NowCheck COVID-19 Antigen METHOD AND SAMPLE COLLECTION

1. PURPOSE AND SCOPE

The purpose and scope of this document is to describe in some detail the procedure used by healthcare professionals for identifying COVID-19 antigens in nasal or naso-pharyngeal swabs from symptomatic residents and asymptomatic staff.

2. HAZARDS

Patient Samples
All patient samples should be treated as potentially infectious and handled appropriately. Standard precautions should be employed. Personal Protective Equipment (e.g. gloves and safety glasses) should be worn when processing all samples and quality control testing.

3. CLINICAL SIGNIFICANCE

COVID-19 is a coronavirus, part of a group of viruses that can cause respiratory infections ranging from the common cold to more serious respiratory diseases. Other coronaviruses include Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). COVID-19 is a relatively new form of coronavirus which was first reported in December 2019 in Wuhan City in China.

COVID-19 is highly contagious and infected individuals must isolate themselves in order to prevent transmission of the virus. Thus testing for COVID-19 is essential for controlling outbreaks of the disease.

There are a number of different types of tests for COVID-19. Most testing is by molecular methods using the reverse-transcriptase polymerase chain reaction. This is based on detection of the genetic material in the virus and while it is the most accurate method, the results are only available 24-48 hrs after testing.

The NowCheck COVID-19 Antigen test is a quicker process which detects the antigens on the surface of the virus. While not as accurate as molecular testing ti gives a result in 15 minutes and can be useful for screening for the virus in certain populations.

4. TEST PRINCIPLE

The COVID-19 Antigens are detected using the technique of lateral flow immunoassay. Within the test strip 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 are used as detectors for SARS-CoV-2 antigen device.

During the test, SARS-CoV-2 antigen in the specimen interacts with monoclonal anti-SARS-CoV-2 antibody conjugated with color particles making the 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.

Both the control line and test line in the result window are not visible before applying any specimens. 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 SARSCoV-2 antigens are not present in the specimen, then no color appears in the test line.

The control line is used as a procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working.

5. LIMITATIONS AND KNOWN INTERFERENCE

- 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 specimens.
- 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 or 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 of time than adults, which may result in differences in sensitivity between adults and children.
- When using VTM, sensitivity can be reduced due to excessive dilution.

6. PERFORMANCE CHARACTERISTICS

Clinical Performance

The performance characteristic for the NowCheck COVID-19 Ag Test for rapid detection of SARS-CoV-2 antigen was established in prospective, single institute, randomized, single-blinded study conducted at a clinical center in india during the 2020 SARSCoV- 2 pandemic situation.

A total of 167 prospective specimens were tested using the NowCheck COVID-19 Ag Test. These specimens consisted of nasopharyngeal swabs from symptomatic/ asymptomatic patients. The performance of the NowCheck COVID-19 Ag Test was compared to a commercialized molecular assay.

Test sensitivity and specificity

The NowCheck COVID-19 Ag Test showed a sensitivity of 89% and a specificity of 98%.

Nasopharygeal swab specimens (N = 167)		Real-time PCR		
		Positive	Negative	Total
NowCheck COVID-19 Antigen Test	Positive	47	2	49
	Negative	6	112	118
	Total	53	114	167

7. ANALYTICAL PERFORMANCE

1. To determine the Limit of Detection (LoD) a study used the “SARS-CoV-2 (2019-nCOV) NCCP 43326/2020/Korea” strain. The titer of cultured virus was confirmed by PCR. The inactivated virus was spiked into the negative nasophayngeal swab. The LoD is 3.12 X 102.2 TCID50/ml.

2019-nCoV Strain Tested	NCCP 43326/2020/Korea								
Virus stock titer	$1 \times 10^{6.2}$ $TCID_{50}$/ml								
Concentration in Dilution tested ($TCID_{50}$/ml)	$1 \times 10^{5.2}$	$1 \times 10^{4.2}$	$5 \times 10^{3.2}$	$2.5 \times 10^{3.2}$	$1.25 \times 10^{3.2}$	$6.25 \times 10^{2.2}$	$3.12 \times 10^{2.2}$	$1.56 \times 10^{2.2}$	$7.8 \times 10^{1.2}$
Cell rate of 5 replicated	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
Call rates of 20 replicates near cut-off	NA	NA	NA	NA	NA	100% (20/20)	100% (20/20)	100% (0/20)	100% (0/20)
Lowest Concentration with Uniform Positivity per Analyte	$3.12 \times 10^{2.2}$ $TCID_{50}$/ml								
Limit of detection (LoD)	$3.12 \times 10^{2.2}$ $TCID_{50}$/ml								

2. 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 X 10^4 $TCID_{50}$/ml	No cross-reactivity
Human Coronavirus 229E	1 X $10^{4.5}$ $TCID_{50}$/ml	No cross-reactivity
Human Coronavirus OC43	1 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Human Coronavirus NL63	1 X 10^4 $TCID_{50}$/ml	No cross-reactivity
Influenza A	3 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Influenza B	3 X 10^5 $TCID_{50}$/ml	No cross-reactivity
RSV A	3 X 10^5 $TCID_{50}$/ml	No cross-reactivity
RSV B	3 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Human Metapneumovirus (hMPV)	1 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Parainfluenza virus	1 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Rhinovirus	1 X 10^5 $TCID_{50}$/ml	No cross-reactivity
Enterovirus	1 X 10^4 $TCID_{50}$/ml	No cross-reactivity
Adenovirus	3 X 10^5 $TCID_{50}$/ml	No cross-reactivity
human immunodeficiency virus lysate	10 µg/ml	No cross-reactivity
Mycobacterium tuberculosis	5 X 10^4 cells/ml	No cross-reactivity
Haemophilus influenzae	5 X 10^4 cells/ml	No cross-reactivity
Mycoplasma pneumoniae	5 X 10^4 cells/ml	No cross-reactivity
Streptococcus pneumonia	5 X 10^4 cells/ml	No cross-reactivity
Streptococcus pyrogens	5 X 10^4 cells/ml	No cross-reactivity
Legionella pneumophila	5 X 10^4 cells/ml	No cross-reactivity
Candida albicans	5 X 10^4 cells/ml	No cross-reactivity
Bordetella pertussis	5 X 10^4 cells/ml	No cross-reactivity
Moraxella catarrhalis	5 X 10^4 cells/ml	No cross-reactivity
Pseudomonas aeruginosa	5 X 10^4 cells/ml	No cross-reactivity
Staphylococcus epidermidis	5 X 10^4 cells/ml	No cross-reactivity
Streptococcus salivarius	5 X 10^4 cells/ml	No cross-reactivity
Chlamydia pneumoniae	1 X 10 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%.

3. The results of endogenous/exogenous interference substance studies and their potential interference are listed in the table below. There was no any interfering activity from any of the substances 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
Anti-inflammatory medication	Acetaminophen	1030 µM
	Acetylsalicylic acid	167 µM
	Ibuprofen	1060 µM
Antibiotics	Mupirocin	3 µM
	Tobramycin	70.6 µM
	Erythromycin (antibiotic)	188 µM
	Ciprofloxacin (antibiotic)	36.2 µM
Nasal sprays or drop	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 Ge	5% (v/v)
	Sodium Cromoglycate	20 mg/ml
	Olopatadine Hydrochloride	10 mg/ml
Oral anaesthetic	Anbesol (Benzocaine 20%)	5% (v/v)
Throat lozenges	Strepsils (flurbiprofen 8.75 mg)	5% (w/v, 50 mg/ml)
	Throat candy (mint)	5% (w/v, 50 mg/ml)
Others	Mucin: bovine submaxillary gland, type I-S	100 µg/ml
	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 g/ml

4. High-dose Hook Effect: The highest concentration of heat and chemical inactivated. SARS-CoV-2 stock available (TCID50 of 1 X 106.2 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 30 minutes.

Type	Virus spiking	Cytopathic Effect	Interpretation
Extraction buffer	0	Non CPE	Virus inactivated
Cell culture media		CPE	Positive control

8. STORAGE STABILITY

Store the kit at room temperature (2-30□C / 36-86°F), out of direct sunlight. Do not freeze the kit. Kit materials are stable until the expiration date printed on the outer box. It is recommended to perform the test immediately after removing the test device from the foil pouch as it is sensitive to humidity and temperature.

9. SPECIMEN REQUIREMENTS

Nasal Swab Sample:

For optimal test performance with a Nasal Swab specimen, use the Swabs supplied in the kit.. It is important to obtain as much secretion as possible. Therefore, to collect a nasal swab sample, insert the sterile swab into the nostril that presents the most secretion under visual inspection. Using gentle rotation, push the Swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the Swab a few times against the nasal wall.

Nasopharyngeal Swab Sample:

It is important to obtain as much secretion as possible. Therefore, to collect a nasopharyngeal swab sample, carefully insert the sterile Swab into the nostril that presents the most secretions under visual inspection. Keep the Swab near the septum floor of the nose while gently pushing the Swab into the posterior nasopharynx. Rotate the Swab several times.

Specimens should be tested immediately after collection.

10. QUALITY CONTROL

Built-in quality control

The Test Strip contains a built-in procedural control feature with a Control line always appearing if the test procedure has run correctly.

This control line will normally appear, irrespective of whether the patient sample si positive or negative for the virus. with a two-color result format providing a simple interpretation for positive and negative results.

If the Control Line does not develop after 15-30 minutes, the test result is considered invalid and should be repeated with another test strip

External Quality Control

External controls are also available to demonstrate that the reagents and assay procedure perform properly.

All control results should be recorded in the Results database and marked as a Quality Control Sample.

11. TEST PROCEDURE

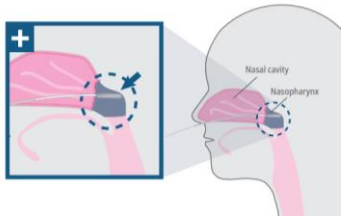
Test strip preparation

1. Remove the test strip from foil pouch.
2. Attach the film provided onto the test strip.

Sample Collection

1. Wash your hands and apply the necessary Personal Protective Equipment

2. Use a sterile swab supplied with the test kit to collect a naso-pharyngeal sample from the nostril of the patient, reaching the surface of the posterior nasopharynx.



3. Using gentle rotation, push the swab until resistance is met at the level of turbinate.

4. Rotate the swab several times, against the nasopharyngeal wall.

5. Withdraw the sterile swab from the nasal cavity.

6. If the samples cannot be tested immediately after collection, 1 mL VTM could be used instead of extraction buffer.

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

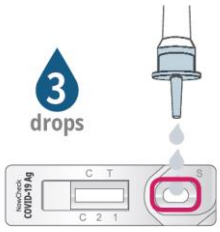
Specimen testing

1. Insert the swab into an extraction buffer tube.

2. While squeezing the buffer tube, stir the swab more than 5 times.

3. Press the nozzle cap tightly onto the tube.

4. Apply 3 drops of extracted specimen to the sample hole of the test device.



5. Read the test result in 15-30 minutes. Do not read after 30 minutes.

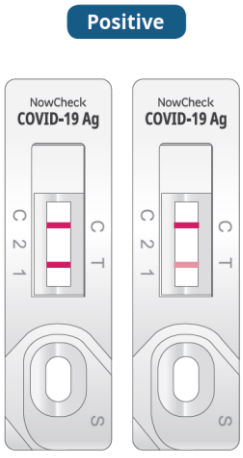
6. Dispose of all specimens and materials used to perform the test as biohazard waste.

Result interpretation

1. **Positive Result:**

   A coloured band appearing in the top section of the result window indicates that the test is working properly. This is called the control band (c).

   The appearance of ANY coloured band in the lower window – called the Test band (T) - indicates a positive test for the COVID-19 antigen.

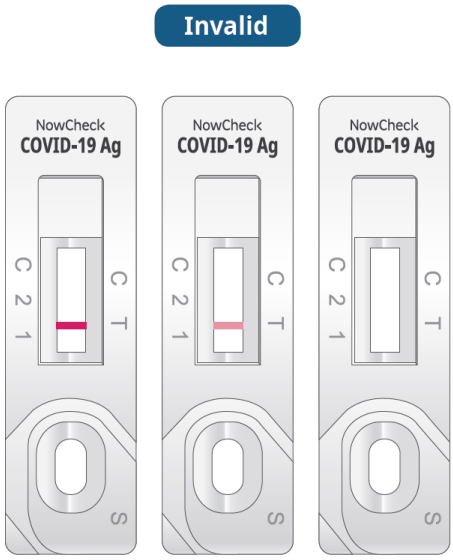


2. **Negative Result:**

   The absence of any sort of band in the lower window indicates a negative test for COVID-19.

3. **Invalid Result**:

   If no Control band appears in the upper part of the test window, but a band appears in the lower Test window the result is invalid and the test should be repeated.



   Result recording

   Enter the result together with the subject details (Name, Unit No), test date and test time, into the APPN database.

12. RESULTS

Enter the result together with the subject details (Name, Unit No), test date and test time, into the APPN database.

13. REFERENCES

This method has been adapted from the NowChek COVID-19 Ag Test (2020).