Ag Test is not affected by the United Kingdom variant (B.1.1.7) and South African variant (B.1.351), and Brazil variant (B.1.1.248). In other words, NowCheck COVID-19 Ag Test can detect all these variants. *In-silico* analysis shows that the nucleocapsid (N) proteins of these variants have very high homology comparing with Wuhan-hu-1. And the analytical sensitivity tests were conducted using both the recombinant N protein and cultured virus of these variants.

Variants	Outbreak Country	*In-silico* Analysis	Analytical Sensitivity Test	
			Recombinant Protein	Cultured Virus
Wuhan-Hu-1	China	N/A	0.0156 µg/ml	3.12 X $10^{2.25}$ $TCID_{50}$/ml
B.1.1.7 (VOC-202012/01)	United Kingdom	99.52%	0.0156 µg/ml	3.12 X $10^{1.32}$ $TCID_{50}$/ml
B.1.351 (501.V2)	South Africa	99.76%	0.0156 µg/ml	3.12 X $10^{2.44}$ $TCID_{50}$/ml
B.1.1.248 (P.1)	Brazil	99.28%	0.0156 µg/ml	N/A

LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
2. The test should be used for the detection of SARS-CoV-2 antigen in human nasopharyngeal swab.
3. Neither the quantitative value nor the rate of SARS-CoV-2 antigen concentration can be determined by this qualitative test.
4. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
5. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
6. The test result must always be evaluated with other data available to the physician.
7. 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.
8. Positive test results do not rule out co-infections with other pathogens.
9. Negative test results are not intended to rule in other coronavirus infection except the SARS-CoV-1.
10. Children tend to shed virus for longer periods than adults, which may result in differences in sensitivity between adults and children.
11. When using VTM, sensitivity can be reduced due to excessive dilution.

EXTERNAL QUALITY CONTROL

1. 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.
2. Quality controls should be treated and tested the same as patient specimens.
3. 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

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

SYMBOL

Symbol	Description
	Manufacturer
	Consult instructions for use
REF	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
LOT	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
CE	Fulfill the requirements of Directive 98/79/EC on *in vitro* diagnostic medical devices
EC REP	Indicates the Authorized Representative in the European Community

Doc. No.: I1901-13E
Issued date : Mar. 29, 2021

IVD CE

Authorized Representative
EC REP MT Promedt Consulting GmbH
Altenhofstrasse 80 D-66386 St. Ingbert Germany
Tel: +49-6894-581020 I Fax: +49-6894-581021

BIONOTE
22, Samsung 1-ro 4-gil, Hwaseong-si, Gyeonggi-do 18449, Republic of Korea
Tel: +82-31-211-0516 I Fax: +82-31-8003-0618 www.bionote.co.kr