PATHKITS

SIMPLE COVID-19 Ag RAPID Test

Product Code : **PKRK001**
Kit Contents (**25 Kits**)
Shell Life: 24 Months

Kit Components	Quantity
Test Strips	25
Extraction Buffer	2 x Bottles
Nasal Swabs	25
Sample Collection Tubes	25
IFU	1

Intended Use

The Simple COVID-19 Ag Rapid Test is a lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasal and nasopharyngeal (NP) swab specimens from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of the onset of symptoms. It is intended for research use only for the identification of infection with SARS-CoV-2, not for use in diagnostic procedures.

Test Principle

The Simple 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 (T 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 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 T 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 T line. If the C line does not develop, the test result is invalid and the specimen must be retested with a new device.

Warnings and Precautions

1. Read these Instructions for Use completely before performing the test. Failure to follow the 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 and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other bloodborne pathogens.
7. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
8. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
9. Handle the negative and positive controls in the same manner as the patient specimens

Reagent Preparation and Storage Instructions

All reagents are ready to use as supplied. Store unused test devices unopened at 2-30°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.

Materials Required but not provided

1. Timer or Stopwatch
2. PPE and other consumables for collection and disposal of samples

Specimen transport and storage:

Test specimens as soon as possible after collecting. If not tested immediately, swab specimens can be stored in a clean and closed container at 2-8°C for up to 24 hours.

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. It is intended for research use only, not for use in diagnostic procedures. The Simple 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.
3. 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.

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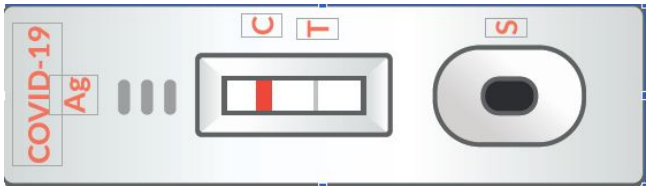
4. A negative or non-reactive result for an individual subject indicates absence of detectable SARS-CoV-2 virus. However, a negative or non-reactive result does not preclude the possibility of SARS-CoV-2 virus infection.
5. A negative or non-reactive result can occur if the quantity of the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the
6. 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.
7. The Simple COVID-19 Ag Rapid Test device detects both viable and non-viable SARSCoV-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.
8. Performance of the test has not been established for monitoring antiviral treatment of SARS-CoV-2 infection.

Assay Procedure

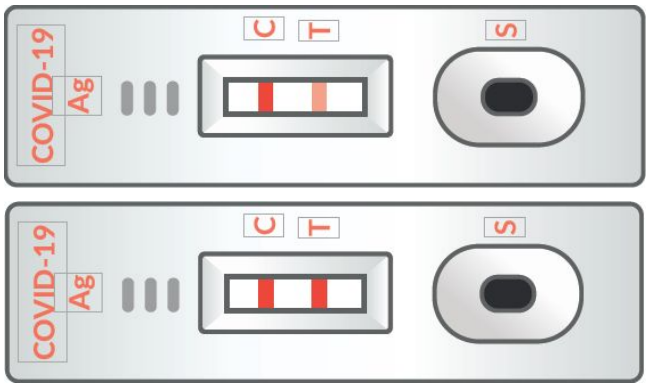
1. Specimen extraction: Add 10-12 drops (~0.35 mL) of the sample extraction buffer into the extraction tube, then keep the tube upright using the provided sample extraction tube rack.
2. To collect a nasopharyngeal (NP) swab specimen, insert the sterile swab into the nostril until it reaches the posterior nasopharynx. Rotate the swab a few times against the nasopharyn geal wall and remove the swab carefully from the nostril.
3. All swab samples: Insert the swab into the extraction tube containing 0.35 mL of the extraction buffer. Swirl the swab at least 10 times. Squeeze the swab several times against the inside of the tube. Remove while squeezing the liquid from the swab.
4. Discard the swab in a safe manner. The extracted specimen in the tube is now ready for testing.
5. Cover the tube with a filter cap and tighten the lid
6. Incubate for 2 mins
7. Remove the test device from the sealed pouch just prior to testing. Lay the test device on a clean, flat surface.
8. Invert the tube and add 2 drops (~80-90 µL) of the test sample into the sample well by gently squeezing the tube.

Interpretation of results

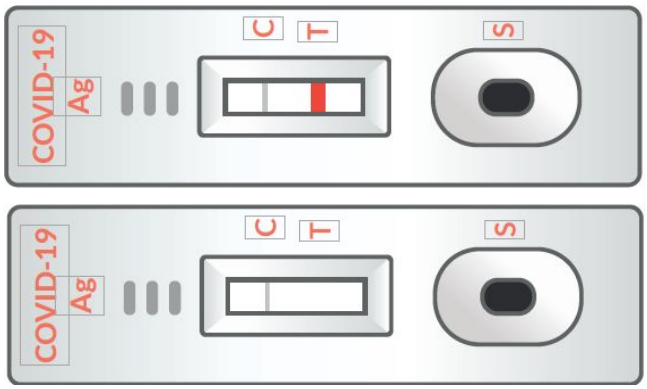
NEGATIVE RESULT: If only the C line develops, the test indicates that no detectable SARS-CoV-2 virus is present in the specimen. The result is negative or non-reactive.



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



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



Technical Assistance

For customer support, please contact our Technical Support:

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31 Jan 2021

No: SARS-CoV-2/Rapid Antigen Test Kit evaluation/2020/KIPMR11

Evaluation report of Rapid Antigen Test using nasopharyngeal samples from COVID-19 suspected patients

Name of the Validation Centre: King Institute of Preventive Medicine and Research, Chennai

Name of the kit: PATHKITS Simple COVID-19 Ag RAPID Test

Country of Origin: India

Lot Nos.: RK/CO/AG/11/20/01, RK/CO/AG/11/20/02, RK/CO/AG/11/20/03

Recommended temperature of storage of kit: 2 to 30 °C

Details of kit components: Test strips, Extraction buffer (2 x vials), nasal swabs, sample collection tubes, Instruction for use. 25 tests per pack

Details of additional equipment required: Timer, PPEs, disposal container, Real Time PCR is needed for validation purpose only

Number of patients recruited: 232

Gold Standard used: NIV Multiplex Single Tube SARS-CoV-2 RT PCR assay

Methodology:

A total of 232 samples, well characterized samples comprising of 151 RT PCR positives and 81 RT PCR negatives were taken for analysis.

These 232 Nasopharyngeal swab samples from 147 laboratory confirmed cases of COVID-19 (hospital inpatients) by RT-PCR and 85 patients from the out-patient department attending the healthcare centers with symptoms of SARS-CoV-2 infection and diagnosis was performed immediately as per the kit manufacturer's instructions.

All the 232 nasopharyngeal swab samples were put in VTM, validated by Real Time RT PCR using NIV Multiplex Single Tube SARS-CoV-2 RT PCR assay, which detects 3 targets such as E, RdRp and ORF of SARSCoV-2. Among the 232 samples, 151 samples were found to be COVID-19 positive and others negative. Among the positive samples, 55 samples were found to have early Ct values (Ct values between 14 to 20) for the target genes, 49 samples were

found to have early Ct values (Ct values between 14 to 20) for the target genes, 49 samples were found to have moderate Ct values *i.e.* 21 to 27 and 47 samples were found to have late Ct values between 28 to 32.

All the collected nasopharyngeal samples were subjected to the detection of SARS-CoV-2 antigen using the PATHKITS Simple COVID-19 Ag RAPID Test kit according to the manufacturer's instruction. Briefly, the swab was inserted into the 0.35 mL of extraction medium on extraction tube swirled well, squeezed the swab followed by closing of the tube was closed and incubation for 2 min. 90 µL of the test sample was added in the well of test strip and observed for appearance of colour in both control and test lines.

The evaluation of rapid antigen test and the real-time RT-PCR results were compared; considering real time RT-PCR as the gold standard test, the sensitivity and specificity of the rapid antigen test were calculated.

Results:

Rapid antigen test	**NIV Multiplex Single Tube SARS-CoV-2 RT PCR assay (Gold standard)**		**Total**
	Positive	**Negative**	
Positive	89	1	**90**
Negative	62	80	**142**
Total	**151**	**81**	**232**

Sensitivity: 58.94%

Specificity: 98.77%

Positive Predictive Value: 98.89%

Negative Predictive Value: 56.34%

Comments on the kit performance: Sensitivity of the kit is observed to be low

Comments on quality of kit packaging: Packaging is good; 25 tests/kit

Name and signature of the In-charge:

(Dr. K. Kaveri)
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