COVID-19
Antigen Saliva Test Kit

COV-P35

latest revision: April 2021

1. Intended use

The COVID-19 Antigen Saliva Test Kit is an in vitro immunoassay. The assay is for the direct and qualitative detection of SARS-CoV-2 viral nucleoprotein antigens from saliva samples. This test is intended for professional use only.

2. Principle

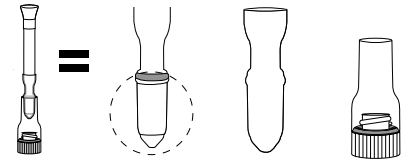
The COVID-19 Antigen Saliva Test Kit detects SARS-CoV-2 viral antigens through visual interpretation of color development. Anti-SARS-CoV-2 antibodies are immobilized on the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to colored particles are immobilized on the conjugated 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



Test Kit | Saliva Collector | Protection Cap | Tube with buffer solution

- Instructions for use

Materials Required but Not Provided

- Clock, timer, or stopwatch

4. Precautions

- For in vitro Diagnostic Use Only.
- 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 Instructions for use, 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 saliva collector, protection cap, tube with buffer solution, instructions for use 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.
- 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.

5. Storage and stability

- Store the COVID-19 Antigen Saliva Test Kit at 2 ~ 30°C 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

Bring testkits, reagents and specimens and/or controls to room temperature (2 ~ 30°C) before use.

1 Remove the testkit from its packing. Label the device with the patient's identification. For the best results, the assay should be performed within two hours.

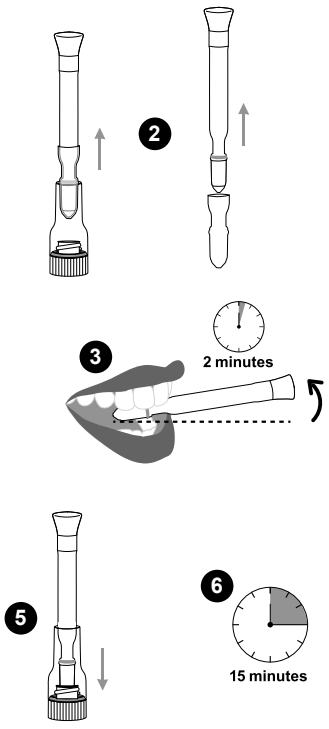
2 Take the testkit out of the tube with extraction buffer. Remove the protection cap.

3 Place the saliva collector(the circle part in the picture) at the back of the mouth. Keep the top of the device pointing up, to be in an angle to the horizontal line. Hold for 2 minutes.

4 Take the saliva collector out of the mouth.

5 Place the saliva collector vertically into the tube with extraction buffer.

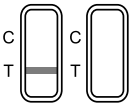
6 Read the results at 15 minutes.



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:

- The color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.
- 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.

EXTERNAL POSITIVE AND NEGATIVE CONTROLS

Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

9. Limitations of the test

- 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."
- Both viable and nonviable SARS-CoV-2 viruses are detectable with The COVID-19 Antigen Saliva Test Kit.
- 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.
- Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
- 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.
- Negative results do not preclude SARS-CoV-2 infection and should be confirmed via molecular assay.

10. Performance characteristics

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)

The limit of detection was determined with a quantified SARS-CoV-2 virus and has been evaluated at 1.25x101.4TCID50/mL.

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

CLINICAL EVALUATION

SITE 1

The study was performed on 66 negative saliva specimens and 20 positive saliva specimens, all confirmed by RT-PCR (Roche Diagnostics Cobas 6800 System) have been tested in the assays. The result is listed below.

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

		RT-PCR		
		Positive	*Negative*	*Total*
COVID-19 Antigen Saliva Test Kit	*Positive*	*18*	*2*	*20*
	Negative	*2*	*64*	*66*
	Total	*20*	*66*	*86*

*Relative Sensitivity 90.0 % (69.9% ~ 97.2%)**
*Relative Specificity: 97.0 % (89.6% ~ 99.2%)**
*Overall Agreement: 95.3 % (88.6% ~ 98.2%)**
**95% Confidence Interval*

SITE 2

443 evaluation samples were collected in this study, 193 positive samples (confirmed by RT-PCR (Roche LightCycler instrument 480-II) with Ct values separated at <31, <28 and <25 were tested. And 250 negative samples(confirmed by RT-PCR (Roche LightCycler instrument 480-II). All the samples were tested by COVID-19 Antigen Saliva Test Kit. The result is listed below.

Table: COVID-19 Antigen Saliva Test Kit vs. RT-PCR (Ct value <31)

		RT-PCR		
		Positive	*Negative*	*Total*
COVID-19 Antigen Saliva Test Kit	*Positive*	*166*	*0*	*166*
	Negative	*27*	*250*	*277*
	Total	*193*	*250*	*443*

*Relative Sensitivity: 86.0 % (80.3% ~ 90.6%)**
*Relative Specificity: 100.0 % (98.5%-100.0%)**
*Overall Agreement: 93.9 % (91.3% ~ 96.0%)**
**95% Confidence Interval*

Table: COVID-19 Antigen Saliva Test Kit vs. RT-PCR (Ct value <28)

		RT-PCR		
		Positive	*Negative*	*Total*
COVID-19 Antigen Saliva Test Kit	*Positive*	*166*	*0*	*166*
	Negative	*27*	*250*	*277*
	Total	*193*	*250*	*443*

*Relative Sensitivity: 94.2 % (89.6% ~ 97.2%)**
*Relative Specificity: 100.0 % (98.5%-100.0%)**
*Overall Agreement: 97.6 % (95.7% ~ 98.7%)**
**95% Confidence Interval*

Table: COVID-19 Antigen Saliva Test Kit vs. RT-PCR (Ct value <25)

		RT-PCR		
		Positive	*Negative*	*Total*
COVID-19 Antigen Saliva Test Kit	*Positive*	*116*	*0*	*166*
	Negative	*2*	*250*	*252*
	Total	*118*	*250*	*368*

*Relative Sensitivity: 98.3 % (94.1% ~ 99.8%)**
*Relative Specificity: 100.0 % (98.5%-100.0%)**
*Overall Agreement: 99.5 % (98.0% ~ 99.9%)**
**95% Confidence Interval*

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.

Microorganismus	***Target Concentration***
Adenovirus 1	*1.0×10^5 TCID50/mL*
Adenovirus 2	*1.0×10^5 TCID50/mL*
Adenovirus 3	*1.0×10^5 TCID50/mL*
Adenovirus 4	*1.0×10^5 TCID50/mL*
Adenovirus 5	*1.0×10^5 TCID50/mL*
Adenovirus 52	*1.0×10^5 TCID50/mL*
Epstein-Barr virus	*5.0×10^5 copies/mL*
Enterovirus EV70	*1.0×10^5 TCID50/mL*
Enterovirus EV71	*1.0×10^5 TCID50/mL*
Enterovirus A16	*1.4×10^5 TCID50/mL*
Enterovirus A24	*1.0×10^5 TCID50/mL*
Enterovirus B1	*1.1×10^5 TCID50/mL*
Echovirus 6	*1.0×10^5 TCID50/mL*
HCoV-229E	*1.0×10^5 TCID50/mL*
HCoV-OC43	*1.0×10^5 TCID50/mL*
HCoV-NL63	*1.0×10^5 TCID50/mL*
MERS-coronavirus	*1.0×10^5 copies/mL*
SARS-coronavirus	*1.0×10^5 copies/mL*
Humanmetapneumovirus	*1.0×10^5 TCID50/mL*
Influenza A (H1N1)pdm09	*1.0×10^5 TCID50/mL*
Influenza A (H3N2)	*1.0×10^5 TCID50/mL*
Influenza B Victoria lineage	*1.0×10^5 TCID50/mL*
Influenza B Yamagata lineage	*1.0×10^5 TCID50/mL*
Norovirus	*1.0×10^5 TCID50/mL*
Parainfluenza virus 1	*1.0×10^5 TCID50/mL*
Parainfluenza virus 2	*1.0×10^5 TCID50/mL*
Parainfluenza virus 3	*1.0×10^5 TCID50/mL*
Parainfluenza virus 4	*1.0×10^5 TCID50/mL*
Respiratory syncytial virus A	*1.0×10^5 TCID50/mL*
Respiratory syncytial virus B	*1.0×10^5 TCID50/mL*
Rhinovirus A30	*1.0×10^5 TCID50/mL*
Rhinovirus A52	*1.0×10^5 TCID50/mL*
Bordetellaparapertussis	*1.8×10^6 cfu/mL*
Bordetellapertussis	*2.0×10^6 cfu/mL*
Candida albicans	*1.0×10^6 cfu/mL*
Chlamydiapneumoniae	*2.0×10^6 EB/ml*
Group C Streptococcus	*2.0×10^6 cfu/mL*
Haemophilusinfluenzae	*1.0×10^6 cfu/mL*
Legionellapneumophila	*2.0×10^6 cfu/mL*
Mycoplasmapneumoniae	*2.0×10^6 cfu/mL*
Mycobacterium tuberculosis	*6.32×10^6 cfu/mL*
Staphylococcus aureus	*2.0×10^6 cfu/mL*
Staphylococcus epidermidis	*2.0×10^6 cfu/mL*
Streptococcus agalactiae	*2.0×10^6 cfu/mLL*
Streptococcus pneumoniae	*2.0×10^6 cfu/mL*
Streptococcus pyogenes	*2.0×10^6 cfu/mL*

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	***Concentration***
3 OTC nasal sprays	*10%*
3 OTC mouthwashes	*10%*
3 OTC throat drops	*10%*
4-acetamidophenol	*10 mg/mL*
Acetylsalicylic acid	*10 mg/mL*
Albuterol	*10 mg/mL*
Chlorpheniramine	*5 mg/mL*
Dexamethasone	*50 µg/ml*
Dextromethorphan	*10 µg/ml*
Diphenhydramine	*5 mg/mL*
Doxylamine succinate	*1 mg/mL*
Flunisolide	*25 µg/ml*
Guaiacol glycerol ether	*20 mg/mL*
Mucin	*1%*
Mupirocin	*250 µg/mL*
Oxymetazoline	*25µg/ml*
Phenylephrine	*10 mg/mL*
Phenylpropanolamine	*1 mg/mL*
Zanamivir	*10 mg/mL*
Adamantanamine	*500 ng/mL*
Oseltamivir phosphate	*10 mg/mL*
Tobramycin	*10 mg/mL*
Triamcinolone	*14 mg/mL*

11. Literature references

- Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular evolution of human coronavirus genomes. Trends Microbiol. 25, 35–48 (2017).
- 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	**Catalogue number**
	Temperature limits
	Read instructions for use
LOT	**Batch code**
IVD	**In-vitro diagnostic device**
	Use before
	Production date
	Manufacturer
	Contains <n> tests
	Not for re-use
EC REP	**Authorised representative in the European Community**
CE	**CE marking in accordance with IVD Medical Devices Directive 98/79/EC**