TRUSTline COVID-19 Ag Rapid Test - Cassette

REF AR0182C IVD

for the qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasopharyngeal (NP) swab specimens

INTENDED USE

The TRUSTline COVID-19 Ag Rapid Test is a lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasopharyngeal (NP) swab specimens from individuals who are suspected of COVID-19. This test is an aid to early diagnosis of SARS-CoV-2 infection along with clinical symptoms and other confirmatory assays. The TRUSTline COVID-19 Ag Rapid Test is intended to be used by trained clinical laboratory personnel specifically instructed and trained in the techniques of in vitro diagnostic procedures.

SUMMARY AND EXPLANATION OF THE TEST

SARS-CoV-2 belongs to the broad family of coronaviruses which are capable of causing illnesses ranging from the common cold to more severe diseases[1]. SARS-CoV-2 infections cause COVID-19 disease. The infected patients have a wide range of clinical symptoms, from little to no symptoms, to fever, tiredness and dry cough, and possibly leading to severe sickness and death. Most patients recover without special treatment. Around 1 out of every 6 patients who get COVID-19 become seriously ill and develop difficulty breathing. Older people and those with underlying medical problems, like high blood pressure, heart problems or diabetes, are more likely to develop serious illness.

Human-to-human transmission of the virus has been confirmed and occur primarily via respiratory droplets from coughs and sneezes within a range of about 6 feet (1.8 m). Viral RNA has also been found in stool samples from patients. It's possible that the virus can be infectious even during the incubation period, but this has not been proven[2].

Currently, the laboratory method for detecting COVID-19 is RT-PCR. However, this method requires sophisticated equipment and highly trained laboratory technicians. Moreover, viral load decreases rapidly 9 or 10 days after onset of symptoms. Presence of SARS-CoV-2 virus could be detected within the first week of the onset of symptoms.

The TRUSTline COVID-19 Ag Rapid Test is an antigen detection test that provides a result in 15 minutes by minimally skilled personnel and without the use of laboratory equipment.

TEST PRINCIPLE

The TRUSTline COVID-19 Ag Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing anti-SARS-CoV-2 antibody conjugated with colloidal gold (antibody conjugates) and 2) a nitrocellulose membrane strip containing a test line (Ag line) and a control line (C line). The test line precoated with antibodies specific to SARS-CoV-2 and the C line is pre-coated with a control line antibody.

The specimen is collected with a swab and the SARS-CoV-2 nucleocapsid antigen is extracted from the swab with extraction buffer. The antigen extracts contact the test strip and then migrate by capillary action across the test strip. SARS-CoV-2 antigen, if present in the extract, will bind to the antibody conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-SARS-CoV-2 antibody, forming a colored Ag line, indicating a COVID-19 positive test result.

The test contains an internal control (C line) which should exhibit a colored line regardless of color development on the Ag line. If the C line does not develop, the test result is invalid and the specimen must be retested with a new device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. Sample extraction tubes
3. Sample extraction tube rack
4. Sample extraction buffer
5. Nozzles
6. Sterile swabs, each sealed in a plastic-paper pouch
7. Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

1. Positive control
2. Negative control

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Clock or timer

WARNINGS AND PRECAUTIONS

1. Read these instructions for use completely before performing the test. Failure to follow these instructions could lead to inaccurate test results.
2. Do not open the sealed pouch unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Wear protective clothing, disposable gloves and goggle while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Follow the US CDC Universal Precautions throughout testing procedures.
8. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. Handle the negative and positive controls in the same manner as the patient specimens.
11. Read testing results 15 minutes after specimen is applied to the sample well. Any results interpreted outside of the 15-minute window should be considered invalid and must be repeated.
12. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 1-30 °C. If stored at 2-8°C, ensure that the test device is brought to room temperature (15-30 °C) before opening. The test device is stable through the expiration date printed on the pouch. Do not freeze or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Specimen collection

Nasopharyngeal (NP)

Nasopharyngeal (NP) swab specimens

To collect a nasopharyngeal (NP) swab specimen, insert the sterile swab into the nostril until it reaches the posterior nasopharynx. Rotate the swab a few times against the nasopharyngeal wall and withdraw the swab from the nasal cavity and proceed to specimen extraction following assay procedure described below.

Specimen transport and storage:

Test specimens as soon as possible after collecting, following assay procedure described below. If not tested immediately, specimens extracted from the swab can be stored at 2-8°C for upto 8 hours before testing.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature (15-30°C) if needed.

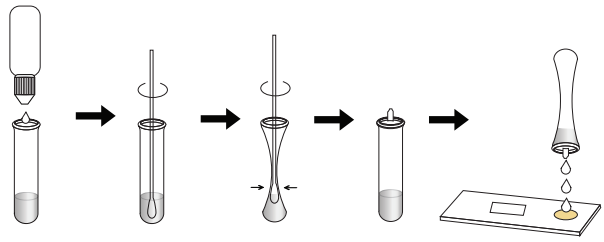
Step 2: **Specimen extraction**
Add 11 drops (~0.3 mL) of the sample extraction buffer into the extraction tube or add the sample extraction buffer into the extraction tube until the meniscus reaches the horizontal line engraved on the tube. Keep the tube upright using the provided sample extraction tube rack.

Step 3: Insert the swab into extraction tube containing 0.3 mL of the extraction buffer. Swirl the swab at least 5 times. Squeeze swab several times while removing it from extraction tube. Remove and discard the swab in a safe manner.

Step 4: Attrach the nozzle on to sample extraction tube containing extracted specimen. The extracted specimen in the tube is now ready for testing.

Step 5: Remove the test device from the sealed pouch just prior to testing. Lay the test device on a clean, flat surface. Label the device with the specimen's ID number.

Step 6: Invert the tube and add 3 drops (~80-90 µL) of the extracted specimen into sample well by gently squeezing the tube.



Step 6: Set up the timer.

Step 7: Read results at 15 minutes. Positive results can be visible in as soon as 3 minutes. Negative results must be confirmed at the end of 15 minutes. Any result interpreted after 15 minutes should be disregarded and must be repeated with a new device.

Discard used devices after interpreting the result following local laws governing the disposal of devices.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. If the C line does not develop after sample application, the result is invalid. Review the entire procedure and repeat the test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to ensure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used
   c. A new shipment of test kits is used.
   d. The temperature during storage of the kits falls outside of 1-30 °C.
   e. The temperature of the test area falls outside of 15-30 °C.
   f. To verify a higher than expected frequency of positive or negative results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C line develops, the test indicates that no detectable SARS-CoV-2 virus is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT:**
In addition to the presence of the C line, if the Ag line develops, the test indicates the presence of SARS-CoV-2 virus. The result is COVID-19 positive or reactive.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the Ag line. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Clinical evaluation

1.1. In-house Evaluation Result

A total of 40 Specimens from COVID susceptible patients were tested with the TRUSTline COVID-19 Ag Rapid Test in comparison with RT-PCR results.Comparison for all patient is shown in the following table.

	TRUSTline COVID-19 Ag Rapid Test		
CMIA & RT PCR	**Positive**	**Negative**	**Total**
Positive	22	3	25
Negative	0	15	15
Total	22	18	40

Relative sensitivity : 88% Relative Specificity : 100%

1.2. External Evaluation Result

A total of 216 Specimens from COVID susceptible patients were tested with TRUSTline COVID-19 Ag Rapid Test in comparison with commerical Rapid Antigen test and RT-PCR results. Comparison of the results for all patient is shown in the following table.

Type of cases	Total	RT-PCR Positive RAT Positive	RT-PCR Positive RAT Negative	RT-PCR Negative RAT Positive	Both Negative
IPD (Covid-19 Cases)	125	43	16	5	61
OPD	91	15	7	0	69
Total	216	58	23	5	130

Relative sensitivity : 71.6% Relative Specificity : 96.3%

2. Analytical Performance

Limit of detection (LoD) of the test device was analyzed by evaluating performance of TRUSTline COVID-19 Ag Rapid Test with recombinant SARS-CoV-2 Nucleocapsid Protein in gradient concentrations (1 ng/ml, 0.5 ng/ml and 0.25 ng/ml) and SARS-CoV-2 virus lysate in varying dilutions (1:100 to 1:800). For each dilution 20 replicates were tested (20 replicates/specimen) and positive rate was tabulated. The LOD was defined as the minimum concentration of analytes that produced ≥ 95% percent positive rate.

2.1. Reactivity with Recombinant SARS-CoV-2 Nucleocapsid Protein (NP)

	Positive rate
Recombinant SARS-CoV-2 NP	**TRUSTline COVID-19 Ag Rapid Test**
1 ng/ml	100% (20/20)
0.5 ng/ml	95% (19/20)
0.25 ng/ml	25% (5/20)

2.2. Limit of Detection: In this study, SARS CoV-2 viral lysate was used. The cultured virus titer is 2.8 x 10^6 $TCID_{50}$ /mL. Up-to 1:200 dilution (i.e. 1.4 x 10^4 $TCID_{50}$ /mL) the positive reactivity was obtained.

Dilution	Positive rate
1:100	100% (20 positive results out of 20 replicates)
1:200	100% (20 positive results out of 20 replicates)
1:400	90% (18 positive results out of 20 replicates)
1:800	40% (8 positive results out of 20 replicates)

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing for the presence of COVID-19 virus in the swab specimen from individual subjects. For optimal test performance, proper sample collection is critical. Failure to follow the procedure may lead to inaccurate results.
2. The Performance of the TRUSTline COVID-19 Ag Rapid Test was established with frozen samples, and test performance may be slightly different with fresh samples.
3. TRUSTline COVID-19 Ag Rapid Test was validated with the swabs provided in this kit. Use of alternate swabs may results in false negative results.
4. As with all antigen tests, performance may decrease as days since symptom onset increases. The amount of antigen in a sample may decrease as the duration of illness increases.
5. The TRUSTline COVID-19 Ag Rapid Test is limited to the qualitative detection of SARS-CoV-2 virus. The intensity of the test line does not have linear correlation with virus titer in the specimen.
6. Sensitivity can differ with various strains of SARS-CoV-2 due to differences of antigen expression. Specimens might contain a new or non-identified strain of SARS-CoV-2 that expresses varying amounts of antigen.
7. A negative or non-reactive result for an individual subject indicates absence of detectable of SARS-CoV-2 virus. However, a negative or non-reactive result does not preclude the possibility of SARS-CoV-2 virus infection.
8. A negative or non-reactive result can occur if the quantity of the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus that are detected are not present in the swab specimen sampled, or the viruses have undergone minor amino acid mutation in the epitope recognized by the antibody utilized in the test.
9. The TRUSTline COVID-19 Ag Rapid Test device detects both viable and non-viable SARS-CoV-2 antigens. Test performance depends on antigen loaded in the sample. A positive test does not rule out the possibility that other pathogens may be present.
10. Performance of the test has not been established for monitoring antiviral treatment of SARS-CoV-2 infection.

REFERENCES

1. Cascella, M., Rajnik, M., Cuomo, A., Dulebohn, S. C., & Di Napoli, R. (2020). Features, evaluation and treatment coronavirus (COVID-19). In Statpearls [internet]. StatPearls Publishing.
2. Healthcare Professionals: Frequently Asked Questions and Answers. (2020, March 22). Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/hcp/faq.html.

Index of Symbols

Consult instructions for use	REF Catalogue number	Use-by date
IVD For *in vitro* diagnostic use only	LOT Batch code	Tests per kit
Temperature limit 1-30 °C	Do not re-use	Keep dry
Manufacturer	Date of manufacture	
Do not use if package is damaged	Keep away from sunlight	

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