COVID-19 Antigen Saliva Test Kit
COV-S35Pen

1. INTENDED USE

The COVID-19 Antigen Saliva Test Kit is an in vitro immunoassay. The kit is for the direct and qualitative detection of SARS-CoV-2 viral nucleoprotein antigens from saliva samples collected from individuals suspected of COVID-19. The kit is in aid of diagnosis of COVID-19.

The COVID-19 Antigen Saliva Test Kit is intended for use by trained healthcare professionals. For laboratory and point of care use. This assay is not intended for home testing (or self-testing).

Results are for the identification of SARS-CoV-2 viral nucleoprotein antigen. Antigen is generally detectable in saliva samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all positive results to the appropriate public health authority.

Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary for patient management.

2. PRINCIPLE

The COVID-19 Antigen Saliva Test Kit 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 the specimen.

During testing, target antigens, if present in the saliva samples, will be released into the extraction buffer individually packed in the kit. Consequently, the extracted antigens will 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, generally indicating that the proper volume of specimen has been added and membrane wicking is working.

3. MATERIALS

Materials Provided

- 20 Individual packaged test
- 1 Package insert

Materials Required but Not Provided

- Clock, timer, or stopwatch

4. PRECAUTIONS

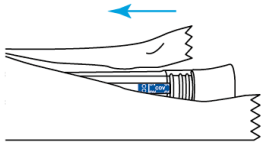
- For In Vitro Diagnostic Use Only.
- Each device is for single use only and cannot be reused
- DO NOT eat, drink, smoke, brush teeth, or chew gum for 30 minutes before collecting saliva.
- Caution should be taken when inserting sponge into the mouth in case of choking.
- DO NOT ingest.
- Read the Package 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 kit when any component including test device, protector, extraction buffer, package insert is missing.
- 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, or may lead to false positive results. Inaccurate or inappropriate specimen collection, storage, and transport may also yield false test results.
- The buffer components include salts and surfactants, the preservative is sodium azide and water is the solvent. Avoid skin or eyes 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.
- There may be false negatives due to new unknown variants of SARS-Cov-2 prior to validation data available

5.STORAGE AND STABILITY

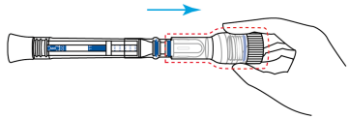
- Store the COVID-19 Antigen Saliva Test Kit at 2~30℃when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

6. TEST PROCEDURE

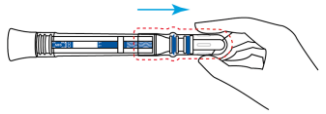
1. Bring devices, reagents and specimens and/or controls to room temperature (15~30℃) before use.
2. Remove the test device from its packing. Label the device with the patient's identification. For the best results, the assay should be performed within two hours.



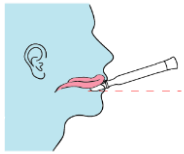
3. Take the test device out of the tube with extraction buffer.



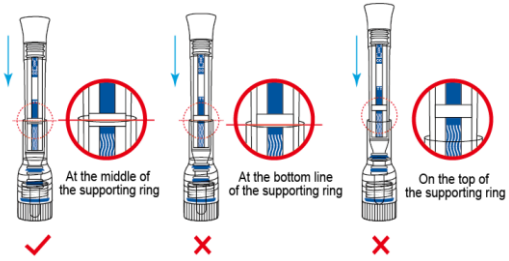
4. Remove the protector.

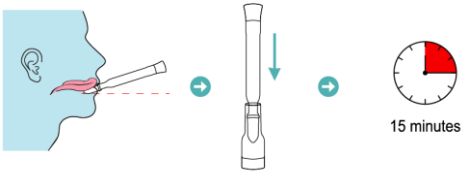


5. Place the saliva collector (the circle part in the picture) into the mouth, near the sublingual gland. And keep the top of the device pointing up, to be an angle to the horizontal line, hold for 2 minutes.



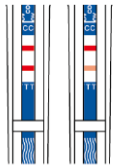
6. Take out the saliva collector of the mouth.
7. Place the test device vertically into the extraction tube until the edge of the extraction tube reach the middle of the supporting ring.



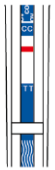


8. Read the results at 15 minutes.

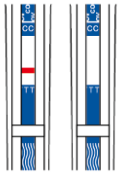
7.RESULT INTERPRETATION



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. 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.

8. QUALITY CONTROL

Internal Procedural Controls

The COVID-19 Antigen Saliva Test Kit 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.

9.LIMITATIONS OF THE TEST

1. The COVID-19 Antigen Saliva Test Kit is for professional in vitro diagnostic use and should only be used for the qualitative detection of the 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 Saliva Test Kit.
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.

10.PERFORMANCE

Analytical Sensitivity (Limit of Detection):

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at $1.25\times10^{1.4}TCID_{50}$/mL.

The limit of detection was also determined with recombinant SARS-CoV-2 nucleoprotein and has been evaluated at 37 pg/mL.

Clinical Evaluation:

The performance of the COVID-19 Antigen Saliva Test Kit was established with 184 specimens collected and enrolled from individual symptomatic patients who were suspected of COVID-19. The specimens were tested fresh by minimally trained operators, and FDA EUA RT-PCR assay for the detection of SARS-CoV-2 was utilized as the comparator method for the study. The results were summarized below:

Table: COVID-19 Antigen Saliva Test Kit vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
COVID-19 Antigen Saliva Test Kit	Positive	40	2	42
	Negative	5	137	142
Total		45	139	184

Relative Sensitivity: 88.9% (76.5% ~ 95.2%)*

Relative Specificity: 98.6% (94.9% ~ 99.6%)*

Overall Agreement: 96.2% (92.4% ~ 98.1%)*

*95% Confidence Interval

The table below shows the positive results broken down by days since symptom onset:

Days Since Symptom Onset	Cumulative Specimens Tested	Cumulative RT-PCR Positive (+)	Cumulative COVID-19 Antigen Rapid Test Device Positive(+)	PPA	95%CI
1	23	5	5	100.0%	56.6%-100.0%
2	50	17	17	100.0%	81.6%-100.0%
3	78	24	24	100.0%	86.2%-100.0%
4	107	32	32	100.0%	89.3%-100.0%
5	123	39	37	94.9%	83.1%-98.6%
6	133	39	37	94.9%	83.1%-98.6%
7	147	39	37	94.9%	83.1%-98.6%
8	170	41	38	92.7%	80.6%-97.5%
9	177	45	40	88.9%	76.5%-95.2%
10	179	45	40	88.9%	76.5%-95.2%
11	181	45	40	88.9%	76.5%-95.2%
12	182	45	40	88.9%	76.5%-95.2%
13	184	45	40	88.9%	76.5%-95.2%

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 Saliva Test Kit.

Adenovirus 1	MERS-coronavirus
Adenovirus 2	SARS-coronavirus
Adenovirus 3	Human metapneumovirus
Adenovirus 4	Influenza A (H1N1)pdm09
Adenovirus 5	Influenza A (H3N2)
Adenovirus 7	Influenza B Victoria lineage
Adenovirus 55	Influenza B Yamagata lineage
Epstein-Barr virus	Norovirus
Enterovirus EV70	Parainfluenza virus 1
Enterovirus EV71	Parainfluenza virus 2
Enterovirus A16	Parainfluenza virus 3
Enterovirus A24	Parainfluenza virus 4
Enterovirus B1	Respiratory syncytial virus A
Echovirus 6	Respiratory syncytial virus B
HCoV-229E	Rhinovirus A30
HCoV-OC43	Rhinovirus B52
HCoV-NL63	*Mycoplasma pneumoniae*
Bordetella parapertussis	*Mycobacterium tuberculosis*
Bordetella pertussis	*Staphylococcus aureus*
Candida albicans	*Staphylococcus epidermidis*
Chlamydia pneumoniae	*Streptococcus agalactiae*
Group C Streptococcus	*Streptococcus pneumoniae*
Haemophilus influenzae	*Streptococcus pyogenes*
Legionella pneumophila	

In silico analysis:

For Human Coronavirus HKU1, homology exists between the SRAS-COV-2 nucleocapsid protein and Human Coronavirus HKU1. Blast results showed 36 sequence IDs, mostly nucleocapsid protein showing homolody. Sequence ID AXT92485.1 had the highest alignment scores and was found to be 36.7% homologous across 82% of the sequence. This is relatively low but cross-reactivity cannot be fully ruled out.

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 the test performance of the COVID-19 Antigen Saliva Test Kit.

Substance	Con.	Substance	Con.
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	25 ug/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 ug/ml	Adamantanamine	500 ng/ml
Dextromethorphan	10 ug/ml	Oseltamivir phosphate	10 mg/ml
Diphenhydramine	5 mg/ml	Tobramycin	10 mg/ml
Doxylamine	1 mg/ml	Triamcinolone	14 mg/ml
Flunisolide	25 ug/ml	Whole blood	4%

Precision:

The COVID-19 Antigen Saliva Test Kit demonstrates the expected test repeatability and reproducibility with three different lots at three different sites in 5 days by three difference operators.

Hook effect:

The study demonstrated that no false negatives occurred on virus level at $1\times10^{6.4}$ $TCID_{50}$/mL and recombinant SARS-CoV-2 nucleocapsid protein level at 1.48mg/mL.

11.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).

12. 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		

IVD

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