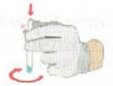
careUS™ COVID-19 antigen

2. Remove the device and extraction tube from its foil pouch immediately before testing.
3. The kit is intended to be used only with nasopharyngeal swab specimens.
4. The kit is not intended for testing liquid samples such as wash or aspirate samples or swabs in transport media as results can be compromised by over dilution
5. Peel of aluminum foil seal.
6. Place the swab into the extraction tube. Rotate the swab vigorously at least 5 times.
7. Remove the swab by rotating against the extraction tube while squeezing the sides of the tube to release the liquid from the swab. Properly discard the swab.
8. Close the tube by pushing the cap firmly onto the tube. Mix thoroughly by flicking the bottom of the tube.
9. Invert the extraction tube and hold the sample vertically above the sample well. Squeeze the tube gently. Allow three drops of sample to fall into the sample well.
10. Read and interpret the test result at 10 – 15 minutes. The test result should not be read and interpreted after 15 minutes.






Interpretation of Results

NOTE: The test result should not be read and interpreted after 15 minutes. Do not interpret the result using any instruments.

Positive: Two distinct colored line appear.

One red-colored line next to "C" and one blue-colored line next to "T" indicate an COVID-19 positive result.

Negative: Only one line appears.

Only one red-colored line next to "C" indicates a negative result.

Invalid: No control line appears.

If the control line "C" is not visible, the result is invalid. It is recommended the specimen be re-tested. If the same invalid result appears, contact the manufacturer or distributor before continuing the test.

Quality control

Internal Quality Control: The device contains a built-in internal procedural control that is included in the test device. A red-colored line appearing in the control region "C" is designed as an internal procedural control. The appearance of the procedural control line indicates that sufficient flow has occurred, and the functional integrity of the test device has been maintained. If the procedural control line does not develop in 10 minutes, the test result is considered invalid, and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact the manufacturer or distributor.

External Quality Control: External control is used to demonstrate that the test device and test procedure perform properly. It is recommended that positive and negative external control swabs are run once with every new lot, shipment, and each new user. External positive and negative control swabs are provided in the kit. The external control should be tested using the nasopharyngeal swab or nasal swab test procedure provided in this package insert or the quick reference instruction card. If the external control results are invalid, please contact the manufacturer or distributor before testing patient specimens.

Performance Characteristics

Clinical characteristics

Method (Nasopharyngeal swab)		Comparator Method	
		Positive	Negative
careUS™ COVID-19 antigen	Positive	30	-
	Negative	1	30
Total		31	30
PPA(Positive Percent Agreement)		30/31*100 = 96.78%	
NPA(Negative Percent Agreement)		40/40*100 = 100.00%	

Positive Percent Agreement was 96.78% (30/31) and negative Percent Agreement was 100.0% (30/30) comparing with comparator method (RT-PCR) in nasopharyngeal swab.

Method (Nasal swab)		Comparator Method	
		Positive	Negative
careUS™ COVID-19 antigen	Positive	4	-
	Negative	0	10
Total		4	10
PPA(Positive Percent Agreement)		4/4*100 = 100.00%	
NPA(Negative Percent Agreement)		10/10*100 = 100.00%	

Positive Percent Agreement was 100.00% (4/4) and negative Percent Agreement was 100.00% (10/10) comparing with comparator method (RT-PCR) in nasal swab.

Analytical Sensitivity: Limit of Detection (LoD)

The LoD concentration of the virus strain was determined by 3 replicates of serial dilution test. The determined LoD concentration of the virus strain was verified with 20 replicates. The result percentile of 20 replicates of the virus strain was calculated as 100%. In this study, LoD of the careUS™ COVID-19 antigen test kit was determined as 8×10^2 $TCID_{50}$/mL.

Stock concentration	Estimated LoD concentration	No. of positive/ No of replicate
1.6×10^5 $TCID_{50}$/mL	8.0×10^2 $TCID_{50}$/mL	20/20

* The same as each sample matrix.

Analytical Specificity: Cross-Reactivity (Exclusivity) and Microbial Interference

Cross reactivity test results confirmed that there was no cross reactivity on the positive and negative panels. A total of 8 bacteria were tested at a target concentration of approximately 10^7 CFU/mL with the exception of *mycoplasma pneumoniae*, which was tested at a test concentration of 1.5×10^3 CFU/mL. The 17 viruses were tested at concentrations between $10^{5.0}$ and $10^{7.9}$ $TCID_{50}$/mL. All negative samples gave negative results at the concentrations of the potentially cross-reactive common organisms tested showing no cross-reactivity with the device. All samples with SARS-CoV-2 strain tested positive showing no microbial interference at the concentrations of the potentially interfering common organisms tested.

Potential Cross-Reactant	
Adenovirus1	Parainfluenza virus type 1
Adenovirus7	Parainfluenza virus type 2
Enterovirus 71, Tainan/4643/1998	Parainfluenza virus type 3
Human coronavirus (OC43)	Parainfluenza virus type 4
Human coronavirus (229E)	Respiratory syncytial virus Type B
Human coronavirus (NL63)	Rhinovirus
Human metapneumovirus (hMPV)	SARS-Coronavirus
Influenza A/Michigan/45/2015	MERS-Coronavirus, Irradiated Lysate
Influenza B/Wisconsin/01/2010	*Bordetella pertussis*
Candida albicans	*Chlamydophila pneumoniae*
Mycoplasma pneumoniae	*Haemophilus influenzae*
Streptococcus pneumoniae	*Legionella pneumophila*
Streptococcus pyogenes, Group A	Pooled human nasal wash

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology.

- The homology between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 nucleocapsid protein is relatively low, at 36.7% across 86.4% of sequences, but cross-reactivity cannot be ruled out.
- The homology between SARS-CoV-2 nucleocapsid protein and *Mycobacterium tuberculosis* total protein (3,991 proteins) is relatively low, homology-based cross reactivity can be ruled out.
- The homology between SARS-CoV-2 nucleocapsid protein and *Pneumocystis jirovecii* total protein (3,745 proteins) is relatively low, homology-based cross-reactivity can be ruled out

Interfering Substances Effect

- To assess substances with the potential to interfere with the performance of the device, positive and negative samples were tested with the addition of potentially interfering substances. All samples tested produced expected results, demonstrating that the device performance was not affected by any of the 30 potentially interfering substances listed in the table below at the concentrations tested.
- The interfering effects of biotin concentrations ranging between 625 ng/mL and 10 ug/mL were tested in a separate study. Biotin concentrations less than or equal to 1.25 ug/mL did not lead to false negative results. Biotin concentration more than 2.5 ug/mL can cause false-negative COVID-19 results.

Potential Interfering Substances	Concentration
Acetaminophen	10 mg/mL
Acetylsalicylic acid	15 mg/mL
Beclomethasone	0.5 mg/mL
Benzocaine	5 mg/mL
Budesonide	2 mg/mL
Chlorpheniramine maleate	5 mg/mL
Dexamethasone	1 mg/mL
Dextromethorphan HBr	2 mg/mL
Diphenhydramine HCl	5 mg/mL
Ephedrine HCl	10 mg/mL
Flunisolide	5 mg/mL
Fluticasone	1 mg/mL
Guaiacol glyceryl ether	20 mg/mL
Histamine dihydrochloride	10 mg/mL
Menthol	10 mg/mL
Mometasone	1 mg/mL
Mucin	2%
Mupirocin	1 mg/mL
OTC throat drop (Halls)	15%
OTC throat drop (Ricola)	15%
OTC nasal spray (Afrin)	15%
OTC nasal spray (Vicks sinex)	15%
OTC nasal spray (Zicam)	15%
Oxymetazoline HCl	10 mg/mL
Phenylephrine HCl	5 mg/mL
Phenylpropanolamine	5 mg/mL
Tobramycin	1 mg/mL
Triamcinolone	1 mg/mL
Whole blood	2%
Zanamivir	1 mg/mL

Hook Effect

All the 3 replicates were tested as positive at the range of test concentration from 1.6×10^5 $TCID_{50}$/mL to 1.6×10^1 $TCID_{50}$/mL and the results indicate there is no hook effect.

References

1) Li, etc., Early transmission Dynamics in Wuhan, China of Novel Coronavirus-Infected Pneumonia, DOI: 10.1056/NEJMoa2001316.
2) Li Tai sheng, Peking Union Medical College Hospital's Proposal for Diagnosis and Treatment of "Novel Coronavirus Infected Pneumonia" (V2.0), Union Medical Journal, 2020. 1. 27.
3) Wei Qiuhua, Disinfection measures for pneumonia epidemic sources of novel coronavirus infection in 2019, Chinese Journal of Disinfection, 2020 (37) 1,59-62.
4) Chao, E.L.; Henshaw, J.L., Occupational Safety and Health Administration:Model Plans and Programs for the OSHA Bloodborne Pathogens and Hazard Communications Standards. OSHA 3186-06R, 2003.

Description of Symbol Used

REF	Catalogue number	Σ	Contains sufficient for <n> tests
LOT	Batch code		Temperature limitation
	Manufacturer		Consult instructions for use
	Use by		Do not use if package is damaged
	Single use	IVD	*In vitro* diagnostic medical device
EC REP	Authorized representative in the EC	CE	CE mark

WELLS BIO, INC.
16, Magokjungang 8-ro 1-gil, Gangseo-gu, Seoul, 07795, Republic of Korea
+82-2-3660-6900 info@wellsbio.net
+82-2-3660-6990 www.wellsbio.net

EC REP MDSS GmbH
Schiffgraben 41, 30175
Hannover, Germany
+49-511-62 62 86 30
www.mdssar.com

CE IVD

REF RCN-M02082 / RCE-M02082 / RCM-M02082

A rapid diagnostic test for the detection of COVID-19 antigen directly from nasal or nasopharyngeal swabs.

careUS™ COVID-19 antigen

Intended use

The careUS™ COVID-19 antigen is a lateral flow immunochromatographic assay intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal or nasal swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider. The device is intended for use by healthcare professionals or trained operators who are proficient in performing tests and trained clinical laboratory personnel.

Summary

Since the first outbreak reported in December 2019, the new virus has been named "SARS-CoV-2". Before the new virus was known, six types of coronavirus were known to cause human infection. There were four types (229E, OC43, NL63, HKU1) causing colds and two types (SARS-CoV, MERS-CoV) resulting severe pneumonia. The newly identified SARS-CoV-2 is the third type that causes severe pneumonia. SARS-CoV-2 has been detected worldwide and designated as a pandemic by the World Health Organization (WHO) due to its highly contagious and devastating impacts on healthcare systems and economy. To effectively control the spread of SARS-CoV-2, it is necessary to screen and detect the infection status rapidly, resulting in reducing further infection. As a rapid diagnostics test with a 15 minutes testing time, the device allows effective screening of COVID-19 infection on a large scale.

Principle

careUS™ COVID-19 antigen test is an immunochro matographic assay for the detection of extracted nucleocapsid protein antigens specific to SARS-CoV-2 in nasal or nasopharyngeal swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider. Control antibody and test antibody are immobilized on the nitrocellulose membrane to form two distinct lines (control line, test line). The nitrocellulose membrane is attached onto a plastic backing card and combined with other reagents and pads to compose a test strip. The test strip is encased inside a plastic cassette. The prepared sample specimen can be added to the sample well of the test cassette to start a test. The SARS-CoV-2 antigens in sample specimen interact with the monoclonal SARS-CoV-2 antibodies that are conjugated to cellulose nano beads (CNB) and they form immune complexes. Immune complexes bind to streptavidin region (test line) on a membrane to generate a colored line as they migrate through the strip by capillary force. If no COVID-19 antigen exists, no test line will appear. The control line will appear as CNB-conjugated chicken IgY binds to the control antibody in the control region. The presence of one colored line in the control line region "C" indicates COVID-19 negative. No appearance of a colored line in the control region "C" indicates an invalid test.

Test kit contents

Contents	Quantity	Description
Test device	20 each	Foil pouched test device containing one test strip which is encased on plastic device cassette.
Extraction tube / cap	20 tubes and caps	The extraction tube contains 400 uL extraction buffer solution.
Specimen collection swab	20 each	Swab for collecting the specimen
Positive control swab	1 each	Recombinant SARS-CoV-2 nucleocapsid protein antigen is dried on the foam-tipped head.
Negative control swab	1 each	Blank Universal Viral Transport media is dried on the foam-tipped head.
Package insert	1 each	Instructions for use

* Materials not supplied

- Pair of gloves
- Timer / Pen
- Biohazard or sharps container
- Sterile gauze or cotton

Limitations

1. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic test should be considered to rule out infection in these individuals.
2. Results from antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
3. This test may complement the diagnostic accuracy of quantitative polymerase chain reaction (qPCR) tests, but it is not meant to compare this test's clinical sensitivity and specificity with those of a molecular test since the performance of these tests is affected by virus titers and patient's immunity against SARS-CoV-2.
4. This test will only indicate the presence of SARS-CoV-2 nucleocapsid protein antigen in the specimen from both viable and non-viable SARS-CoV-2 virus.
5. The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
6. Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
7. This device has been evaluated for use with human specimen material only.
8. False-negative results may occur if the concentration of the target antigen in the clinical specimen is below the detection limits of the device.
9. This device is a qualitative test and does not provide information on the viral concentration present in the specimen.
10. This test cannot rule out diseases caused by other bacterial or viral pathogens.
11. The prevalence of infection will affect the test's predictive values.
12. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely during peak activity when the prevalence of the disease is high. False-positive test results are more likely during the periods of low SARS-CoV-2 activity when prevalence is moderate to low.
13. The amount of antigen in a sample may decrease as the duration of illness increases. Specimens collected after day 5 of illness are more likely to be negative compared to a RT-PCR assay.

Precautions

1. For professional use and *in vitro* diagnostic use only.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. Immediately use after opening the test device in the pouch.
4. In order to obtain accurate results, the test must follow this package insert.
5. Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false-positive result. Avoid touching any bleeding areas of the nasopharynx when collecting specimens.
6. Do not interpret the test result before 10 minutes and after 15 minutes starting the test.
7. Do not use if the test device package is damaged.
8. Do not use the kit contents beyond the expiration date.
9. Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
10. Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
11. Dispose of used contents as biohazardous wastes in accordance with regional requirements.
12. Nitrile or latex gloves and appropriate personal protective equipment should be worn when performing this test.
13. If the extraction buffer contacts the skin or eye, flush with copious amounts of water.
14. Handle all specimens as though they contain infectious agents.
15. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
16. Reagents contain sodium azide, which is harmful if inhaled, swallowed, or exposed to skin. Contact with acids produces a very toxic gas. If there is contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
17. Do not interchange kit contents from different lots.
18. Do not re-use any contents in the kit as they are single-use only.

Storage and Stability

1. Store the test kit as packaged between 2 ~ 30°C.
2. The reagents and materials in the careUS™ COVID-19 antigen test are stable until the expiration date printed on the outer packaging. Do not use beyond the expiration date.
3. The test cassette must remain in the sealed pouch until use.
4. Do not freeze any contents of the kit.

Specimen Type

Acceptable specimen types are nasopharyngeal and nasal swab which are collected from nasopharynx using nasopharyngeal and nasal swabs. It is essential that correct specimen collection and preparation methods be followed. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to obtain accurate test results.

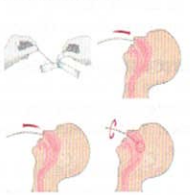
Specimen Collection and Test Procedures

Sample Collection Procedure

Procedural Notes

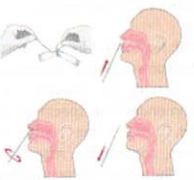
- Use only recommended nasopharyngeal and nasal swab for specimen collection.
- Collect the specimen wearing safety gloves and appropriate personal protective equipment to avoid contamination.
- Process the test of the sample immediately after collection.
- Do not touch the tip (specimen collection area) of the swab.

Nasopharyngeal Swab Sample Collection Procedure



1. Remove a specimen collection swab from the pouch.
2. Place the swab into one of patient's nostrils until it reaches the posterior nasopharynx.
3. Slowly rotate 3-5 times the swab over the surface of the posterior nasopharynx.
4. Remove the swab from the nostril.

Nasal Swab Sample Collection Procedure



1. Remove a specimen collection swab from the pouch.
2. Insert the swab into one of patient's nostrils up to 1 inch from the edge of the nostril.
3. Slowly roll the swab 5 times over the surface of the surface of the nostril. Using the same swab, repeat this collection process in the other nostril.
4. Remove the swab from the nostril.

Test Procedure

Procedural Notes

1. Allow test cassettes, reagents, and specimens to warm up to room temperature (15~30°C) before testing.