StrongStep®
SARS-CoV-2 Antigen Rapid Test

Specimen: Anterior nasal swab

Language: English	**Version:** 07
Effective Date: 2021-10	**REF:** 500200

For use by self-testing

INTENDED USE

StrongStep® SARS-CoV-2 Antigen Rapid Test Cassette employs immunochromatography technology to detect the SARS- CoV-2 nucleocapsid antigen in human anterior nasal swab specimen. This test is single use only and intended for self-testing. It is recommended to use this test within 5 days of symptom onset. It is supported by the clinical performance assessment.

INTRODUCTION

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

PRINCIPLE

The StrongStep® SARS-CoV-2 Antigen Test employs immunochromatographic test. Latex conjugated antibodies (Latex-Ab) corresponding to SARS-CoV-2 are dry-immobilized at the end of nitrocellulose membrane strip. SARS-CoV-2 antibodies are bond at the Test Zone (T) and Biotin-BSA is bond at the Control Zone (C). When the sample is added, it migrates by capillary diffusion rehydrating the latex conjugate. If present in sample, SARS-CoV-2 antigens will bind with the conjugated antibodies forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by SARS-CoV-2 antibodies generating a visible red line. If there are no SARS-CoV-2 antigens in sample, no red line is formed in the Test Zone (T). The streptavidin conjugate will continue to migrate alone until it is captured in the Control Zone(C) by the Biotin-BSA aggregating in a blue line, which indicates the validity of the test.

KIT COMPONENTS

Content	Purpose
Sealed foil pouch packed test devices	Each device contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions.
Dilution Buffer vials	0.1 M Phosphate buffered saline (PBS) and 0.02% sodium azide.
Extraction Tubes	For specimens preparation use.
Packs of swab	For specimen collection.
Workstation	Place for holding buffer vials and tubes.
Package insert	For operation instruction.

Specifications(tests/kit) Content	1	2	3	4	5	7	10	15	20	25
Test devices	1	2	3	4	5	7	10	15	20	25
Buffer (0.7mL/vial)	1	2	3	4	5	7	10	15	20	25
Tube	1	2	3	4	5	7	10	15	20	25
Swab (optional)	1	2	3	4	5	7	10	15	20	25
Workstation	1	1	1	1	1	1	1	1	1	1
Package insert	1	1	1	1	1	1	1	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use.
Any necessary personal protective equipment	

PRECAUTIONS

- This kit is for IN VITRO diagnostic use only.
- Read the instructions carefully before performing the test.
- This product does not contain any human source materials.
- Do not use kit contents after the expiration date.
- Wear gloves during the whole procedure.

STORAGE AND STABILITY

The sealed pouches in the test kit may be stored between 2-30℃ for the duration of the shelf life as indicated on the pouch.

SPECIMEN COLLECTION AND STORAGE

An anterior nasal swab sample can be collected or by an individual performing a self-swab. Children under 18 years of age, should be performed by their adult supervision. Adults aged 18 and over can perform the anterior nasal swab by themselves. Please follow your local guidelines for specimen collection by children.

- Insert one swab into one nostril of the patient. The swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nostril. Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected.
- Use the same swab, repeat this process for the other nostril to ensure that an adequate sample is collected from both nasal cavities.

It is recommended that specimens be processed as soon as possible after collection. Specimens can be held in container up to 1 hour at room temperature (15°C to 30°C), or up to 24 hours when refrigerated (2°C to 8°C) before processing.

PROCEDURE

Bring test devices, specimens, buffer and/or controls to room temperature (15-30°C) before use.

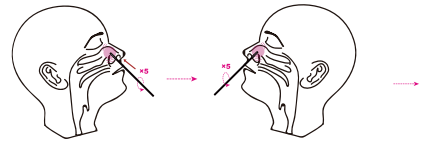
- Place the collected specimen Extraction tube in the designated area of the workstation.
- Squeeze all the Dilution Buffer into the extraction tube.
- Put the specimen swab into the tube. Vigorously mix the solution by rotating the swab forcefully against the side of the tube for least 15 times (while submerged). Best results are obtained when the specimen is vigorously mixed in the solution.
- Allow the swab to soak in the Extraction Buffer for one minute prior to the next Step.
- Squeeze out as much liquid as possible from the swab by pinching the side of the flexible extraction tube as the swab is removed. At least 1/2 of the sample buffer solution must remain in the tube for adequate capillary migration to occur. Put the cap onto the extracted tube.
- Discard the swab in a suitable biohazardous waste container.
- The specimens extracted can retain at room temperature for 30 minutes without affecting the result of the test.
- Remove the test device from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within 30 minutes.
- Add 3 drops (approximately 100 µL) of extracted sample from the Extraction Tube to the round sample well on the test device.
  Avoid trapping air bubbles in the sample well (S), and do not drop any solution in observation window.
  As the test begins to work, you will see color move across the membrane.
- Wait for the colored band(s) to appear. The result should be read by visual at 15 minutes. Do not interpret the result after 30 minutes.

- **Put the test tube containing the swab and the used test device into theattached biohazard bag and seal it, and then discard it in a suitablebiohazard waste container. Then throw away the remaining items**
- **Wash your hands or reapply hand sanitizer.**

Discard used Extraction Tubes and Test Devices in suitable biohazardous waste container.

Swab sample processing

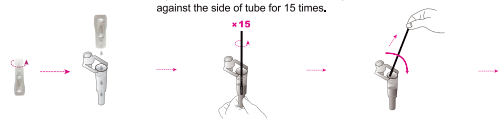
- Insert one swab into one nostril of the patient. The swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nostril. Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected.
- Use the same swab, repeat this process for the other nostril to ensure that an adequate sample is collected from both nasal cavities.



Squeeze all the Dilution Buffer into the extraction tube.

Put the nasal swab into the tube and squeeze the tube while roll the swab against the side of tube for 15 times.

Squeeze the swab against the upper part of tube and discard the swab.



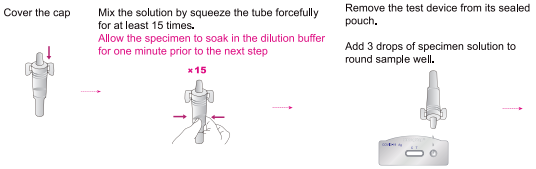
Test operation

Cover the cap

Mix the solution by squeeze the tube forcefully for at least 15 times.
Allow the specimen to soak in the dilution buffer for one minute prior to the next step

Remove the test device from its sealed pouch.

Add 3 drops of specimen solution to round sample well.

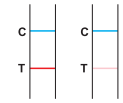


Wait for 15 minutes reading result.

Note:Result after 15 minutes may not be accurate.

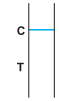
INTERPRETATION OF RESULTS

POSITIVE RESULT :



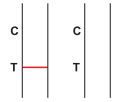
Two colored bands appear within 15minutes. One blue band appears in the Control Zone (C) and another red band appears in the Test Zone (T). The test result is positive and valid. No matter how faint the red band appears in the Test Zone (T), the test result should be considered as positive result.

NEGATIVE RESULT :



One blue band appears in the Control Zone (C) within 15 minutes. No red band appears in the Test Zone (T). The test result is negative and valid.

INVALID RESULT :



No blue band appears in the Control Zone (C) within 15 minutes. The test result is invalid. Repeat the test with a new test device.

QUALITY CONTROL

Internal procedural controls are included in the test. A blue band appearing in the control region (C) is considered as an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONSOF THE TEST

1. The kit is intended to use for the qualitative detection of SARS-CoV-2 antigens from Nasal .
2. This test detects both viable (live) and non-viable SARS-CoV-2. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
3. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
4. Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
5. Test results must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
6. Positive test results do not rule out co-infections with other pathogens.
7. Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
8. Negative results from patients with symptom onset beyond seven days, should be treated as presumptive and confirmed with an local FDA authorized molecular assay, if necessary, for clinical management, including infection control.
9. Specimen stability recommendations are based upon stability data from influenza testing and performance may be different with SARS-CoV-2. Users should test specimens as quickly as possible after specimen collection.
10. The sensitivity for RT-PCR assay in diagnosis of COVID-19 is only 50%-80% due to poor sample quality or disease time point at the recoverd phase,etc.SARS-CoV-2 Antigen Rapid Test Device's sensitivity is theoretically lower because of its methodology.
11. In order to get enough virus, it is suggested to use two or more swabs to collect different sites of sample and extract all the sampled swab in the same tube.
12. Positive and negative predictive values are highly dependent on prevalence rates.
13. Positive test results are more likely to represent false positive results during periods of little / no SARS-CoV-2 activity when disease prevalence is low.False negative test results are more likely when prevalence of disease caused by SARS-CoV-2 is high.
14. Monoclonal antibodies may fail to detect, or detect with less sensitivity,SARS-CoV-2 influenza viruses that have undergone minor amino acid changes in the target epitope region.
15. The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection and performance may differ in asymptomatic individuals.
16. 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.
17. Sensitivity of the test after the first five days of the onset of symptoms has been demonstrated to decrease as compared to a RT-PCR assay.
18. It is suggested to use StrongStep® SARS-CoV-2 IgM/IgG antibody rapid test (cat# 502090) to detect the antibody to increase the sensitivity of diagnosis of COVID-19.
19. It is not recommend to use Virus Transportation media(VTM) specimen in this test, if customers insist to use this sample type, customers should validate themselves.
20. The StrongStep® SARS-CoV-2 Antigen Rapid Test was validated with the swabs provided in the kit. Use of alternative swabs may result in false results.
21. Frequent testing is necessary to increase the sensitivity of diagnosis of COVID-19.
22. No drop off in sensitivity when compared with the wild type with respect to the following variants - VOC1 Kent, UK, B.1.1.7 and VOC2 South Africa, B.1.351.
23. Keep out of reach of children.
24. Positive results indicate that viral antigens were detected in the sample taken, please Self-quarantine and inform your family doctor promptly.

PERFORMANCE CHARACTERISTICS

Table 1. CLINICAL PERFORMANCE

Swabs	PCR Comparator		
		Positive	Negative	Total
StrongStep® SARS-CoV-2 Antigen Rapid Test	Positive	112	0	112
	Negative	4	207	211
	Total	116	207	323

Positive Percent Agreement: (PPA)= 96.55%% (91.41%~99.05%.)*
Negative Percent Agreement: (NPA)= 100.0% (98.23%~100.00%)*
Kappa: 0.9729（0.9269～0.9889.highly consistent)*
*95% Confidence Interval

ANALYTICAL PERFORMANCE

a) Limit of Detection (LoD):

The Limit of Detection (LoD) of the test was determined using limiting dilutions of heat-inactivated SARS-CoV-2. It is a preparation of SARS-Related Coronavirus-2 (SARS-CoV-2), isolate in China CDC, that has been inactivated by heating at 65°C for 30 minutes. The material was supplied frozen at a concentration of $TCID_{50}$ of 5.00 x10^5/mL.

To determine the SARS-CoV-2 to reflect the assay when using direct swabs. In this study a NP swab was spiked with approximately 50 µL of the virus dilution in saline. The spiked swab was added to the SARS-CoV-2 Test extractant concurrently to a NP swab containing NP matrix. The swabs were processed concurrently according to the package insert.

The LoD was determined in three steps:

1. LoD Screening

10-fold dilutions of the heat inactivated virus were made in saline and processed for each study as described above. These dilutions were tested in triplicate. The concentration demonstrating 3 of 3 positives was chosen for LoD range finding.

Based on this testing, the concentration chosen was $TCID_{50}$ of 5.00 x10^2/mL.

2. LoD Range Finding

Five (5) doubling dilutions were made of the $TCID_{50}$ of 5.00 x10^2/mL concentration in saline processed for the study as described above. These dilutions were tested in triplicate. The concentration demonstrating 3 of 3 positives was chosen for LoD confirmation.

Based on this testing the concentration chosen was $TCID_{50}$ of 2.50 x10^2/mL.

3. LoD Confirmation

The concentration $TCID_{50}$ of 2.50 x10^2/mL dilution was tested for a total of twenty (20) results. Nineteen (19) of twenty (20) results were positive.

Conclusion:

Based on this testing the concentration was confirmed as:

LoD: $TCID_{50}$ 2.50 x10^2/mL

b) Cross-Reactivity:

Cross-reactivity of the StrongStep® SARS-CoV-2 Antigen Rapid Test was evaluated by testing various microorganisms (10^6 CFU/mL), viruses (10^5 PFU/mL) and negative matrixes that may potentially cross-react with the StrongStep® SARS-CoV-2 Antigen Rapid Test. Each organism and virus were tested in triplicate. Based on the data generated by this study, the StrongStep® SARS-CoV-2 Antigen Rapid Test does not cross-react with the organisms or viruses tested.

SARS	
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus NL63	Parainfluenza virus 1-4
Influenza A & B	Enterovirus
MERS-coronavirus	Respiratory syncytial virus
Human coronavirus	Rhinovirus
Bordetella pertussis	Haemophilus influenzae
Mycoplasma pneumoniae	Streptococcus pneumoniae
Chlamydia pneumoniae	Streptococcus pyogenes
Legionella pneumophila	Candida albicans
Mycobacterium tuberculosis	Pooled human nasal wash – representative of normal respiratory microbial flora
Pneumocystis jirovecii (PJP)	
Pseudomonas aeruginosa	Staphylococcus epidermis
Staphylococcus salivarius	

c) Interfere substance:

Potential interfering substances of the StrongStep® SARS-CoV-2 Antigen Rapid Test was evaluated by testing various substances with concentration below that may potentially interfere with the StrongStep® SARS-CoV-2 Antigen Rapid Test. Each substance were tested in triplicate. Based on the data generated by this study, the StrongStep® SARS-CoV-2 Antigen Rapid Test does not interfere with the substances tested.

Analyte	Concentration	Analyte	Concentration
Respiratory Specimens	/	Histamine hydrochloride	400mg/L
Mucin	3.4%	Phenylephrine	400mg/L
Blood (human)	50%	Oxymetazoline	1mg/L
Alpha-interferon（Nasal gel）	400mg/L	Sodium chloride (with preservatives)	9%
Zanamivir	400mg/L	Beclomethasone	1mg/L
Ribavirin	400mg/L	Dexamethasone	5mg/L
Oseltamivir	500mg/L	Flunisolide	5mg/L
Peramivir	400mg/L	Triamcinolone acetonide	5mg/L
Lopinavir	500mg/L	Budesonide	5mg/L
Ritonavir	400mg/L	Mometasone	5mg/L
Arbidol	400mg/L	Fluticasone	5mg/L
Levofloxacin	400mg/L	Mints	400mg/L
Azithromycin	400mg/L	Dicaine	400mg/L
Ceftriaxone	400mg/L	Human Anti-mouse Antibody (HAMA)	200ug/L
Meropenem	400mg/L	Biotin	1200ug/L
Tobramycin	400mg/L	Escherichia coli	1×10^6 CFU/mL
Staphylococcus aureus	1×10^6 CFU/mL	Proteus vulgaris	1×10^6 CFU/mL
Proteus mirabilis	1×10^6 CFU/mL	Helicobocton Pyloni	1×10^6 CFU/mL

d) Hook Effect:

The highest concentration of heat-inactivated SARS-CoV-2 stock available ($TCID_{50}$ of 5.00 x 10^5/mL) was tested. There was no Hook effect detected.

GLOSSARY OF SYMBOLS

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

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