COV-S23

COVID-19 Antigen Rapid Test Device (Nasopharyngeal/Oropharyngeal Swab)

INTENDED USE

The COVID-19 Antigen Rapid Test Device is an in vitro immunoassay. The assay is for the direct and qualitative detection of SARS-CoV-2 viral nucleoprotein antigens from nasopharyngeal secretions and Oropharyngeal secretions. This test is intended for professional use only.

PRINCIPLE

The COVID-19 Antigen Rapid Test Device detects SARS-CoV-2 viral antigens through visual interpretation of color development. Anti-SARS-CoV-2 antibodies are immobilized on the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to colored particles are immobilized on the conjugated pad. A sample is added to the extraction buffer which is optimized to release the SARS-CoV-2 antigens from specimen.

During testing, the extracted antigensbind to anti-SARS-CoV-2 antibodies conjugated to colored particles. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by the anti-SARS-CoV-2 antibodies at the testregion. Excess colored particles are captured at the internal control zone.

The presence of a colored band in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

MATERIALS

Materials Provided

- Individually packed test devices
- Extraction tube
- Individually packed swabs
- Package insert
- Negative control(If required)
- Extraction buffer
- Nozzle with filter
- Tube stand
- Positive control(If required)

Materials Required but Not provided

- Clock, timer or stopwatch
- Transfer pipette

PRECAUTIONS

- For *in vitro* Diagnostic Use Only.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed.Erroneous result may occur if test reagents or components are improperly stored.
- Do not use the Extraction Buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- Avoid skin contact with buffer.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.
- Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens are NOT recommended, except in a BSL3 laboratory using BSL3 work practices.

STORAGE AND STABILITY

- Store The COVID-19 Antigen Rapid Test Device at 2~30℃when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

SPECIMEN COLLECTION AND STORAGE

-Nasopharyngeal swab (NP swab):

1) Remove the swab from its packing
2) Insert the swab into the nostril parallel to the palate, and gently pushing the swab into the posterior nasopharynx.-Rotating against the nasal wall.(to ensure swab contains cells as well as mucus) 3) Process the swab as soon as possible after collecting the specimen

-Oropharyngeal swab (OP swab):

1) Remove the swab from its packing
2) Insert the swab completely from the mouth into the throat, centering on the red part of the throat wall and maxillary tonsils, and rub the bilateral throat tonsils and throat wall moderately. Avoid touching the tongue and remove the swab
3) Process the swab as soon as possible after collecting the specimen

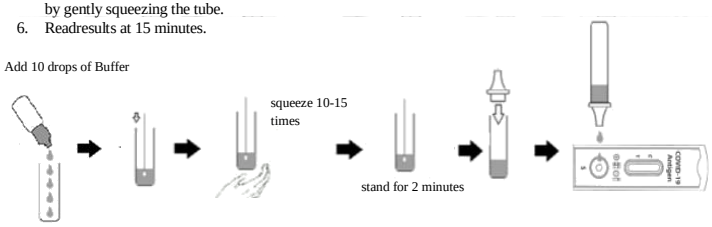
Note:

1. Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit further testing.
2. Swabs specimens should be tested as soon as possible after collection. Use freshly collected specimens for best test performance.
3. If not tested immediately, swab specimensmay be stored at 2-8 ℃ for 24 hours after collection.
4. Do not use specimens that are obviously contaminate with blood, as it may interfere with the flow of sample with the interpretation of test results.

TEST PROCEDURE

Bring devices, reagents and specimens and/or controls to room temperature (15~30℃) before use.

1. For each specimen, open the foil pouch just before testing and remove the test device, and put it on a clean, level surface. Label the tube with the patient identification. For best results, the assay should be performed within one hour.
2. Gently mix extraction buffer. Add 10 drops into the extraction tube.
3. Insert the swab into the extraction tube. Mix well and squeeze the swab 10-15 times bycompressing the walls of the tube against the swab.
   Stand for 2 minutes.
4. Roll the swab head against the inner wall of the tube as you remove it. Try to release as much liquid as possible. Dispose of the used swab in accordance with your biohazard waste disposal protocol.
5. Insert nozzle into sample extraction tube. Invert the tube and add 2 dropsof solution into the sample well by gently squeezing the tube.
6. Readresults at 15 minutes.



RESULT INTERPRETATION

POSITIVE:Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

Internal Procedural Controls

The COVID-19 Antigen Rapid Test Device has built-in (procedural) controls.Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the colored band located at the "C" region is present before reading the result.

External Positive and Negative Controls

Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The COVID-19 Antigen Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of SARS-CoV-2 antigen. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable SARS-CoV-2 viruses are detectable with The COVID-19 Antigen Rapid Test Device.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. Negative results do not preclude SARS-CoV-2 infection and should be confirmed via molecular assay.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Detection):

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at $2x10^{2.4}TCID_{50}/mL$.

The limit of detection was also determined with recombinant SARS-CoV-2 nucleoprotein and has been evaluated at 0.4 ng/mL.

Clinical Evaluation:

Clinical evaluation was performed to compare the results obtained by COVID-19 Antigen Rapid Test and RT-PCR. The results were summarized below:

Table: COVID-19 Rapid Test vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
COVID-19 Antigen Rapid Test	Positive	50	9	59
	Negative	3	1027	1030
Total		53	1036	1089

Relative Sensitivity: 94.3 % (84.6% ~ 98.1%)*

Relative Specificity: 99.1 % (98.4% ~ 99.5%)*

Overall Agreement: 98.9 % (98.1% ~ 99.4%)*

*95% Confidence Interval

Cross Reactivity:

Cross reactivity with the following organisms has been studied. Samples positive for the following organisms were found negative when tested with the COVID-19 Antigen Rapid Test Device (Nasopharyngeal/Oropharyngeal Swab).

HCoV-HKU1	Influenza A (H5N1)	Coxsackie virusA16
HCoV-OC43	Influenza A (H7N9)	Norovirus
HCoV-NL63	Influenza A (H7N7)	Mump virus
HCoV-229E	Influenza B Victoria lineage	*Legionella pneumophila*
Measles virus	Influenza B Yamagata lineage	*Mycoplasma pneumoniae*
Streptococcus pneumoniae	Respiratory syncytial virus	*Chlamydia pneumoniae*
Epstein-Barr virus	Adenovirus	*Streptococcus pyogenes*
Bordetella parapertussis	Parainfluenza 1/2/3 virus	*Streptococcus agalactiae*
InfluenzaA (H1N1)pdm09	Human metapneumovirus	Group C *Streptococcus*
Influenza A (H3N2)	Rhinovirus	*Staphylococcus aureus*

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the respiratory tract, were evaluated at the concentrations listed below. None of them were found to affect test performance of The COVID-19 Antigen Rapid Test Device.

Substance	Concentration	Substance	Concentration
3 OTC nasal sprays	10%	Guaiacol glyceryl ether	20 mg/ml
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Mupirocin	250 μg/ml
4-acetamidophenol	10 mg/ml	Oxymetazoline	10 mg/ml
Acetylsalicylic acid	20 mg/ml	Phenylephrine	10 mg/ml
Albuterol	20 mg/ml	Phenylpropanolamine	20 mg/ml
Chlorpheniramine	5 mg/ml	Relenza ® (zanamivir)	20 mg/ml
Dexamethasone	5 mg/ml	Rimantadine	500 ng/ml
Dextromethorphan	10 mg/ml	Tamiflu ® (oseltamivir)	100 mg/ml
Diphenhydramine	5 mg/ml	Tobramycin	40 mg/ml
Doxylaminesuccinate	1 mg/ml	Triamcinolone	14 mg/ml
Flunisolide	3 mg/ml		

LITERATURE REFERENCES

1. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular evolution of human coronavirus genomes. Trends Microbiol. 25, 35–48 (2017).
2. Ithete, N. L. et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg. Infect. Dis. 19, 1697–1699 (2013).

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marking according to IVD Medical Devices Directive 98/79/EC		

CE

EC REP

Lotus NL B.V.
Koningin Julianaplein 10, le Verd,
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ECOTEST®

COVID-19 IgG/IgM

Rapid Test Device

(Whole Blood/Serum/Plasma)

COV-W23M

INTENDED USE

The COVID-19 IgG/IgM Rapid Test Device is an *in vitro* immunoassay for the direct and qualitative detection of anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG in human whole blood, serum, or plasma as an aid in the diagnosis of COVID-19.The test is for professional use only.

INTRODUCTION

Coronaviruses are a large family of viruses that are common in many different species of animals, including camels, cattle, cats, and bats.
The two highly pathogenic viruses, SARS-CoV and MERS-CoV, cause severe respiratory syndrome in humans, and the other four human coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1) induce only mild upper respiratory diseases in immunocompetent hosts, although some of them can cause severe infections in infants, young children and elderly individuals[1,2,3].
COVID-19 is the disease associated with SARS-CoV-2, which was identified in China at the end of 2019. Coronaviruses cause respiratory and intestinal infections in animals and humans[1].
The virus is transmitted mainly via respiratory droplets that people sneeze, cough, or exhale. The incubation period for COVID-19 is currently estimated at between two and 14 days. Common symptoms of COVID-19 infection include fever, cough and respiratory symptoms such as shortness of breath and breathing difficulties. More serious cases develop severe pneumonia, acute respiratory distress syndrome, sepsis and septic shock that can lead to the death of the patient. People with existing chronic conditions seem to be more vulnerable to severe illness.
Detection of IgM indicates recent infection and can be used for early diagnosis of infection. IgG antibodies gradually appear and increase in the late stage of infection, and the COVID-19 IgG/IgM Rapid Test Device is a simple lateral flow immunoassay for the direct detection of anti-SARS-CoV-2 IgG/IgM antibody. It will provide a presumptive diagnosis of COVID-19.

PRINCIPLE

The COVID-19 IgG/IgM Rapid Test Device detects anti-SARS-CoV-2 IgG/IgM antibody through visual interpretation of color development.
Anti-human IgG and anti-human IgM are used to detect specific antibodies in the human whole blood, serum, or plasma specimen. When specimen is added to the sample well, specific IgM and/or IgG antibodies, if present, will bind to the SARS-CoV-2 antigens conjugated to colored particles on the conjugate pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-human IgM and/or anti-human IgG antibodies immobilized on the test region(s). Excess colored particle are captured at the internal control region.
The presence of a red band(s) on the test region(s) indicates a positive result for the particular IgG and/or IgM antibodies, while its absence indicates a negative result. A red band at the control region (C) serves as a procedural control, indicating that membrane wicking is working.

REAGENTS AND MATERIALS

Materials Provided

- Individually packed test devices
- 5uLdisposable pipettes
- 10uLdisposable pipettes
- Buffer
- Package insert

Materials Required but Not provided

- Clock, timer or stopwatch
- Transfer pipette

PRECAUTIONS

- For *in vitro* Diagnostic Use Only.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
- Do not use the Buffer if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Care should be taken to store specimens as indicated in the document (refer to SPECIMEN COLLECTION AND STORAGE).
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- Avoid skin contact with all components containing sodium azide which is a skin irritant.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.

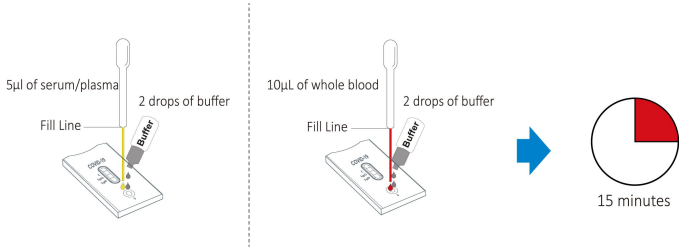
STORAGE AND STABILITY

- Store the COVID-19 IgG/IgM Rapid Test Device at 2~30℃when not in use.
- **DO NOT FREEZE.**
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days. For long term storage, serum or plasma specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 3 days after collection. Do not freeze whole blood specimens.
- Containers containing anticoagulants such as EDTA, citrate, heparin or oxalate should be used for whole blood storage.
- Bring specimens to room temperature prior to testing. Frozen serum or plasma specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

TESTPROCEDURE

Specimen Collection:
Allow the test device, specimen, buffer, and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test device on a clean and level surface. Label the test with patient or control identification.
   For Serum or Plasma Specimens:
   a) Using the provided 5μL disposable pipette, draw the specimen up to the Fill Line, and transfer all the specimen (appr. 5 μL) into the specimen well of the test device, then add 2 drops of buffer and start the timer.
   For Whole Blood Specimens:
   a) Using the provided 10μL disposable pipette, draw the specimen up to the Fill Line, and transfer all the specimen (appr. 10 μL) into the specimen well of the test device, then add 2 drops of buffer and start the timer.
3. Wait for the colored line(s) to appear. Read results at 15 minutes.
   Note: Specimens can also be applied using a micropipette.



RESULT INTERPRETATION

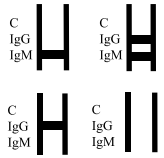
For COVID-19 IgG/IgM Test:

IgM Positive:*The colored line in the control region (C) changes from blue to red, and a colored line appears in the IgM test region. The result is positive for COVID-19 virus specific-IgM antibodies.

IgG Positive:*The colored line in the control region (C) changes from blue to red, and a colored line appears in the IgG test region. The result is positive for COVID-19 virus specific-IgG antibodies.

IgM and IgG Positive:*The colored line in the control region (C) changes from blue to red, and two colored lines should appear in IgG and IgM test regions. The color intensities of the lines do not have to match. The result is positive for IgM and IgG antibodies.

Negative: The colored line in the control region (C) changes from blue to red. No line appears in IgM or IgG test regions.



Invalid: Control line (C) is still completely or partially blue, and fails to completely change from blue to red. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

NOTE:

1. The color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

Internal Procedural Controls
The COVID-19 IgG/IgM Rapid Test Device has built-in (procedural) controls. Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the blue band should be always located at the "C" region before testing, and the red band should be always present before result interpretation.
External Positive and Negative Controls
Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The COVID-19 IgG/IgM Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of anti-SARS-CoV-2 IgM and anti-SARS-CoV-2 IgG. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
3. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
4. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
5. A high dose "hook effect" may occur where the color intensity of test band decreases as the concentration of anti-SARS-CoV-2 IgG/IgM increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the test band.
6. Negative results do not preclude COVID-19 and should be confirmed via other methods such us molecular assay.

PERFORMANCE CHARACTERISTICS

Clinical Evaluation:

79 specimens were collected from patients exhibiting pneumonia or respiratory symptoms. 83 specimens were also collected from convalescent patients. 227 negative specimens were collected in the study.

For IgM detection:

Method		PCR+	PCR-	Total
COVID-19 IgG/IgM Rapid Test	IgM+	74	2	76
	IgM-	5	225	230
Total		79	227	306

Relative sensitivity: 93.7% (86.0%-97.3%)*

Relative specificity: 99.1% (96.8%-99.8%)*

Overall agreement: 97.7% (95.4%-98.9%)*

*95% Confidence Interval

For IgG detection:

Method		Convalescent samples	PCR-	Total
COVID-19 IgG/IgM Rapid Test	IgG+	82	3	85
	IgG-	1	224	225
Total		83	227	310

Relative sensitivity: 98.8% (93.5%-99.8%)*

Relative specificity: 98.7% (96.2%-99.5%)*

Overall agreement: 98.7% (96.7%-99.5%)*

*95% Confidence Interval

Cross Reactivity

There was no cross-reactivity with any of the unrelated infections tested. No inhibition was observed with any of the specimens.

Anti-HAV IgM +	Chagas IgG+
Anti-HEV IgM +	Anti-Syphilis +
HBsAg +	Anti-Chlamydia +
Anti-HCV +	Anti-Tuberculosis +
Anti-HIV+	Typhoid IgM +
Anti-Rubella IgM +	Lyme disease+
Anti-CMV IgM +	P. falciparum +
Anti-HSV-I IgM +	P. vivax +
Anti-HSV-II IgM +	Toxoplasmosis +
EBV IgG +	HAMA +
Anti-Dengue virus +	RF +
Anti-Yellow fever +	ANA+
Anti-Zika virus +	
Anti-Chikungunya +	

Interfering Substances

The assay performance of COVID-19 IgG/IgM Rapid Test is not affected by substances at concentrations listed below.

Interfering substances	Concentration of analyate
Blood analytes	
Albumin	5 g/dL
Bilirubin	5 mg/dL
Hemoglobin	20 g/dL
Triglycerides	500 mg/dL
Anticoagulants	
EDTA	3.4 µmol/L
Heparin	3000 U/L
Sodium citrate	5 mg/mL
Potassium oxalate	2 mg/mL
Abnormal blood sample	
Visual hemolysis	NA
Icteric	NA
Lipemic	NA
Common medicines	
Acetylsalicylic acid	3.62 mmol/L
Ascorbic acid (Vitamin C)	342 µmol/L
Amoxicillin	206 µmol/L
Fluconazole	245 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Nadolol	3.88 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.04 µmol/L
Anti-malarial medicines	
Quinine	148 µmol/L
Anti-tuberculosis medicines	
Rifampicin	78.1 µmol/L
Isoniazid	292 µmol/L
Ethambutol	58.7 µmol/L
Common consumables	
Coffee (caffeine)	308 µmol/L
Alcohol (ethanol)	86.8 mmol/L

LITERATURE REFERENCES

1. Masters, P. S. & Perlman, S. in Fields Virology Vol. 2 (eds Knipe, D. M. & Howley, P. M.) 825–858 (Lippincott Williams & Wilkins, 2013).
2. Su, S. et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 24, 490–502 (2016).
3. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular evolution of human coronavirus genomes. Trends Microbiol. 25, 35–48 (2017).
4. Kan, B. et al. Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. J. Virol. 79, 11892–11900 (2005).
5. Ithete, N. L. et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg. Infect. Dis. 19, 1697–1699 (2013).
6. "Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)" https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marking according to IVD Medical Devices Directive 98/79/EC		

ECOTEST®

CE

EC REP

Lotus NL B.V.

Address: Koningin Julianaplein 10, le Verd, 2595AA,The Hague,Netherlands

COV-S23 COVID-19 Antigen Rapid Test Device (Nasopharyngeal/Oropharyngeal Swab)

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PRINCIPLE

The COVID-19 Antigen Rapid Test Device detects SARS-CoV-2 viral antigens through visual interpretation of color development. Anti-SARS-CoV-2 antibodies are immobilized on the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to colored particles are immobilized on the conjugated pad. A sample is added to the extraction buffer which is optimized to release the SARS-CoV-2 antigens from specimen.

During testing, the extracted antigens bind to anti-SARS-CoV-2 antibodies conjugated to colored particles. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by the anti-SARS-CoV-2 antibodies at the test region. Excess colored particles are captured at the internal control zone.

The presence of a colored band in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

MATERIALS

Materials Provided

- Individually packed test devices
- Tube stand
- Package insert
- Negative control(If required)
- Extraction buffer
- Individually packed swabs
- Positive control(If required)

Materials Required but Not provided

- Clock, timer or stopwatch
- Transfer pipette

PRECAUTIONS

- For *in vitro* Diagnostic Use Only.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
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- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
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- Avoid skin contact with buffer.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.
- Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2 specimens are NOT recommended, except in a BSL3 laboratory using BSL3 work practices.

STORAGE AND STABILITY

- Store The COVID-19 Antigen Rapid Test Device at 2~30℃when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

SPECIMEN COLLECTION AND STORAGE

-Nasopharyngeal swab (NP swab):

1) Remove the swab from its packing
2) Insert the swab into the nostril parallel to the palate, and gently pushing the swab into the posterior nasopharynx. Rotating against the nasal wall.(to ensure swab contains cells as well as mucus)
3) Process the swab as soon as possible after collecting the specimen

-Oropharyngeal swab (OP swab):

1) Remove the swab from its packing
2) Insert the swab completely from the mouth into the throat, centering on the red part of the throat wall and maxillary tonsils, and rub the bilateral throat tonsils and throat wall moderately. Avoid touching the tongue and remove the swab
3) Process the swab as soon as possible after collecting the specimen

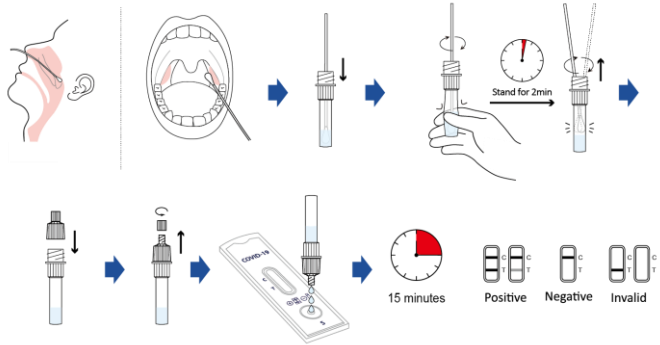
Note:

1. Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit further testing.
2. Swabs specimens should be tested as soon as possible after collection. Use freshly collected specimens for best test performance.
3. If not tested immediately, swab specimens may be stored at 2-8℃ for 24 hours after collection.
4. Do not use specimens that are obviously contaminate with blood, as it may interfere with the flow of sample with the interpretation of test results.

TEST PROCEDURE

Bring devices, reagents and specimens and/or controls to room temperature (15~30℃) before use.

1. For each specimen, open the foil pouch just before testing and remove the test device, and put it on a clean, level surface. Label the tube with the patient identification. For best results, the assay should be performed within one hour.
2. Take off the extraction tube cap and insert the swab into the extraction tube. Mix well and squeeze the swab 10-15 times by compressing the walls of the tube against the swab.
   Stand for 2 minutes.
3. Roll the swab head against the inner wall of the tube as you remove it. Try to release as much liquid as possible. Dispose of the used swab in accordance with your biohazard waste disposal protocol.
4. Place the blue cap back to the extraction tube. Unscrew the white cap and add 3 drops of solution into the sample well by gently squeezing the tube.
5. Read results at 15 minutes.



RESULT INTERPRETATION

POSITIVE:Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.

2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

Internal Procedural Controls

The COVID-19 Antigen Rapid Test Device has built-in (procedural) controls.Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the colored band located at the "C" region is present before reading the result.

External Positive and Negative Controls

Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The COVID-19 Antigen Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of SARS-CoV-2 antigen. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable SARS-CoV-2 viruses are detectable with The COVID-19 Antigen Rapid Test Device.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. Negative results do not preclude SARS-CoV-2 infection and should be confirmed via molecular assay.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Detection):

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at $2x10^{2.4}TCID_{50}/mL$.

The limit of detection was also determined with recombinant SARS-CoV-2 nucleoprotein and has been evaluated at 0.4 ng/mL.

Clinical Evaluation:

Clinical evaluation was performed to compare the results obtained by COVID-19 Antigen Rapid Test and RT-PCR. The results were summarized below:

Table: COVID-19 Rapid Test vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
COVID-19 Antigen Rapid Test	Positive	125	9	134
	Negative	3	1027	1030
Total		128	1036	1164

Relative Sensitivity: 97.7 % (93.3% ~ 99.2%)*

Relative Specificity: 99.1 % (98.4% ~ 99.5%)*

Overall Agreement: 99.0 % (98.2% ~ 99.4%)*

*95% Confidence Interval

Cross Reactivity:

Cross reactivity with the following organisms has been studied. Samples positive for the following organisms were found negative when tested with the COVID-19 Antigen Rapid Test Device (Nasopharyngeal/Oropharyngeal Swab).

HCoV-HKU1	Influenza A (H5N1)	Coxsackie virusA16
HCoV-OC43	Influenza A (H7N9)	Norovirus
HCoV-NL63	Influenza A (H7N7)	Mump virus
HCoV-229E	Influenza B Victoria lineage	*Legionella pneumophila*
Measles virus	Influenza B Yamagata lineage	*Mycoplasma pneumoniae*

Streptococcus pneumoniae	Respiratory syncytial virus	*Chlamydia pneumoniae*
Epstein-Barr virus	Adenovirus	*Streptococcus pyogenes*
Bordetella parapertussis	Parainfluenza 1/2/3 virus	*Streptococcus agalactiae*
InfluenzaA (H1N1)pdm09	Human metapneumovirus	Group C *Streptococcus*
Influenza A (H3N2)	Rhinovirus	*Staphylococcus aureus*

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the respiratory tract, were evaluated at the concentrations listed below. None of them were found to affect test performance of The COVID-19 Antigen Rapid Test Device.

Substance	Concentration	Substance	Concentration
3 OTC nasal sprays	10%	Guaiacol glycerol ether	20 mg/mL
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Mupirocin	250 μg/mL
4-acetamidophenol	10 mg/mL	Oxymetazoline	25μg/ml
Acetylsalicylic acid	10 mg/mL	Phenylephrine	10 mg/mL
Albuterol	10 mg/mL	Phenylpropanolamine	1 mg/mL
Chlorpheniramine	5 mg/mL	Zanamivir	10 mg/mL
Dexamethasone	50 μg/ml	Adamantanamine	500 ng/mL
Dextromethorphan	10 μg/ml	Oseltamivir phosphate	10 mg/mL
Diphenhydramine	5 mg/mL	Tobramycin	10 mg/mL
Doxylamine	1 mg/mL	Triamcinolone	14 mg/mL
Flunisolide	25 μg/ml		

LITERATURE REFERENCES

1. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular evolution of human coronavirus genomes. Trends Microbiol. 25, 35–48 (2017).
2. Ithete, N. L. et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg. Infect. Dis. 19, 1697–1699 (2013).

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marking according to IVD Medical Devices Directive 98/79/EC		

IVD

CE

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