RAPID SARS-COV-2 ANTIGEN SCREENING TEST CARD

FOR THE QUALITATIVE ASSESSMENT OF SARS-COV-2 VIRUS ANTIGEN

IN NASOPHARYNGEAL SWAB SPECIMENS

REF 1N40C6

For In Vitro Diagnostic Use Only

INTENDED USE

Rapid SARS-COV-2 Antigen Screening Test Card is an immunochromatography based one step in vitro test. It is designed for the rapid qualitative determination of SARS-COV-2 virus antigen in nasopharyngeal swabs taken by a healthcare professional from members of the public as part of a screening programme.

SUMMARY

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. Viable virus has been isolated between -6 and 20 days from symptom onset (Fontana et al., 2020). The median incubation time for SARS-COV-2 has been calculated as 3 days based on modelling, to 5 to 5.2 days based on clinical research (Di Wu et al., 2020).

Mild, asymptomatic or presymptomatic cases may not seek medical support and may go undiagnosed [1]. The viral load in asymptomatic and symptomatic infections is the same [2].

It has been reported that infectiousness peaks a day before symptom onset, and declines within a week [3 5], with no live virus excreted beyond nine days of illness [6]. Pre-symptomatic cases may excrete virus for up to five to six days before they present symptoms [7 8].

Mathematical models have shown that presymptomatic cases at 46 % or above could maintain the SARS-COV-2 pandemic [9]. Screening of individuals is fundamental to the control of infections in the population or work groups.

PRINCIPLE

Rapid SARS-COV-2 Antigen Screening Test Card is an immunochromatographic lateral flow device that employs the principle of double antibody sandwich method. Colloidal gold conjugated anti-SARS-CoV-2 antibodies are dry-immobilized on the test device. When the specimen is added, it migrates by capillary diffusion through the strip to re-hydrate the gold conjugate complexes. If present at or above the limit of detection, SARS-CoV-2 viral antigens will react with the gold conjugate complexes to form particles, which will continue to migrate along the strip until the Test Zone (T) where they are captured by the immobilized anti-SARS-CoV-2 antibodies to form a visible red line. If there are no SARS-CoV-2 viral antigens in the specimen, no red line will appear in the Test Zone (T). The gold conjugate complexes will continue to migrate alone until being captured by immobilized antibody in the Control Zone (C) to form a red line, which indicates the validity of the test.

MATERIALS PROVIDED

1. Rapid SARS-COV-2 Antigen Screening Test Card
2. Sterilized swab
3. Extraction tube
4. Sample extraction buffer
5. Instructions for use

MATERIALS REQUIRED BUT NOT SUPPLIED

Clock or timer, specimen collection container, biohazard waste container. personal protection equipment.

STORAGE

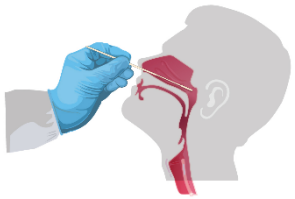
1. Store the test device at 4 ºC to 30 ºC in the original sealed pouch. Do Not Freeze.
2. Kit contents are stable until the expiration date printed on the outer box based on the proper storage conditions.
3. The test device should remain in its original sealed pouch until ready for use. After opening, the test device should be used immediately. Do not reuse the device.

PRECAUTIONS

1. For **professional *in vitro*** diagnostic use only.
2. The product is strictly for medical professional use only and not intended for personal use.
3. Do not use the product beyond the expiration date.
4. Do not use the product if the pouch is damaged or the seal is broken.
5. Handle all specimens as potentially infectious.
6. Follow standard Lab procedure and biosafety guidelines for handling and disposal of potentially infectious material.
7. Inadequate or inappropriate specimen collection, storage, and transport may yield inaccurate test results.
8. Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures. Wear protective clothing such as laboratory coats, disposable gloves, and eye protection when specimens are collected and evaluated. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. Standard precautions and institutional guidelines should always be followed in handling, storing, and disposing of all specimens and all items contaminated with blood or other body fluids.

SPECIMEN COLLECTION

Proper specimen collection, storage, and transport are critical to the performance of this test. Specimens should be tested as soon as possible after collection. The training in specimen collection is highly recommended because of the importance of specimen quality. For optimal test performance, use the swabs supplied in the kit.

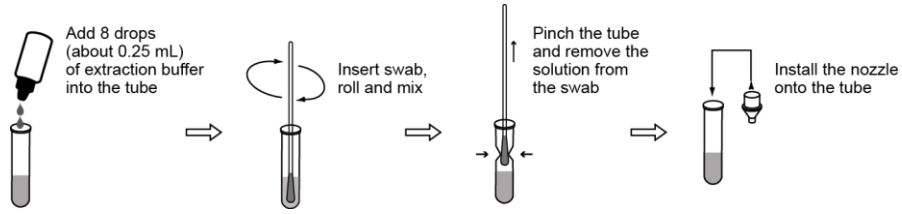


1. Carefully insert the swab into the nostril of the patient, reaching the surface of posterior nasopharynx that presents the most secretion.
2. Swab over the surface of the posterior nasopharynx. Rotate the swab several times.
3. Withdraw the swab from the nasal cavity.

EXCALIBUR
Healthcare Services

SPECIMEN PREPARATION

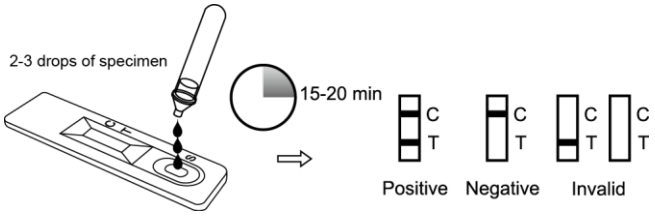
1. Add **8 drops (about 0.25 mL)** of extraction buffer into the extraction tube.
2. Place the swab with specimen into the extraction tube. Roll the swab three to five (3-5) times. **Leave the swab in the extraction buffer for 1 minute**.
3. Pinch the extraction tube with fingers and remove the solution from the swab as much as possible. Dispose of the used swab in accordance with your biohazard waste disposal protocol.
4. Install the nozzle cap onto the sample extraction tube tightly. Use extraction solution as test specimen.



PROCEDURE

1. Bring the kit components to room temperature before testing.
2. Open the pouch and remove the card. Once opened, the test card must be used immediately. Label the test card with patient identity.
3. Invert the extraction tube and add **2-3 drops (50-75 µL)** of test specimen into the specimen well (S) by gently squeezing the extraction tube. 4. Read the results at **15-20 minutes**.

Note: Results after 20 minutes may not be accurate.



INTERPRETATION OF RESULTS

Positive:

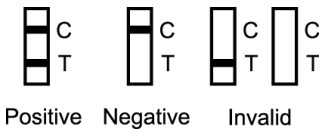
If two coloured bands appear within 15-20 minutes with one coloured band in the Control Zone (C) and another in the Test Zone (T), the test result is positive and valid. No matter how faint the coloured band is in the Test Zone (T), the result should be considered as positive. A positive result does not rule out coinfections with other pathogens.

Negative:

If one coloured band appears in the Control Zone (C) and no coloured band appears in the Test Zone (T) within 15-20 minutes, the test result is negative and valid. A negative result does not exclude SARS-COV-2 viral infection and should be confirmed by molecular diagnostic method if COVID-19 disease is suspected.

Invalid result:

The test result is invalid if there is no coloured band in the Control Zone (C) within 15-20 minutes. Repeat the test with a new test device.



QUALITY CONTROL

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.
2. Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which are not provided with this test kit are commercially available.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The limit of detection (LoD) for the Rapid SARS-COV-2 Antigen Screening Test Card was established in an analytical sensitivity study performed with one virus strain and one recombinant nucleocapsid protein. The LoD was confirmed in the following table.

No.	Item	Limit of Detection
1	SARS-COV-2, Virus	1.3 x10^2 $TCID_{50}$/mL
2	SARS-COV-2, Recombinant nucleocapsid protein	1 ng/mL

Cross Reactivity

The cross reactivity of the Rapid SARS-COV-2 Antigen Screening Test Card was evaluated with a total of 27 microorganisms. None of the microorganisms tested in the following table gave a positive result.

Microorganisms	Concentrations	Microorganisms	Concentrations
Human coronavirus 229E	2.0 x 10^6 $TCID_{50}$/mL	MERS-coronavirus	1.0 x 10^6 $TCID_{50}$/mL
Human coronavirus OC43	2.0 x 10^6 $TCID_{50}$/mL	Chlamydia pneumoniae	2.0 x 10^6 IFU/mL

Human coronavirus NL63	2.0 x 10^6 $TCID_{50}$/mL	Streptococcus pneumoniae	2.0 x 10^6 CFU/mL
Parainfluenza virus 1	2.0 x 10^6 $TCID_{50}$/mL	Streptococcus pyogenes	2.0 x 10^6 CFU/mL
Parainfluenza virus 2	2.0 x 10^6 $TCID_{50}$/mL	Bordetella pertussis	2.0 x 10^6 CFU/mL
Parainfluenza virus 3	2.0 x 10^6 $TCID_{50}$/mL	Mycobacterium tuberculosis	2.0 x 10^6 CFU/mL
Enterovirus EV71	2.0 x 10^6 $TCID_{50}$/mL	Legionella pneumophila	2.0 x 10^6 CFU/mL
Respiratory syncytial virus	2.0 x 10^6 $TCID_{50}$/mL	Mycoplasma pneumoniae	2.0 x 10^6 U/mL
Rhinovirus	2.0 x 10^6 $TCID_{50}$/mL	Haemophilus influenzae	2.0 x 10^6 CFU/mL
Influenza A virus (H1N1)	2.0 x 10^6 $TCID_{50}$/mL	Candida albicans	2.0 x 10^6 CFU/mL
Influenza A virus (H3N2)	2.0 x 10^6 $TCID_{50}$/mL	Staphylococcus aureus	2.0 x 10^6 CFU/mL
Influenza B virus (Yamagata)	2.0 x 10^6 $TCID_{50}$/mL	Pseudomonas aeruginosa	2.0 x 10^6 CFU/mL
Influenza B virus (Victoria)	2.0 x 10^6 $TCID_{50}$/mL	Escherichia coli	2.0 x 10^6 CFU/mL
Adeno virus	2.0 x 10^6 $TCID_{50}$/mL		

Interference

1. Microorganism

Rapid SARS-COV-2 Antigen Screening Test Card has tested samples with common microorganism. The results showed that these microorganisms had no effect on the specificity of the assay up to the listed concentration.

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Adeno virus	2.0 x 10^6 $TCID_{50}$/mL		

2. Endogenous Substances

Rapid SARS-COV-2 Antigen Screening Test Card has tested samples with common endogenous substances. The results showed that these substances had no effect on the specificity of the assay up to the listed concentration.

Substances	Concentrations	Substances	Concentrations
Whole Blood	1% v/v	Homeopathic (Alkalol)	10% v/v
Mucin	2% w/v	CVS Nasal Drops (Phenylephrine)	15% v/v
Tobramycin	0.0004% w/v	Afrin (Oxymetazoline)	15% v/v
Ricola (Menthol)	0.15% w/v	CVS Nasal Spray (Cromolyn)	15% v/v
Chloraseptic (Benzocaine)	0.15% w/v	Fluticasone Propionate	5% v/v
Mupirocin	0.25% w/v	Zicam	5% w/v
Tamiflu (Oseltamivir Phosphate)	0.5% w/v		

Accuracy

The accuracy of Rapid SARS-COV-2 Antigen Screening Test Card was established with 236 nasopharyngeal swabs collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19. The following table summarizes the accuracy of the Rapid SARS-COV-2 Antigen Screening Test Card compared to RT-PCR.

		RT-PCR		
		Positive	Negative	Total
Rapid SARS-COV-2 Screening Antigen Test Card	Positive	30	4	34
	Negative	2	200	202
	Total	32	204	236

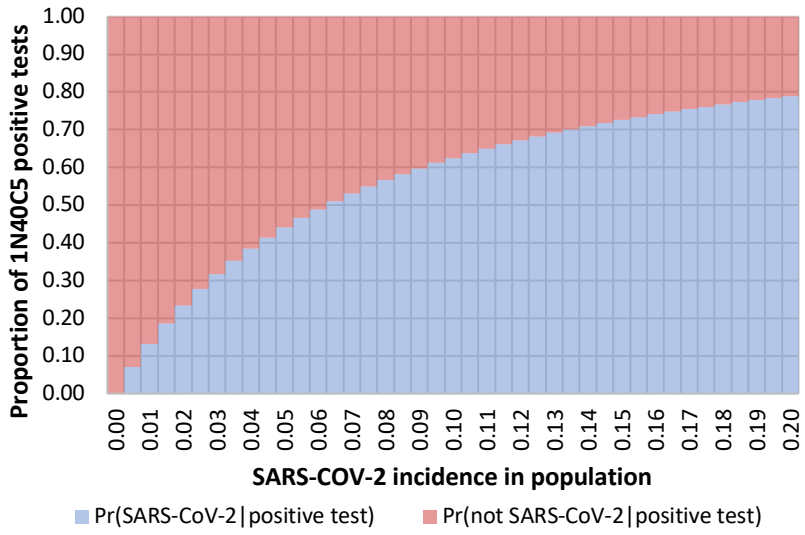
The sensitivity was 93.75% (95%CI: 85.36%~99.99%). The specificity was 98.04% (95%CI: 96.14%~99.94%). The accuracy was 97.46% (95%CI: 95.45%~99.47%).

THE EFFECT OF VARYING INCIDENCE OF SARS-COV-2 IN POPULATIONS

The purpose of presumptive diagnosis by a clinical professional is to reduce cost and the volume of testing in the laboratory. It does not change the test, the symptoms nor the virus.

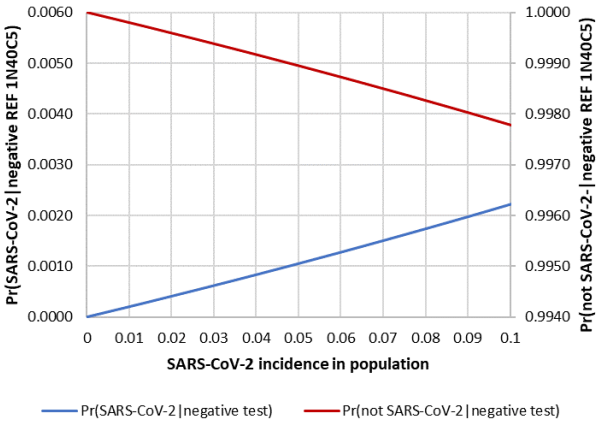
The consequence of screening with RAPID SARS-COV-2 ANTIGEN SCREENING TEST CARD is that at low incidences the number of false positives can be significantly higher than the true positives. All positives with RAPID SARS-COV-2 ANTIGEN SCREENING TEST CARD *must* be confirmed with RT-PCR or an equivalently accurate method.

Figure 1 The proportion of true positives and false positives with varying incidence of SARS-COV-2 with RAPID SARS-COV-2 ANTIGEN SCREENING TEST CARD



The effect of varying incidence on the true negatives and false negatives is shown in Figure 2

Figure 2 The proportion of true negatives and false negatives with varying incidence of SARS-COV-2 with RAPID SARS-COV-2 ANTIGEN SCREENING TEST CARD



LIMITATIONS

1. The test is limited to the qualitative detection of SARS-COV-2 viral antigen in nasopharyngeal swab specimens. The exact concentration of SARS-COV-2 viral antigen cannot be determined by this assay.

2. Proper specimen collection is critical, and failure to follow the procedure may give inaccurate results. Improper specimen collection, storage or repeated freezing and thawing of specimens can lead to inaccurate results.
3. A negative test result may occur if the level of antigen in a specimen is below the limit of detection of the test.
4. There is an increased number of false-positive results with LFD. These are highest at low incidence. This could cause unnecessary economic hardship to individuals and anxiety. Confirm all LFD positive results for SARS CoV 2 with RT-PCR
5. Negative test results do not rule out other potential non-SARS-COV-2 viral infections. Negative results should be confirmed by molecular diagnosis if COVID-19 disease is suspected.
6. Positive test results do not rule out co-infections with other pathogens.
7. Monoclonal antibodies may fail to detect, or detect with less sensitivity, SARS-COV-2 viruses that have undergone minor amino acid changes in the target epitope region.
8. The amount of antigen in a sample may decrease as the duration of illness increases. Specimens collected after day 5-7 of illness are more likely to be tested negative compared to a RT-PCR assay.
9. The Rapid SARS-COV-2 Antigen Screening Test Card can detect both viable and non-viable SARS-COV-2 material. The Rapid SARS-COV-2 Antigen Screening Test Card for rapid detection of SARS-COV-2 performance depends on antigen load and may not correlate with other diagnostic methods performed on the same specimen.
10. The kit was validated with the included swab. Use of other swabs my affect results.
11. Specimen stability recommendations are based upon stability data from influenza testing and performance may be different with SARS-COV-2. Users should test specimens as quickly as possible after specimen collection, and within two hours after specimen collection.
12. The validity of Rapid SARS-COV-2 Antigen Screening Test Card has not been proven for dentification/confirmation of tissue culture isolates and should not be used in this capacity.

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MANUFACTURER – in event of a complaint

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