LIMING BIO

StrongStep® System Device for SARS-CoV-2 & Influenza A/B Combo Antigen Rapid Test

Specimen: Nasal/Oropharyngeal swab	
Language: English	**Version:** 01
Effective Date: 2020-09	**REF:** 500220

For use by clinical laboratories or healthcare workers only

For Medical Professional Use Only

INTENDED USE

The StrongStep® System Device for SARS-CoV-2 & Influenza A/B Combo Antigen Rapid Test is a rapid immunochromatographic assay for the detection of SARS- CoV-2 virus antigen as well as influenza type A and type B antigens in human Nasal / Oropharyngeal swab. The assay is used as an aid in the diagnosis of COVID-19 as well as acute influenza type A and type B viral infections.

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.

Influenza is a highly contagious, acute, viral infection of the respiratory tract. The causative agents of the disease are immunologically diverse, single-strand RNA viruses known as influenza viruses. There are three types of influenza viruses: A, B, and C. Type A viruses are the most prevalent and are associated with most serious epidemics. Type B viruses produce a disease that is generally milder than that caused by type A. Type C viruses have never been associated with a large epidemic of human disease. Both type A and B viruses can circulate simultaneously, but usually one type is dominant during a given season.

PRINCIPLE

The StrongStep® System Device for SARS-CoV-2 & Influenza A/B Combo Antigen Rapid Test employs immunochromatographic test. There are three strips in the device that detect SARS-CoV-2, influenza type A and influenza type B respectively. Latex conjugated antibody (Latex-Ab) corresponding to SARS-CoV-2, or Influenza A, or Influenza B is dry-immobilized at the end of each nitrocellulose membrane strip. SARS-CoV-2, or Influenza A, or Influenza B antibodies are bond at the Test Zone (T) and Biotin-BSA are bond at the Control Zone (C) on each strip. When the sample is added, it migrates by capillary diffusion rehydrating the latex conjugate. If present in sample, SARS-CoV-2, or Influenza A, or Influenza B 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, or Influenza A, or Influenza B antibodies generating a visible red line. If there are no SARS-CoV-2, or Influenza A, or Influenza B antigens in sample, no red line is formed in the Test Zone (T) of each strip . 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

20 Individually packed test devices	Each device contains three strips with colored conjugates and reactive reagents pre-spreaded at the corresponding regions. The extraction buffer are also included in the top of the device which filled in a tube.
20 packs of swab (2 swabs/pack)	For specimen collection.
1 workstation	For holding test device.
1 Package insert	For operation instruction.
1 Positive control swab (on request only)	Non-infectious, recombinant protein of SARS-CoV-2, Influenza A and Influenza B antigens with less than 0.1% sodium azide.

MATERIALS REQUIRED BUT NOT PROVIDED

TimerFor timing use. Any necessary personal
protective equipment

PRECAUTIONS

- This kit is for IN VITRO diagnostic use only.
- This kit is for medical professional 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.
- Handle all specimens as potentially infectious.
- Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infective material. When the assay procedure is complete, dispose specimens after autoclaving them at 121℃ for at least 20 minutes. Alternatively, they can be treated with 0.5% Sodium Hypochlorite four hours before disposal.
- Do not pipette reagent by mouth and no smoking or eating while performing assays.
- 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

Nasal Swab Sample:

- 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.
- Withdraw the swab from the nasal cavity and put the swab front end into extraction tube , against the tube and break off the swab at the break point, let the swab tip fall into the tube.

Oropharyngeal Swab Sample:

- Ask patient to open mouth and press tongue with tongue depressor if necessary. Use another swab into the oropharynx and scrap left and right side pharynx mucous membrane 2 times.
- Withdraw the swab from the mouth and put the swab front end into extraction tube , against the tube and break off the swab at the break point, let the swab tip fall into the tube.

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.

Use the swab supplied in the kit, alternative swabs may adversely affect test perfo rmance, Users should validate their swab before use it.

It is recommended that swab specimens be processed as soon as possible after collection. Swabs can be held in any clean, dry plastic tube or sleeve 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

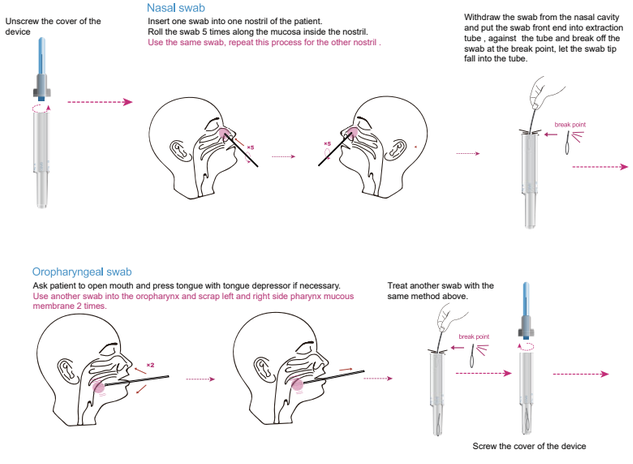
Bring tests, specimens and/or controls to room temperature (15-30°C) before use.

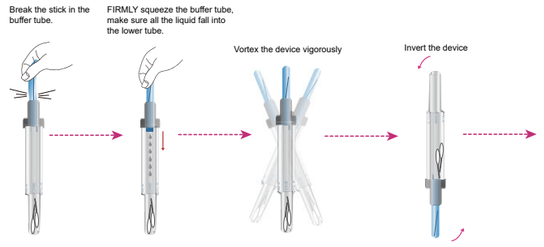
- Bring the kit components to room temperature before testing. Open the pouch and remove the test device.
- Once opened, the test device must be used immediately.
- Label the test device with patient identity. • Unscrew the cover of the device.
- Put the swab into the tube, break the swab at the breakpoint, let the sampled swab fall into the tube and discard the upper stick. •

Treat another swab with the same method above. •

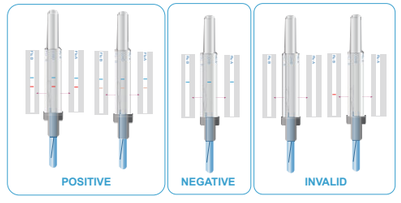
Screw the cover of the device.

- Break the stick in the buffer tube.
- FIRMLY squeeze the buffer tube, make sure all the liquid fall into the lower tube.
- Vortex the device vigorously.
- Invert the device, put the device into the workstation, let the sample buffer migrate onto the test strip.
- At the end of 15 minutes read the results. A strong positive sample may show result earlier.



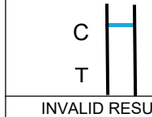


RESULT



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

1. 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.
2. External positive procedural controls may provided(on request only) in the kit to ensure that the tests are functioning properly. Use the swabs supplied in the kit as negative procedural control. Also, the Controls may be used to demonstrate

proper performance by the test operator. To perform a positive or negative control test, treat the positive and negative swabs as the specimen, follow the instructions above to handle the control swabs and read the results at 15 minutes.

LIMITATIONS OF THE TEST

1. The kit is intended to use for the qualitative detection of SARS-CoV-2 antigen as well as influenza type A and type B antigens from Nasal/Oropharyngeal swab.

2. This test detects both viable (live) and non-viable virus. 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, an d 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-CoV-2, non-influenza A, non-influenza B 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. StrongStep® System Device for SARS-CoV-2 & Influenza A/B Combo Antigen Rapid Test sensitivity is theoretically lower because of its methodology.

In order to get enough virus, it is suggest to use two or more swabs to collect different sites of sample and extract all the sampled swab in the same tube.

11.Positive and negative predictive values are highly dependent on prevalence rates.Positive test results are more likely to represent false positive results during periods of little/no SARS-CoV-2/influenza activity when disease prevalence is low .False negative test results are more likely when prevalence of disease caused by SARS-CoV-2/influenza is high.

12. 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.

13.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.

14.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.

15. 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.

16. 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.

17. The StrongStep® System Device for SARS-CoV-2 & Influenza A/B Combo Antigen Rapid Test was validated with the swabs provided in the kit. Use of alternative swabs may result in false results.

18.Frequent testing is necessary to increase the sensitivity of diagnosis of COVID-19.

PERFORMANCE CHARACTERISTICS

Table 1. CLINICAL PERFORMANCE

	PCR Comparator		Total
SARS-CoV-2 Antigen Test		Positive	Negative	
	Positive	36	2	38
	Negative	3	71	74
	Total	39	73	112

Positive Percent Agreement: (PPA)= 92.31% (79.13%～98.38%)*
Negative Percent Agreement: (NPA)= 97.26% (90.45%～99.67%)*
Kappa: 0.9011(0.8164～0.9858)*

	PCR Comparator		Total
Influenza type A Antigen Test		Positive	Negative	
	Positive	29	5	34
	Negative	7	137	144
	Total	36	142	178

Positive Percent Agreement: (PPA)= 80.56% (63.98%～91.81%)*
Negative Percent Agreement: (NPA)= 96.48% (91.97%～98.85%)*
Kappa: 0.7867(0.6712～0.9021)*

	PCR Comparator		Total
Influenza type B Antigen Test		Positive	Negative	
	Positive	17	2	19
	Negative	6	153	159
	Total	23	155	178

Positive Percent Agreement: (PPA)=73.91% (51.59%～89.77%)*
Negative Percent Agreement: (NPA)= 98.71% (95.42%～99.84%)*
Kappa: 0.7843(0.6407～0.9280)*
***95% Confidence Interval**

ANALYTICAL PERFORMANCE

a) Limit of Detection (LoD):

SARS-CoV-2

The Limit of Detection (LoD) of the test was determined using limiting dilutions of heat-inactivat ed 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 sup plied 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. T he 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 stud y as described above. These dilutions were tested in triplicate. The concentration demonstratin g 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 pro cessed for the study as described above. These dilutions were tested in triplicate. The concent ration 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

Analytical Sensitivity with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level
			$TCID_{50}$/mL
California/04/09*	A	H1N1	4.4 x 10^3
			EID_{50}/mL
A/Anhui/1/2013*	A	H7N9	7.90 x 10^6
			pfu/mL**
Hong Kong	A	H3N2	6.60 x 10^{-1}
Beijing/32/92	A	H3N2	3.30 x 10^0
Shanghai/11	A	H3N2	6.70 x 10^0
USSR	A	H1N1	2.00 x 10^2
Puerto Rico/8/34	A	H1N1	2.60 x 10^2
New Jersey	A	H1N1	2.70 x 10^2
Taiwan	A	H1N1	3.30 x 10^2
Tokyo/3/67	A	H2N2	3.40 x 10^2
Bayern	A	H1N1	6.60 x 10^2
Sichuan	A	H3N2	6.60 x 10^2
Beijing/352/89	A	H3N2	7.70 x 10^2
NWS/33	A	H1N1	1.00 x 10^3
Texas/77	A	H1N1	3.30 x 10^3
Fort Monmouth/1/47	A	H1N1	6.70 x 10^3
Aichi	A	H3N2	3.20 x 10^3
Shangdong	A	H3N2	8.40 x 10^3
Maryland/91	A	H1N1	1.00 x 10^4
Japan/305/57	A	H2N2	1.30 x 10^4
Sydney	A	H3N2	2.00 x 10^4
Bangkok	A	H3N2	3.30 x 10^4
Wuhan	A	H3N2	3.30 x 10^4
Beijing/353/89	A	H3N2	3.30 x 10^5
Singapore/86	A	H1N1	6.60 x 10^5
Texas/91	A	H1N1	1.60 x 10^7
Panama	B	/	1.00 x 10^0
Ann Arbor	B	/	3.30 x 10^2
Singapore	B	/	3.30 x 10^2
Lee	B	/	6.60 x 10^2
Maryland	B	/	6.60 x 10^3
Yamagata/16/88	B	/	6.70 x 10^3
Harbin	B	/	1.40 x 10^4
Stockholm	B	/	3.30 x 10^5

b) Cross-Reactivity:

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

SARS	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus 229E	Human Metapneumovirus (hMPV)
Human coronavirus OC43	Parainfluenza virus 1-4
Human coronavirus NL63	Enterovirus
MERS-coronavirus	Respiratory syncytial virus
Human coronavirus HKU1	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)	

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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