Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva) Package Insert

A RAPID TEST FOR THE QUALITATIVE DETECTION OF NOVEL CORONAVIRUS ANTIGENS IN HUMAN SALIVA.

For professional In Vitro Diagnostic Use Only.

INTENDED USE

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva) is an *in vitro* diagnostic test for the qualitative detection Nucleoprotein and spike glycoprotein of Coronavirus Disease 2019 in human Oropharyngeal saliva, using the rapid immunochromatographic method as an aid in the diagnosis of SARS-Cov-2 infections. The identification is based on the monoclonal antibodies specific for the novel coronavirus antigen. It will provide information for clinical doctors to prescribe correct medications.

SUMMARY

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.

Severe acute respiratory syndrome - coronavirus- 2 (SARS-CoV-2) is an enveloped, non-segmented Positive sense rna virus. It is the cause of the Coronavirus-0 disease (COVID-19) common to humans is contagious. SARS-CoV-2 has several structural proteins, including spike (S), envelope (E), membrane (M) and nucleocapsid(N).

At present, there are many variants of the Novel coronavirus (SARS-CoV-2), and the N501Y mutation and its approximate variants have attracted attention because their mutation position is located in the spike glycoprotein's receptor-binding domain of the virus, thereby changing the virus infected efficiency. In silico analysis demonstrated that the N501Y mutation did not alter the primary and tertiary protein structure of the spike protein RBD domain. Therefore, its antigenicity remains unchanged.

PRINCIPLE

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva) is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to Novel coronavirus.

The test strip is composed of the following three parts, namely sample pad, reagent pad and reaction membrane. The reagent membrane contains the colloidal-gold conjugated with the monoclonal antibodies against Novel coronavirus; the reaction membrane contains the secondary antibodies for Novel coronavirus, and the polyclonal antibodies against the mouse globulin, which are pre-immobilized on the membrane.

When the saliva sample is received by the test, the conjugated solution from the reagent pad gets dissolved and migrates along with the saliva. When the Novel coronavirus is present in the saliva sample, a complex is formed between the anti-Novel coronavirus conjugate and the virus will be caught / detected by the specific anti- Novel coronavirus monoclonal coated on the T region. Whether the sample contains the virus or not, the solution continues to migrate to encounter another reagent (an anti-mouse IgG antibody) that binds the remaining conjugates, thereby producing a red line on the region C.

The Novel Coronavirus (SARS-Cov-2) rapid antigen test (saliva) can detect both the SARS-Cov-2 nucleoprotein as well as the SARS-Cov-2 spike protein. By ELISA, we determined that the antibody we use binds to amino acids 511-531 of the SARS Cov-2 spike protein.

The detectability of genetic SARS-CoV-2 variants was tested by examining the sensitivity toward recombinant SARS-Cov-2 spike proteins (319 to 541aa). In these tests, the Novel Coronavirus (SARS-Cov-2) antigen-rapid test achieved the same values when detecting the B.1.1.7 (UK) and B.1.351 (SA) variants as when detecting the standard variant.

REAGENTS

The reagent membrane contains the colloidal-gold conjugated with the monoclonal antibodies against Novel coronavirus; the reaction membrane contains the secondary antibodies for Novel coronavirus, and the polyclonal antibodies against the mouse globulin, which are pre-immobilized on the membrane.

PRECAUTIONS

- For *in vitro* diagnostic use only.
- Do not use after the expiration date.
- Ensure foil pouch containing test device is not damaged before opening for use.
- Perform test at room temperature 15 to 30°C.
- Wear gloves when hanging the samples, avoid touching the reagent membrane and sample window.
- All samples and used accessories should be treated as infectious and discarded according to local regulations.
- Avoid using bloody samples.

STORAGE AND STABILITY

Store the Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva) at room temperature or refrigerated (2-30°C). Do not freeze. All reagents are stable until the expiration dates marked on their outer packaging and buffer vial.

SPECIMEN COLLECTION AND PREPARATION

1. Specimen collection:

The oral fluid specimen should be collected using the collection tools provided with the kit. Follow the detailed Directions for Use below. No other collection tools should be used with this assay. Oral fluid collected at any time of the day may be used.

2. Specimen preparation:

When the saliva is collected, follow the direction to prepare the specimen with buffer provided with the kit.

MATERIALS

Materials provided

- Test device
- Dropper
- Extraction buffer
- Package Insert
- Nozzle
- Extraction tube
- Tube stand*
- Saliva collection cup/bag
- Plastic bag

*The 20-test package contains the tube stand，the 1-test and 5-test package use the test box itself as tube stand.

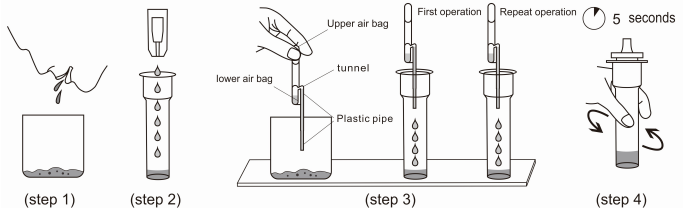
Materials required but not provided

- Timer

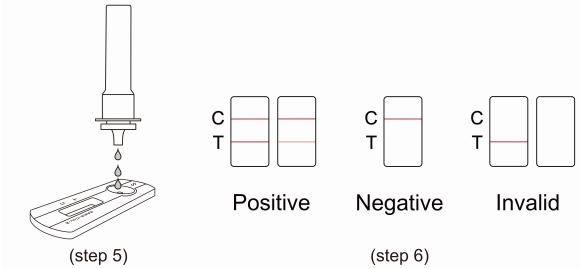
DIRECTIONS FOR USE

Allow the test device, specimen, extraction buffer to equilibrate to room temperature (15-30°C) prior to testing. Do not place anything in the mouth including food, drink, gum, tobacco, water and mouthwash products for at least 10 minutes prior to collection of oral fluid specimen.

1. Spit enough saliva into the saliva collect cup/bag.
2. Take out an extraction tube and a bottle of extraction buffer, remove the extraction buffer bottle cap, add all the extraction buffer into the extraction tube.
3. Draw enough saliva from the cup with the dropper, make sure the liquid level does not exceed the tunnel between the lower air bag and the plastic pipe, transfer all saliva fulling in the plastic pipe into the extraction tube first time. Repeat operation above to add another dropper of saliva into extraction tube.
4. Take out a nozzle and close into the extraction tube, gently shake the extraction tube vertically for about 5 seconds to allow saliva mix well with extraction buffer. Fold the used cup/bag in half and discard it into the plastic bag as medical waste in accordance with local regulations.



5. Remove the test device from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed immediately after opening the foil pouch. Put the test device on a clean and flat surface. Transfer 3 drops of sample into the sample well of test device vertically, start the timer.
6. Read the result at 10~20 minutes. Don't interpret the result after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE: Two red lines appear. One red line appears in the control region(C), and one red line in the test region(T). The shade of color may vary，but it should be considered positive whenever there is even a faint line.

NEGATIVE: Only one red line appears in the control region(C), and no line in the test region(T). The negative result indicates that there are no Novel coronavirus particles in the sample or the number of viral particles is below the detectable range.

INVALID: No red line appears in the control region(C). The test is invalid even if there is a line on test region(T). Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the test procedure and repeat the test using a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

LIMITATIONS

- The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva) is an acute-phase screening test for qualitative detection. Sample collected may contain antigen concentration below the reagent's sensitivity threshold, so a negative test result does not exclude infection with novel coronavirus.
- The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva) detects viable and non-viable novel coronavirus antigen. Test performance depends on antigen load in the sample and may not correlate with cell culture performed on the same sample. A positive test does not rule out the possibility that other pathogens may be present, therefore, the results must be compared with all other available clinical and laboratory information to make an accurate diagnosis.
- A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if poor quality specimen is obtained.
- Performance of the test has not been established for monitoring antiviral treatment of novel coronavirus.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results are not intended to rule in other coronavirus infection except the SARS-Cov-2.
- Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children List.
- The concentration of virus in saliva is greatly affected by factors such as meals, diet, smoking, breath fresheners, etc. Therefore, please strictly follow this manual before collecting samples

A negative result may occur if the concentration of antigen in a specimen is below the detection limit of the test or if the specimen was collected or transported improperly, therefore a negative test result does not eliminate the possibility of SARS-Cov-2 infection, and should be confirmed by viral culture or PCR.

PERFORMANCE CHARACTERISTICS

Clinical Evaluation

Clinical evaluation was performed to compare the results obtained by Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva) and PCR test result.

The performance has been determined using 405 Oropharyngeal saliva from individual symptomatic patients with Suspected COVID-19. Oropharyngeal saliva was made refer to the instructions for use of the specimen collection and preparation. All samples were selected and then tested sequentially in a blinded fashion. The performance of the test device was matched with the results of a compared to commercial RT-PCR reagent. The test device showed a sensitivity of 92.9% and a specificity of 99.58%. The results were summarized below:

Table: Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva) vs. PCR

Method		2019-nCoV Nucleic Acid Test Kit (RT-PCR)		Total Results
The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test device (saliva)	Results	Positive	Negative	
	Positive	157	1	158
	Negative	12	235	247
Total Results		169	236	405

Clinical sensitivity = 157/169= 92.9 % (95%CI*:87.89% to 96.00%)
Clinical specificity =235/236=99.58% (95%CI*:97.39% to >99.99%)
Accuracy: (157+235)/ (157+1+12+235) *100%=96.79% (95%CI* 94.53% to 98.17%)
*Confidence Interval

Limit of Detection (LOD)

2019-nCoV Strain Tested	Realy Tech product				
Stock 2019-nCoV Concentration	1 X 10^5 $TCID_{50}$/mL				
Dilution	1/100	1/200	1/400	1/800	1/1600
Concentration in Dilution tested ($TCID_{50}$/ml)	1X10^3	5X10^2	2.5X 10^2	1.25X10^2	62.5
Call rates of 20 replicates near cut-off	100(20/20)	100(20/20)	100(20/20)	95(19/20)	10(2/20)
Limit of detection (LOD) per Virus Strain	1.25 X 10^2 $TCID_{50}$/mL				

Cross Reaction

The Cross reaction study conducted By using The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva) to test sample in which add each concentration of pathogens listed in the following table to the specimens prepared negative samples and 3XLOD positive samples. The results show that the pathogenic listed in the following table have no cross-reaction that would create false positive or negative results for SARS-Cov-2 antigen.

Virus/Bacteria/Parasite	Strain	Concentration
MERS-coronavirus	N/A	72μg/mL
Adenovirus	Type 1	1.5 x $10^6$$TCID_{50}$/mL
	Type 3	7.5 x $10^6$$TCID_{50}$/mL
	Type 5	4.5 x $10^6$$TCID_{50}$/mL
	Type 7	1.0 x $10^6$$TCID_{50}$/mL
	Type 8	1.0 x $10^6$$TCID_{50}$/mL
	Type 11	2.5 x $10^6$$TCID_{50}$/mL
	Type 18	2.5 x $10^6$$TCID_{50}$/mL
	Type 23	6.0 x $10^6$$TCID_{50}$/mL
	Type 55	1.5 x $10^6$$TCID_{50}$/mL
Influenza A	H1N1 Denver	3.0 x $10^8$$TCID_{50}$/mL
	H1N1 WS/33	2.0 x $10^8$$TCID_{50}$/mL
	H1N1 A/Mal/302/54	1.5 x $10^8$$TCID_{50}$/mL
	H1N1 New Caledonia	7.6 x $10^8$$TCID_{50}$/mL
	H3N2 A/Hong Kong/8/68	4.6 x $10^8$$TCID_{50}$/mL
Influenza B	Nevada/03/2011	1.5 x $10^8$$TCID_{50}$/mL
	B/Lee/40	8.5 x $10^8$$TCID_{50}$/mL
	B/Taiwan/2/62	4.0 x $10^8$$TCID_{50}$/mL

Respiratory syncytial virus	N/A	2.5 x 10^6TCID$_{50}$/mL
Legionella pneumophila	Bloomington-2	1 x 10^5 PFU/mL
	Los Angeles-1	1 x 10^5 PFU/mL
	82A3105	1 x 10^5 PFU/mL
Rhinovirus A16	N/A	1.5 x 10^6TCID$_{50}$/mL
Mycobacterium tuberculosis	K	1 x 10^5PFU/mL
	Erdman	1 x 10^5PFU/mL
	HN878	1 x 10^5PFU/mL
	CDC1551	1 x 10^5PFU/mL
	H37Rv	1 x 10^5PFU/mL
Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	1 x 10^5PFU/mL
	178 [Poland 23F-16]	1 x 10^5PFU/mL
	262 [CIP 104340]	1 x 10^5PFU/mL
	Slovakia 14-10 [29055]	1 x 10^5PFU/mL
Streptococcus pyrogens	Typing strain T1 [NCIB 11841, SF 130]	1 x 10^5PFU/ml
Mycoplasma pneumoniae	Mutant 22	1 x 10^5PFU/ml
	FHstrainofEatonAgent [NCTC 10119]	1 x 10^5PFU/ml
	36M129-B7	1 x 10^5PFU/ml
Coronavirus	229E	1.5 x10^6TCID$_{50}$/mL
	OC43	1.5 x10^6TCID$_{50}$/mL
	NL63	1.5 x10^6TCID$_{50}$/mL
	HKU1	1.5 x10^6TCID$_{50}$/mL
Human etapneumovirus (hMPV) 3 Type B1	Peru2-2002	1.5 x10^6TCID$_{50}$/mL
Human Metapneumovirus (hMPV) 16 Type A1	IA10-2003	1.5 x10^6TCID$_{50}$/mL
Parainfluenza virus	Type 1	1.5 x10^6TCID$_{50}$/mL
	Type 2	1.5 x10^6TCID$_{50}$/mL
	Type 3	1.5 x10^6TCID$_{50}$/mL
	Type 4A	1.5 x10^6TCID$_{50}$/mL

Interfering Substances Reaction

When tested using the Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva), there was no interference between the device reagents and the Potential interference substances listed in below table that would create false positive or negative results for SARS-Cov-2 antigen.

Substance	Concentration	Substance	Concentration
Mucin	100μg/mL	Acetylsalicylic acid	3.0 mM
Whole Blood	5% (v/v)	Ibuprofen	2.5 mM
Biotin	100μg/mL	Mupirocin	10 mg/mL
Neo-Synephrine (Phenylephrine)	5%(v/v)	Tobramycin	10μg/mL
Afrin Nasal Spray (Oxymetazoline)	5%(v/v)	Erythromycin	50uM
Saline Nasal Spray	5%(v/v)	Ciprofloxacin	50uM
Homeopathic	5%(v/v)	Ceftriaxone	110mg/mL
Sodium Cromoglycate	10 mg/mL	Meropenem	3.7μg/mL
Olopatadine Hydrochloride	10 mg/mL	Tobramycin	100μg/mL
Zanamivir	5 mg/mL	Histamine Hydrochloride	100μg/mL
Oseltamivir	10 mg/mL	Peramivir	1mmol/mL
Artemether-lumefantrine	50uM	Flunisolide	100μg/mL
Doxycycline hyclate	50uM	Budesonide	0.64nmol/ L
Quinine	150uM	Fluticasone	0.3ng/mL
Lamivudine	1 mg/mL	Lopinavir	6μg/mL
Ribavirin	1 mg/mL	Ritonavir	8.2mg/mL
Daclatasvir	1 mg/mL	Abidor	417.8ng/mL
Acetaminophen	150uM	Pooled human mouth wash	N/A

Dose Hook Effect

Test SARS-CoV-2 wild strain culture medium (concentration 1X10^7 TCID$_{50}$/ml) and multiple dilution sample with The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Device (saliva), no hook effect was found.

SYMBOL

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer	EC REP	Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse		Consult instruction for use
LOT	Batch code	CE	Meet the requirements of EC Directive 98/79/EC

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