COVID-19 Antigen Rapid Test
(Nasal / Nasopharyngeal / Oropharyngeal Swab – Cassette)
Product code: CCOV-201

INTENDED USE

The CHIL® COVID-19 antigen rapid test is an immunochromatographic in-vitro test for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal, nasopharyngeal and oropharyngeal swabs directly from people. This instruction for use (IFU) must be read and followed carefully prior to use. The reliability of assay results cannot be guaranteed in case of any discrepancies from the instructions for use. This product is intended exclusively for professional use in the laboratory and at the point-of-care.

INTRODUCTION

COVID-19 (Novel Corona Virus Disease) is an infectious disease caused by the most recently discovered coronavirus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have experienced aches and pains, nasal congestion, runny nose, sore throat or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but not developing any symptoms and do not feel sick. Most people (about 80%) recover from the disease without needing special treatment. Around 1 out of every 6 people who get COVID-19 becomes seriously ill and develops difficulty breathing. Older people, and those with underlying medical problems like high blood pressure, heart problems or diabetes, are more likely to develop a severe illness. Overall, about 2% of people who infected with SARS-CoV-2 have lost their lives. People with fever, cough, and difficulty breathing should seek medical attention. People can catch COVID-19 from others who have the virus. The disease can spread from person to person through small droplets from the nose or mouth which are spread when a person with COVID-19 coughs or exhales. These droplets land on objects and surfaces around the person. Other people then catch COVID-19 by touching these objects or surfaces, then touching their eyes, nose or mouth. People can also catch COVID-19 if they breathe in droplets from a person with COVID-19 who coughs out or exhales droplets. Most estimates of the incubation period for COVID-19 range from 1-14 days.

PRINCIPLE

This kit by immune chromatography test, the sample will be under the capillary action to move forward along the test cassette. If the sample contains SARS-CoV-2 N antigens, these antigens will be with colloidal gold labeled coronavirus monoclonal antibody. This immune complex will be membrane fixed by coronavirus monoclonal antibody capture, form the purple line which reveals a positive result. If the line does not show any color, the negative result will be displayed. The test cassette also contains a quality control line (C), which shall appear in purple regardless of whether there is a test line.

PRODUCT CONTENTS

Test cassette contains SARS-CoV-2 antibody-coated in the test region and Goat anti-Chicken IgY polyclonal antibody-coated in the control region on the membrane. The test cassette consists of the SARS-CoV-2 antibody, Chicken IgY, and Colloidal gold conjugate coated in the conjugate pad. The specimen extraction solution is a phosphate solution.

MATERIALS SUPPLIED

1. Swab **3.** Tube with antigen extraction buffer
2. Test cassette **4.** Instruction for Use (IFU)

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Waste for biological hazard materials
- Personal protective equipment

STORAGE AND STABILITY

The kit is stored at 2-30℃ and stable for 12 months in its aluminium sealed pouch. The test cassette should be used within 15 minutes after removed from its aluminium pouch, at the specified environment (temperature 2°C - 35°C, humidity 40% - 60%).

WARNINGS AND PRECAUTIONS

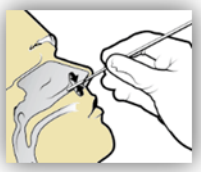
1. For professional *in vitro* diagnosis only. Do not use after the expiry date.
2. The test is a disposable in vitro diagnostic test, which is only used for the detection of human anterior nasal swab, nasopharyngeal swab, or oropharyngeal swab specimens. The application should be carried out strictly according to the instructions. Do not use expired and damaged products.
3. The test should be kept away from moisture. Tests or specimens stored at low temperatures should be balanced to room temperature before they can be used.
4. Tests should be used as quickly as possible after removal from aluminium pouches, to avoid exposure to air for too long and the possibility of affecting test results due to humidity.
5. Do not use specimens that have been placed for too long or contaminated. Please operate in accordance with the laboratory testing procedures for infectious diseases. Waste after use should be treated in accordance with infectious substances and should be discarded. Discard used test tubes and test device in suitable biohazards waste container.
6. Use a clean swab for every patient to avoid cross-contamination.
7. Incorrect operation may affect the accuracy of the results, such as, insufficient specimen amount, insufficient specimen mixing, inaccurate detection time, etc.
8. The components in different batches of the kit cannot be used by mixing.
9. Recommend using "fresh specimen" for testing, if testing not available on the same day, specimen collected by swab should be kept in -20 ℃ for storage and transfer in dry condition.
10. Universal transport medium (UTM) or viral transport medium (VTM) can be used but If you use universal transport medium (UTM) or viral transport medium (VTM), specimen could be diluted so probability of the detection can be diminished.
11. There should be appropriate biosafety assurance procedures for those substances containing and suspected sources of infection. The following are relevant considerations:

- Handle specimens and tests with gloves.
- Do not suck specimens with your mouth.
- Do not smoke, eat, drink, cosmetic or handle contact lenses while handling these items.
- Disinfect the surfaces after usage of the test.
- Disinfect and treat all specimens, Tests, and potential pollutants in accordance with relevant local regulations.
- Each component of the Test remains stable until the expiry date under proper handling and storage conditions. Do not use the expired Test kit.

SPECIMEN COLLECTIONS AND PREPARATION

Oropharyngeal Swab

Nasopharyngeal Swab



Anterior Nasal Swab

1. Throat (Oropharyngeal) and Anterior Nasal or Deep Nose (Nasopharyngeal) Swabs:

After collecting the oropharyngeal swab sample with the same swab, you can collect the anterior nasal sample or nasopharyngeal swab sample. Then place swab which containing the total sample, in the

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extraction tube. Rotate the swab for about 10 seconds and press head of swab against the tube wall to release the antigens in the swab.

2. Only Throat (Oropharyngeal) Swab:

Have the patient's head slightly tilted back, mouth open, and "ah" sound, exposing both sides of the pharyngeal tonsils. Gently rub the swab against the pharyngeal tonsils on both sides of the patient at least 3 times, and then rub the swab against the posterior pharyngeal wall at least 3 times. Place the swab specimen into the extract tube, rotate the swab for about 10 seconds, and press the swab head against the tube wall to release the antigens in the swab.

3. Only Anterior Nasal Swab

Hold the swab halfway up the handle and gently insert the swab 1,5-2,5 cm (0,6-1 inch) into one of the patient's nostrils, depending on the size of the person's nose. Slowly rotate the swab around the inside wall of the nostril 5 times. Make sure to collect any nasal sample that may be present on the swab. Using the same swab, repeat this collection process in the other nostril. Take approximately 15 seconds to collect the sample; place the swab specimen into the extract tube, rotate the swab for about 10 seconds, and press the swab head against the tube wall to release the antigens in the swab.

4. Only Deep Nose (Nasopharyngeal) Swab:

Allow the patient's head to relax naturally, and slowly rotate the swab against the nostril wall into the nostril of the patient to the nasal palate, and then slowly rotate it out while wiping. Wipe the other nostril with the same swab, using the same method; place the swab specimen into the extract tube, rotate the swab for about 10 seconds, and press the swab head against the tube wall to release the antigens in the swab.

5. After transferring the sample to the extraction tube, dispose of the swab in medical waste. Do not touch the swab that touched the extraction buffer to the mucosa again. Close the cap of the tube.

6. The specimens should be used in the test as soon as possible after collected (within half an hour).

7. Specimens should not be inactivated.

TEST PROCEDURE

Do not open the pouch until you are ready to perform a test, and the single-use test is suggested to be used under low environment humidity (RH≤70%) within 1 hour.

1. Allow all kit components and specimens to reach room temperature between 18℃ - 26℃ prior to testing.

2. Remove the test cassette from the pouch and place it on a clean dry surface.

3. Open the pouch and take out the test cassette and identify the test cassette for each patient.

4. Put the swab specimen into the extraction tube, rotate the swab for about 10 seconds, and press the swab head against the tube wall to release the antigen in the swab. Squeeze the swab over the head to remove as much liquid as possible from the swab. Dispose of swabs according to the biohazard waste disposal procedure.

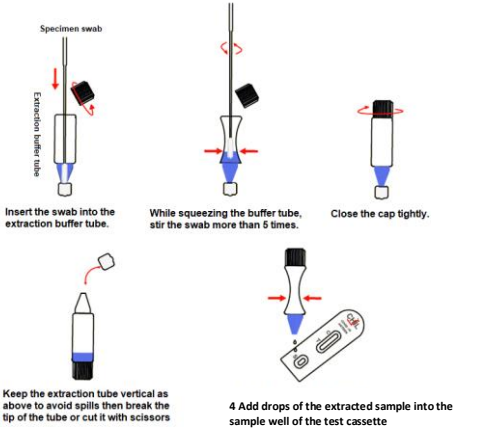
5. Close the cap of the extraction tube.

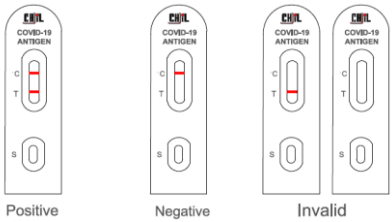
6. Keep the tube vertical as the breaking part will be upward to avoid specimen solution spills. Make sure that the liquid in the conical part goes down, if necessary, you can tap it gently so that the liquid goes down.

7. Keep the tube vertical as the breaking part will be upward to avoid specimen solution spills. Break the top part of the tube (If there is a problem cut tip of the tube with scissors, then disinfect the scissors) and add 4 drops tp the sample well of the test cassette and start the timer.

8. Optimum reading time of results is 20 minutes. The results after 30 minutes are no longer valid.

See the application illustration below;





INTERPRETATION OF TEST RESULTS

Positive: If both the quality control line (C) and the test line (T) appears, it means that SARS-CoV-2 antigen has been detected and the result is positive.

Negative: If there is only a quality control line (C), the test line (T) is colorless, it means that SARS-CoV-2 antigen has not been detected and the result is negative.

Invalid: If the quality control line (C) is not observed, it will be invalid regardless of whether there is a test line (T), and the test shall be conducted again.

Note: Test line (T) color intensity may differ according to density of the collected specimen. However, within the specified observation time, regardless of the density of the colored line, even a very weak line should be interpreted as a positive result.

QUALITY CONTROL

Internal procedural control is included in the test cassette. A colored line appearing in the control line region (C) is an internal valid procedural control, it confirms adequate membrane wicking. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The result of the product should not be taken as a confirmed diagnosis, but for clinical reference only. Diagnosis should be made along with RT-PCR results, clinical symptoms, epidemic conditions, and further clinical data.

2. In the early stage of infection, the test result might be negative due to the the low SARS-CoV-2 antigen level or antigen has not yet appeared in the specimen.

3. Due to the limitation of the detection method, the negative result cannot exclude the possibility of an infection. The positive result should not be taken as a confirmed diagnosis.

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4. This Test can only qualitatively detect SARS-CoV-2 antigens in human anterior nasal and/or nasopharyngeal and/or oropharyngeal swab. It cannot determine the certain antigen level in the specimens.
5. The accuracy of the test depends on the specimen collection process. Improper specimen collection, improper specimen transportation, and storage or freezing and thawing of the specimen would affect the test results.
6. It is needed when diluting swab specimens with the matched extraction buffer supplied. Using other extraction buffers may cause wrong results.
7. The extraction buffer and test cassette must be equilibrated to room temperature (18°C - 26°C) before the use, otherwise, the results may be incorrect.
8. Sensitivity may decrease if the specimen was not tested directly. Please test the specimen as soon as possible.
9. Cross-reactions may exist due to the N protein in SARS which has high homology with the new coronavirus (SARS-CoV-2). However, the interpretation of the results is not affected during seasons without SARS infection.
10. **Analysis of the possibility of false-negative results:**
    - Inappropriate specimen collection, using another non-matching solution, extensive specimen transfer time (more than half an hour), the extensive volume of solution added when eluted the swab, non- standardized elution operation, low virus titer in the specimen. All the above may all lead to false-negative results.
    - Mutations in viral genes may lead to changes in the antigen epitope, leading to false-negative results.
11. **Analysis of the possibility of false-positive results:**
    - Inappropriate specimen collection, using another non-matching solution, non- standardized elution operation may lead to false-positive results.
    - Cross-contamination of specimens may lead to false-positive results.
12. **Analysis of the possibility of an invalid result:**
    - If the specimen volume is less or over than required, the chromatography cannot be carried out successfully.
    - The test would be invalid if the package was crashed. The packaging status must be checked carefully prior to the application.

PERFORMANCE CHARACTERISTICS

1. Reference Control Material Study

1.1. The coincidence rate of positive controls
Tested with 100 positive controls, the results were all positive, and the coincidence rate (+ / +) was 100/100.

1.2. The coincidence rate of negative controls
Tested with 200 negative controls, the results were all negative, and the coincidence rate (- / -) was 200/200.

1.3. Repeatability
Tested with repeatable control 1 for 10 times, the results were all positive and consistent.
Tested with repeatable control 2 for 20 times, the results were all positive and consistent.

1.4. Reference Control Material with 100980 catalogue number, supplied from NIBSC UK is used.

2. Limit Detection: Limit of detection is 30 TCID50/mL

COVID-19 Titer	$3.84x10^5$ TCID50/mL								
Dilution	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600
Concentration in Dilution tested (TCID50/mL)	$3.84x10^3$	$1.92x\ 10^3$	$9.6x10^2$	$4.8x10^2$	$2.4x10^2$	$1.2x10^2$	6x 10	3x 10	1.5x10
Detection rates of 5 replicates	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	80% (4/5)
Detection rates of 20 replicates near cut-off	NA	NA	NA	NA	NA	100% (20/ 20)	100% (20/20)	95% (19/20)	75% (15/20)

Lowest Concentration with Uniform Positivity per Analyte	30 TCID50/mL
Limit of detection(LOD) per inactivated virus culture	30 TCID50/mL

3. Cross-Reactivity

This cross reaction study is performed to verify the influence of common respiratory pathogens on the detection performance of CHIL COVID-19 Antigen Test. The following respiratory pathogens are selected for cross-reactivity tests: Influenza A virus H1N1, influenza B virus, *Mycoplasma pneumoniae*, Rhinovirus A, Rotavirus, Large intestine *Escherichia*, respiratory syncytial virus, adenovirus, etc. The concentration of bacterial specimens is set to 10^6 cfu/ml (cfu: colony-forming units) or higher, and the concentration of virus specimens is set to 10^5 pfu/ml (pfu: plaque-forming unit) or higher. The test results are shown in the table below.

Pathogen	Concentrations	CHIL COVID-19 Antigen Rapid Test
HKU1	10^5 pfu/mL	Negative
OC43	10^5 pfu/mL	Negative
NL63	10^5 pfu/mL	Negative
229E	10^5 pfu/mL	Negative
MERS-coronavirus	10^5 pfu/mL	Negative
Human Metapneumovirus	10^5 pfu/mL	Negative
Influenza A virus H1N1	10^5 pfu/mL	Negative
Influenza A virus H3N2	10^5 pfu/mL	Negative
Influenza A virus H5N1	10^5 pfu/mL	Negative
Influenza A virus H7N9	10^5 pfu/mL	Negative
Influenza B virus	10^5 pfu/mL	Negative
Rhinovirus A	10^5 pfu/mL	Negative
Rhinovirus B	10^5 pfu/mL	Negative
Rhinovirus C	10^6 pfu/mL	Negative
Adenovirus 1	10^5 pfu/mL	Negative
Adenovirus 2	10^5 pfu/mL	Negative
Adenovirus 3	10^5 pfu/mL	Negative
Adenovirus 4	10^5 pfu/mL	Negative
Adenovirus 5	10^5 pfu/mL	Negative
Adenovirus 7	10^5 pfu/mL	Negative
Adenovirus 55	10^5 pfu/mL	Negative
Enterovirus A	10^5 pfu/mL	Negative
Enterovirus B	10^5 pfu/mL	Negative
Enterovirus C	10^5 pfu/mL	Negative
Enterovirus D	10^5 pfu/mL	Negative

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EB Virus	10^5 pfu/mL	Negative
Measles virus	10^5 pfu/mL	Negative
Human cytomegalovirus	10^5 pfu/mL	Negative
Rotavirus	10^5 pfu/mL	Negative
Norovirus	10^5 pfu/mL	Negative
Mumps virus	10^5 pfu/mL	Negative
Varicella-zoster virus	10^5 pfu/mL	Negative
Respiratory syncytial virus	10^5 pfu/mL	Negative
Mycoplasma pneumoniae	10^6 cfu/mL	Negative
Escherichia coli	10^6 cfu/mL	Negative

Experiment results on twenty SARS CoV-2 antigen negative specimens of healthy people have confirmed as all negative for the concentration of microorganisms or viruses mentioned above. These tested pathogens have no cross reaction effect on the detection performance of the COVID-19 Antigen Rapid Test.

4. Interference Substances

Interference testing is performed to evaluate CHIL COVID-19 antigen rapid test performance with common substances. The study is designed as to add the following substances to negative and weakly positive specimens to evaluate the interference effect on the COVID-19 Antigen Rapid Test results (see the table below).

Substances	Concentrations	CHIL COVID-19 Antigen Rapid Test Results on Negative specimens	CHIL COVID-19 Antigen Rapid Test Results on Positive specimens
Mucin	200 mg/ml	Negative	Positive
Hemoglobin	10 mg/ml	Negative	Positive
Histamine Hydrochloride	4.0mg/L	Negative	Positive
Human albumin	60 mg/ml	Negative	Positive
α- interferon	2 ng/ml	Negative	Positive
Lopinavir	2 µg/ml	Negative	Positive
Tobramycin	10 mg/L	Negative	Positive
Ribavirin	40 mg/L	Negative	Positive
Tramadol	12 µg/ml	Negative	Positive
Azithromycin	5 µg/ml	Negative	Positive
Meropenem	10 mg/ml	Negative	Positive
Oseltamivir	1000 ng/ml	Negative	Positive
Benzocaine	1.5 mg/ml	Negative	Positive
Peramivir	20 µg/ml	Negative	Positive

The test results show that the above mentioned common substances have no interference effect on the detection performance of the COVID-19 Antigen Rapid Test.

5. High Dose Hook Effect

SARS-CoV-2 cultured virus was spiked into sample. SARS-CoV-2 cultured virus did not show hook-effect at $9.73x10^6$ TCID50/mL.

6. Clinical Study

Analysis of coincidence rate of COVID-19 Antigen Rapid Test and PCR Test in nasal specimens.

		PCR Test		Total
		Positive	Negative	
CHIL COVID-19 Antigen Rapid Test	Negative	4	464	468
	Positive	401	2	403
Total		405	466	871

Sensitivity: 99.01% (95%CI: 97.49% - 99.73%)*
Specificity: 99.57% (95%CI: 98.46% - 99.95%)*
Accuracy: 99.54% (95%CI: 98.83% - 99.88%)*
*95 % Confidence Interval: (%-%)
Disease prevalence accepted as 5%.

Analysis of coincidence rate of COVID-19 Antigen Rapid Test and PCR Test in anterior nasal specimens.

		PCR Test		Total
		Positive	Negative	
CHIL-COVID 19 Antigen Rapid Test	Negative	6	244	250
	Positive	98	2	100
Total		104	246	350

Sensitivity: 94.23% (87.87% to 97.85%)*
Specificity: 99.19% (97.09% to 99.90%)*
Accuracy: 99.19% (97.09% to 99.90%)*
*95 % Confidence Interval: (%-%)
Disease prevalence accepted as 5%.

REFERENCE

1, Peaper DR, Landry ML. Rapid diagnosis of influenza: state of the art. Clin Lab Med. 2014;34(2):365-385. doi:10.1016/j.cll.2014.02.009
2. Patel J, Sharma P. Design of a novel rapid immunoassay for simultaneous detection of hepatitis C virus core antigen and antibodies. Arch Virol. 2020;165(3):627-641. doi:10.1007/s00705-019-04518-0
3. Chafekar A, Fielding BC. MERS-CoV: Understanding the Latest Human Coronavirus Threat. Viruses. 2018;10(2):93. Published 2018 Feb 24. doi:10.3390/v10020093
4. https://www.medcalc.org/calc/relative_risk.php

	Consult instruction for use		Keep dry
2°C 30°C	Store between	LOT	Batch number
	For single use	IVD	In vitro diagnostic medical device
	Manufacturer	Σ	Contains sufficient for <n> tests
	Expire date	CE	CE Marking
	Caution! Consult instruction for use for warnings except written on package.		

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