Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (swab) Package Insert

A RAPID TEST FOR THE QUALITATIVE DETECTION OF NOVEL CORONAVIRUS ANTIGENS IN NASOPHARYNGEAL SWAB.

For professional In Vitro Diagnostic Use Only.

INTENDED USE

The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (swab) is an *in vitro* diagnostic test for the qualitative detection of novel coronavirus antigens in Nasopharyngeal swab, using the rapid immunochromatographic method. 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 (swab) 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 sample is received by the test, the conjugated solution from the reagent pad gets dissolved and migrates along with the nasal sample. When the Novel coronavirus is present in the 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.

Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (swab) 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 taking 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 Cassette (swab) 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.

The collected clinical nasopharyngeal swab should be used immediately. If the test cannot be performed immediately, the swab can be stored in a clean airtight container, and can be stored in a normal temperature environment(15 to 25℃) for 2 hours, and at the same time stably stored for 24 hours under refrigerated environment.

SPECIMEN COLLECTION AND PREPARATION

1. Specimen collection:

It is applicable to the diagnosis of the Novel coronavirus from the samples of Nasopharyngeal swab. Using freshly collected samples for optimal test performance. Inadequate sample collection or improper sample handling may yield a false-negative result.

Completely insert the sterilized swab supplied in this kit into the nasal basin, and swab several times to collect the epidermal cells of the mucus.

2. Specimen preparation:

1) Take out 1 bottle of Sample Extraction Buffer, remove the bottle cap, add all the extraction buffer into the extraction tube.

2) Insert the swab into the extraction tube which contains Sample Extraction Buffer. Rotate the swab inside the tube using a circular motion to roll the side of the extraction tube so that liquid is expressed and reabsorbed from the swab, remove the swab. The extracted solution will be used as test sample.

MATERIALS

Materials provided

- Test Device
- Sterilized Swab
- Extraction Tube
- Package Insert
- Nozzle with filter
- Sample Extraction Buffer
- Tube Stand*
- Negative control(swab)
- Positive control(swab)

*The 25-test package contains the tube stand，the 5-test package use the test box itself as tube stand.

Materials required but not provided

- Timer

DIRECTIONS FOR USE

Allow the test, specimen, extraction buffer to equilibrate to room temperature (15-30°C) prior to testing.

For clinical specimen preparation

1. **C**ompletely insert the sterilized swab supplied in this kit into the nasal basin, and swab several times to collect the epidermal cells of the mucus.
2. Unscrew the whole cap of the specimen collection tube, take out 1 bottle of Sample Extraction Buffer, remove the bottle cap, add all the extraction buffer into the extraction tube.
3. Place the sterilized swab specimen in the sample extraction buffer. Rotate the swab for approximately 10 seconds while pressing the head against the inside of the tube to release the antigen in the swab.
4. Remove the sterilized swab while squeezing the sterilized swab head against the inside of Buffer as you remove it to expel as much liquid as possible from the swab. Discard the sterilized swab in accordance with your biohazard waste disposal protocol.
5. Screw on and tighten the cap onto the specimen collection tube, then **shake the specimen collection tube vigorously** to mix the specimen and the sample extraction buffer. See illustration 4.

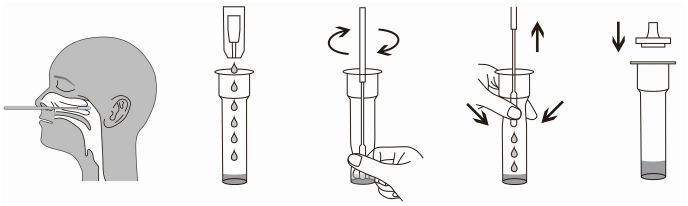
For control swab preparation

1 Unscrew the whole cap of the specimen collection tube, take out 1 bottle of Sample Extraction Buffer, remove the bottle cap, add all the extraction buffer into the extraction tube.

2 Place the control swab in the sample extraction buffer. Rotate the swab for approximately 10 seconds while pressing the head against the inside of the tube to release the antigen in the swab.

3 Remove the control swab.

4 Screw on and tighten the cap onto the specimen collection tube, then **shake the specimen collection tube vigorously** to mix the specimen and the sample extraction buffer. See illustration.

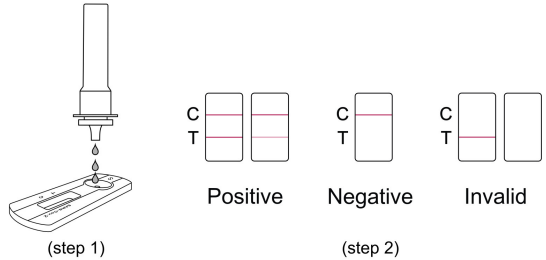


(step 1) (step 2) (step 3) (step 4) (step 5)

When the sample is ready, take the following procedures to complete the test:

1, 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.

2, Transfer 3 drops of sample into the sample well of test device vertically, start the timer, read the result at 10~20 minutes. Don't interpret the result after 20 minutes.



(step 1) (step 2)

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 Cassette (swab) 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 Cassette (swab) 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.
- 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.

QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control.

Positive Control and Negative control swab are supplied with this kit; it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance. Please refer to specimen collection and preparation to prepare the control swab and test according to direction for use, when test positive control, the result should be positive and when test negative control, the test result should be negative.

PERFORMANCE CHARACTERISTICS

Clinical Evaluation

Clinical evaluation was performed to compare the results obtained by Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (swab) and PCR. The results were summarized below:

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

Method		2019-nCoV Nucleic Acid Test Kit (RT-PCR)		Total Results
The Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (Swab)	Results	Positive	Negative	
	Positive	201	0	201
	Negative	8	450	458
Total Results		209	450	659

Clinical sensitivity = 201/209=96.17 % (95%CI* 92.51% to 98.17%)

Clinical specificity = 450/450＞99.9% (95%CI* 98.98% to 100%)

Accuracy: (201+450)/ (201+0+8+450) *100%=98.79% (95%CI* 97.58% to 99.43%)

*Confidence Interval

Clinical sensitivity by days post onset

The clinical sensitivity of the test device stratified by days post onset of symptoms is described as follow:

Days post onset of symptoms	Positive	Negative	Tested number	Detectable rate
0-3	160	0	160	100%
4-7	19	2	21	90.48%
>7	22	6	28	78.57%
Total	201	8	209	/

In conclusion, the patients should test the COVID-19 as soon as possible when he or her has the manifestations include fever, fatigue and dry cough, nasal congestion, runny nose, sore throat, myalgia and diarrhea.

PRECISION

Precision of test Device was established using in-house reference panels containing negative specimens and positive specimens. There were no differences observed inter-batch/within-batch, between-sites, between performers and between-days.

Limit of Detection (LOD)

2019-nCoV Strain Tested	Realy Tech product				
Stock 2019-nCoV Concentration	1 X 10^6 $TCID_{50}$/mL				
Dilution	1/100	1/200	1/400	1/800	1/1600
Concentration in Dilution tested ($TCID_{50}$/ml)	1X10^4	5X10^3	2.5X 10^3	1.25X10^3	6.25X10^2
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 10^3 $TCID_{50}$/mL				

Cross Reaction

The test results are below the corresponding concentration of the substances in the table below, which has no effect on the negative and positive test results of this reagent, and there is no cross-reaction.

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$$TCID_{50}$/mL
Respiratory syncytial virus	N/A	2.5 x $10^6$$TCID_{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 $10^6$$TCID_{50}$/mL
Mycobacterium tuberculosis	K	1 x 10^5PFU/mL
	Erdman	1 x 10^5PFU/mL
	HN878	1 x 10^5PFU/mL
	CDC1551	1 x 10^5PFU/mL
	H37Rv	1 x 10^5PFU/mL
Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	1 x 10^5PFU/mL
	178 [Poland 23F-16]	1 x 10^5PFU/mL
	262 [CIP 104340]	1 x 10^5PFU/mL
	Slovakia 14-10 [29055]	1 x 10^5PFU/mL
Streptococcus pyrogens	Typing strain T1 [NCIB 11841, SF 130]	1 x 10^5PFU/mL
Mycoplasma pneumoniae	Mutant 22	1 x 10^5PFU/mL
	FHstrainofEatonAgent [NCTC10119]	1 x 10^5PFU/mL
	36M129-B7	1 x 10^5PFU/mL
Coronavirus	229E	1.5 x$10^6$$TCID_{50}$/mL
	OC43	1.5 x$10^6$$TCID_{50}$/mL
	NL63	1.5 x $10^6$$TCID_{50}$/mL
	HKU1	1.5 x $10^6$$TCID_{50}$/mL
Human etapneumovirus (hMPV) 3 Type B1	Peru2-2002	1.5 x $10^6$$TCID_{50}$/mL
Human Metapneumovirus (hMPV) 16 Type A1	IA10-2003	1.5 x $10^6$$TCID_{50}$/mL
Parainfluenza virus	Type 1	1.5 x $10^6$$TCID_{50}$/mL
	Type 2	1.5 x $10^6$$TCID_{50}$/mL
	Type 3	1.5 x $10^6$$TCID_{50}$/mL
	Type 4A	1.5 x $10^6$$TCID_{50}$/mL

Interfering Substances Reaction

When tested using the Novel Coronavirus (SARS-Cov-2) Antigen Rapid Test Cassette (swab), 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
Sodium Cromoglycate	10 mg/mL	Ceftriaxone	110mg/mL
Olopatadine Hydrochloride	10 mg/mL	Meropenem	3.7μg/mL
Zanamivir	5 mg/mL	Tobramycin	100μg/mL
Oseltamivir	10 mg/mL	Histamine Hydrochloride	100μg/mL
Artemether-lumefantrine	50uM	Peramivir	1mmol/mL
Doxycycline hyclate	50uM	Flunisolide	100μg/mL
Quinine	150uM	Budesonide	0.64nmol/ L
Lamivudine	1 mg/mL	Fluticasone	0.3ng/mL
Ribavirin	1 mg/mL	Lopinavir	6μg/mL
Daclatasvir	1 mg/mL	Ritonavir	8.2mg/mL
Acetaminophen	150uM	Abidor	417.8ng/mL
Pooled human nasal wash	N/A	/	/

SYMBOLS

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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