Saliva SARS-Cov-2(2019-nCoV) Antigen Combined Test Kit （Nanocarbon Assay）

INTENDED USE

One Step COVID-19 Antigen Test is a lateral flow, one-step immunoassay for the qualitative detection of N/S antigen in Saliva sample. This product is used to obtain a visual, qualitative results and is intended for professional use.

This assay provides only a preliminary analytical test result. A more specific alternate method must be used in order to obtain a confirmed analytical result. Virus nucleic acid test is the preferred confirmatory method.

PRINCIPLE

One Step COVID-19 Antigen Test is a rapid chromatographic immunoassay based on the principle of sandwich antibody and antigen binding.
During testing, COVID-19 N/S antigen, if the Virus antigens present in the saliva specimen it will bind to the antibody conjugates to form a complex. And migrates upward by capillary. The complex will then be captured by the immobilized antibody coated at the Test line region on the NC membrane. A visible colored line will show up in the test line region. The colored line will not form in the test line region if there is no virus antigen in the sample.
A virus antigen-positive saliva specimen will generate a colored line in the test line region because of antigen binding, while a virus antigen-negative saliva specimen or a specimen containing a very low concentration will not generate a colored line in the test line region.
To serve as a procedural control, a colored line will always appear at the control line region (C line), indicating that proper volume of specimen has been added and membrane wicking has occurred.

STORAGE AND STABILITY

Store as packaged in the sealed pouch at 2-30℃. The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

PRECAUTIONS

- For *in vitro* diagnostic use only. For professional use only.
- Do not use after the expiration date.
- The test Cassette should remain in the sealed pouch and until use.
- Saliva specimen may be potentially infectious. Proper handling and disposal methods should be established.
- The used test Cassette should be discarded according to federal, state and local regulations.

Materials

Materials Provided

- Test devices
- Drying agent

Materials Required But Not Provided

- Specimen collection container
- Timer

SPECIMEN COLLECTION AND PREPARATION

Saliva Assay

The saliva specimen must be collected in a clean and dry container. Saliva collected at any time of the day may be used. Saliva specimen exhibiting visible particles should be centrifuged, filtered, or allowed to settle to obtain clear specimen for testing.

Or just put the test into the mouth for several minute to get enough saliva for direct test.

Specimen Storage

Saliva specimens may be stored at 2-8ºC for up to 48 hours prior to assay. For long-term storage, specimens may be frozen and stored below -20ºC. Frozen specimens should be thawed and mixed before testing.

TEST PROCEDURE

Review “Specimen Collection” and “Precautions” instructions. Test cassette, saliva specimen should be brought to room temperature (20-30ºC) prior to testing. Do not open pouches until ready to perform the assay**.**

1. Remove the test cassette from its package and set to the clean and level surface.
2. Method I: Directly put the absorbing rod beneath your tongue to absorb saliva for several minutes, take out the test and place it on a clean and flat table, and start the timing.
3. Method II: Or put your tongue on your upper jaw and bow your head to make saliva secrete naturally into a disposable plastic cup.
4. Insert the absorbing rod vertically into the disposable plastic cup containing saliva (at least 2ml). Require the absorbing rod to be immersed in saliva for at least 2 minutes. During the period, the absorbing rod can be changed slightly to suck up the liquid. When the liquid appears in the observation window, take out the kit and place it on a clean and flat table, and start the timing.
5. Read result at 15 minutes after the addition of samples. Do not read result after 30 minutes.

Method I　　Method II

INTERPRETATION OF RESULTS

Positive: In any window, two lines appear. one black line should be in the control region (C), and another apparent black line should be in the test region (T). This positive result indicates that there are COVID-19 N/S antigens in the saliva present in the saliva specimen.

Negative: Both windows, only one black line appears in the control region (C). No line appears in the test region (T). This negative result indicates that there is no COVID-19 N/S antigen present in the saliva specimen or the antigen concentration is too low.

Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test using a new test device. If the problem persists, discontinue using the lot immediately and contact your local distributor.

Note: The shade of black in the test line region (T) will vary, but it should always be considered as positive whenever there is even a faint black line.

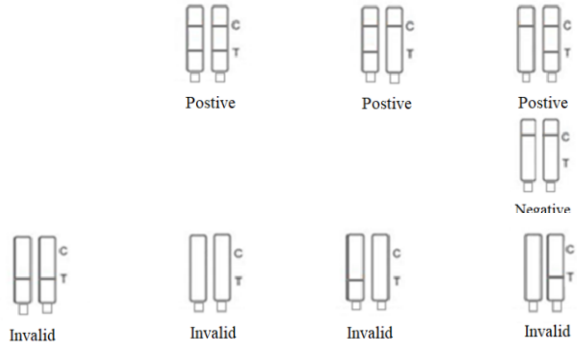
QUALITY CONTROL

Internal Quality Control: Internal procedural controls are included in the test. A collared line appearing in the control region (C) is an internal positive control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative procedural control. If the test is working properly, the background on the result area should be white to black and not interfere with the ability to read the read result.

External Quality Control: Controls are not included in this kit. However, in compliance with Good Laboratory Practice (GLP) positive/negative controls are recommended.[1]

LIMITATIONS

1. One Step COVID-19 Antigen Test provides only a preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Virus nucleic acid test is the preferred confirmatory method.
2. One Step COVID-19 Antigen Test is a qualitative screening assay and cannot determine either the concentration of the antigen in the saliva or the level of the virus.
3. It is possible that technical or procedural errors, as well as other interfering substances in the saliva specimen may cause erroneous results.
4. Adulterants, such as bleach and/or alum, in saliva specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another saliva specimen.
5. A Positive Result indicates presence of the antigen but does not indicate level of the virus in saliva.
6. A Negative Result may not necessarily indicate virus-free saliva. Negative results can be obtained when virus is present but below the cutoff level of the Test.



EXPECTED VALUES

One Step COVID-19 Antigen Test has been compared with a leading commercial RT-PCR test. The correlation between these two systems is no less than 95%.

PERFORANCE CHARACTERISTICS

Sensitivity, Specificity and Accuracy

One Step COVID-19 Antigen Test has been evaluated with specimens obtained from the patients. RT-PCR is used as the reference method for the One Step COVID-19 Antigen Test. Specimens were considered positive if RT-PCR indicated a positive result. Specimens were considered negative if RT-PCR indicated a negative result.

One Step COVID-19 Antigen Test	Reference RT-PCR Assay		
	Positive	Negative	Total
Positive	186	0	186
Negative	14	56	70
Total	200	56	256
Positive Agreement: 186/200	93%(95%CI:83%-95%)		
Negative Agreement: 56/56	100%(95%CI:93%-100%)		

Limit of Detection (Analytical Sensitivity)

One Step COVID-19 Antigen Test limit of detection (LOD) was determined by evaluating different concentrations of heat inactivated SARS-CoV-2 virus. Presumed negative natural saliva specimens were eluted in PBS. Saliva were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Inactivated SARS-CoV-2 virus was diluted in this natural saliva matrix pool to generate virus dilutions for testing. Contrived saliva samples were prepared by absorbing 20 microliters of each virus dilution onto the saliva. The contrived saliva samples were tested according to the test procedure. The LOD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The One Step COVID-19 Antigen Test LOD in natural saliva matrix was confirmed as $1.25x10^2 TCID_{50}$/ml.

Limit of Detection (LoD) Study Results

Concentration $TCID_{50}$/ml	Number Positive/Total	% Detected
$1.25x10^2$	20/20	100

Cross Reactivity (Analytical Specificity) and Microbial Interference

The following organisms were tested at $1.0X10^8$ ORG/ml and all found to be negative when tested with the One Step COVID-19 Antigen Test:

Human coronavirus 229E	Haemophilus influenzae
Human coronavirus OC43	Streptococcus pneumoniae
Human coronavirus NL63	Bordetella pertussis
Adenovirus	Mycoplasma pneumoniae
Human Metapneumovirus (hMPV)	Chlamydia pneumoniae
Parainfluenza virus 1-4	Legionella pneumophila
Influenza A & B	Mycobacterium tuberculosis
Enterovirus	Candida albicans
Respiratory syncytial virus	MERS-coronavirus
Rhinovirus	Human coronavirus HKU1

High Dose Hook Effect

No high dose hook effect was observed when tested with the concentrations of 1.0-100 ug/ml of N and S protein solutions with the One Step COVID-19 Antigen Test.

Endogenous Interfering Substances

The following substances were evaluated with the One Step COVID-19 Antigen Test at the concentrations listed below and were found not to affect test performance.

Mucin	Rheumatoid Factor (RF)
Bilirubin	Antinuclear Antibodies (ANA)
Hemoglobin	Lipoproteins

BIBLIOGRAPHY

1. Westagard JO, Barry PL, Hunt MR, Groth T. A multi-rule Shewhart for quality control in clinical chemistry, Clinical Chemistry 1981;27:493-501
2.Siliva Chiappin, Giorgia Antoneli, et al. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clinica Chimica Acta 383(2007)30-40
3.Jose J.Ceron, Elsa Lamy, et al. Use of Saliva for Diagnosis and Monitoring the SARS-COV-2: A general Perspective. J.Clin.Med,2020.9.1491

ORDERING AND CONTACT INFORMATION

Ningbo Beautiful Life Medical Biotechnology Development Co, Ltd.
Address: Room 305, Building 4, NO.7 Chuangye Avenue, Free Trade West Zone Ningbo, China
Website: www.nbmlrs.com
E-Mail: marketing@shmlrs.cn
Tel: 86-574- 86818897

Distributor in Europe:
LINUS Handelshaus GmbH
Hellmühler Weg 13
D - 16359 Biesenthal
linushandelshaus@web.de

European Representative:
Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd
2595AA, Den Haag, Netherlands

Index of Symbol