OnSite® COVID-19 Ag Rapid Test

REF R0182C CE

Instructions for Use

10108900
Barcode for RTR Use Only

INTENDED USE

The *OnSite* COVID-19 Ag Rapid Test is a lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid antigens in nasopharyngeal (NP) or nasal swab specimens from individuals suspected of COVID-19, within the first seven days of the onset of symptoms. The test is intended for use by healthcare providers or personnel trained in rapid test procedure, as an aid in identifying SARS-CoV-2 infection.

The *OnSite* COVID-19 Ag Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out other bacterial or viral infections.

Negative results from patients with symptom onset beyond seven days should be confirmed with a molecular assay. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The product is intended to be used in any laboratory and non-laboratory environment that meets the requirements specified in the Instructions for Use and local regulations. For *in vitro* diagnostic use only.

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 resulting in a wide range of clinical symptoms, ranging from asymptomatic to fever, tiredness and dry cough, and possibly leading to severe sickness and even death. Most patients recover without special treatment. According to recent data, approximately 15-20% of infected individuals become seriously ill and develop difficulty breathing[2]. The elderly and those with underlying medical problems, such as high blood pressure, heart problems or diabetes are more likely to develop serious illness[2].

Human-to-human transmission of the virus has been confirmed and occurs primarily via respiratory droplets from coughs and sneezes within a range of about six feet (1.8 m)[3]. Viral RNA has also been found in stool samples from patients. It is possible that the virus can be infectious even during the incubation period, but this has not yet been proven[4].

The current laboratory method for detecting COVID-19 is PCR. However, this method requires sophisticated equipment and highly trained laboratory technicians. The *OnSite* COVID-19 Ag Rapid Test is an easy-to-use and cost-efficient assay that can be performed at point-of-care settings.

The *OnSite* COVID-19 Ag Rapid Test detects the presence of antigens from the SARS-CoV-2 virus within the first seven days of the onset of symptoms. Test results should be interpreted at 15 minutes. Results should not be interpreted after 20 minutes. Minimally skilled personnel can perform the test, without the use of cumbersome laboratory equipment.

TEST PRINCIPLE

The *OnSite* COVID-19 Ag Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing anti-SARS-CoV-2 antibodies 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 is pre-coated with anti-SARS-CoV-2 antibodies and the C line is pre-coated with control antibodies.

The specimen is collected with a nasopharyngeal or nasal swab and the SARS-CoV-2 antigen is extracted from the swab with extraction buffer. Alternatively, samples stored in viral transport medium (VTM) can be directly tested. When applied to the sample well, the extracted specimen migrates across the test strip by capillary action. SARS-CoV-2 antigen, if present in the extract, binds to the antibody conjugates and the immunocomplex is then captured on the membrane by the pre-coated anti-SARS-CoV-2 antibody, forming a colored Ag line that indicates 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 (2 bottles, 5 mL each)
5. Nozzles
6. Individually sealed pouches containing a sterile swab
7. Instructions for Use

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Positive control
2. Negative control
3. Nasal swabs

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock, watch or other timing device
2. Disposable gloves, biohazard disposal container

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

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 and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after testing.
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.
10. Read test results 15 minutes after specimen is applied to the sample well. Consider any results read after 20 minutes invalid and repeat test.
11. 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 devices unopened at 2-30°C. If stored at 2-8°C, ensure that the device is brought to room temperature before opening. The cassette device is stable until the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as potentially infectious, and handle them with standard biosafety procedures.

1. Remove mucus from patient's nose.
2. **Collection of swab specimens:**

a. Nasopharyngeal (NP) swab specimens
Carefully insert the sterile swab provided with the kit into the patient's nostril that presents the most secretion, keeping it near the septum floor of the nose while gently pushing into the posterior nasopharynx as shown in Figure A. Rotate the swab several times then remove it from the nasopharynx. Withdraw the swab from the nasal cavity and proceed to specimen extraction following Assay Procedure described below.

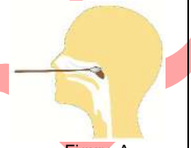


Figure A

b. Nasal swab specimens
Carefully insert a sterile swab into the patient's nostril. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril) as shown in Figure B. Rotate the swab 5 times against the nasal wall then remove it from the nostril. Using the same swab, repeat the process in the second nostril to ensure that sample is collected from both nasal cavities. Withdraw the swab from the nasal cavity and proceed to specimen extraction following Assay Procedure described below.


Figure B

3. **Specimen transport and storage:**
Test specimens as soon as possible after collection, following the assay procedure below. If not tested immediately, specimens extracted from the swab can be stored at 2-8°C for up to 8 hours before testing. Specimens in VTM can be stored frozen until use.

ASSAY PROCEDURE

Assay procedure for testing swab specimens:

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

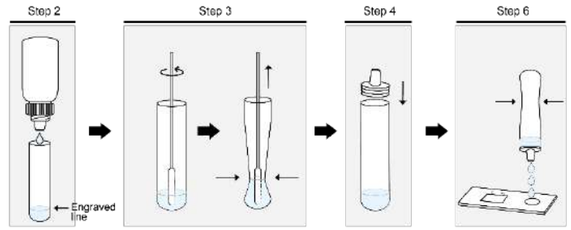
Step 2: Add sample extraction buffer into the extraction tube until the meniscus reaches the horizontal line engraved on the tube (~0.3 mL, 9-10 drops). Keep the tube upright using the provided sample extraction tube rack.

Step 3: Insert the swab into the extraction buffer in the tube. Swirl the swab at least 5 times. Squeeze the tube against the submerged swab several times to facilitate extraction of the specimen. Remove and discard the swab in a safe manner.

Step 4: Attach the nozzle onto the sample extraction tube containing extracted specimen. The extracted specimen in the tube is now ready for testing.

Step 5: Remove the cassette device from the sealed pouch just prior to testing. Lay the 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 the sample well of the cassette device by gently squeezing the tube.



Step 7: Set up the timing device.

Step 8: Read results at 15 minutes. Positive results can be visible in as soon as 3 minutes. Results read after 20 minutes should be considered invalid and must be repeated with a new device. Discard used devices as biohazardous waste following local laws governing the disposal of devices.

Assay Procedure for VTM specimens

Specimens collected and stored in VTM can be tested by directly pipetting 300µl of the VTM specimen into the buffer-filled extraction tube from step 2, mixing by pipetting up and down 5 times, and proceeding to step 4 above.

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 2-30°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.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C line develops, the test did not detect SARS-CoV-2 virus antigen in the specimen. The result is negative or non-reactive.

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2. **POSITIVE RESULT:** If both the C line and Ag line develop, SARS-CoV or SARS-CoV-2 virus (antigen) is present in the specimen. The result is positive or reactive. Some specimens might produce a faint band, but every visible test line band indicates a positive result independently of the band intensity.

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 Performance

1.1. Clinical performance in nasopharyngeal swab specimens

The clinical performance of the *OnSite* COVID-19 Ag Rapid Test was evaluated at five clinical sites in Asia and South America, in nasopharyngeal (NP) swabs specimens collected from subjects suspected of COVID-19 and from healthy individuals. Two NP swabs were collected from each subject, one for testing by the *OnSite* COVID-19 Ag Rapid Test and the other one for testing by commercially available real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2, used as the reference method for this study. The overall performance of the *OnSite* COVID-19 Ag Rapid Test in this study is shown on the table below:

RT-PCR Test (Reference)	*OnSite* COVID-19 Ag Rapid Test Result		
	Positive	Negative	Total
Positive	103	11	114
Negative	0	487	487
Total	103	498	601

Relative Sensitivity: 90.4% (95% CI: 83.4-95.1%); Relative Specificity: 100% (95% CI: 99.3-100%); Overall Agreement: 98.2% (95% CI: 96.8-99.1%)

1.2. Clinical performance in nasal swab specimens

The clinical performance of the *OnSite* COVID-19 Ag Rapid Test was evaluated at at five clinical sites in Europe, Asia and South America, in in nasal swab specimens collected from subjects suspected of COVID-19 and from healthy individuals. Two swabs were collected from each subject, one nasal swab for testing by the OnSite COVID-19 Ag Rapid Test and one NP swab for testing by commercially available real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2, used as the reference method for this study. The combined performance of the OnSite COVID-19 Ag Rapid Test in these studies is shown on the table below:

RT-PCR Test (Reference)	*OnSite* COVID-19 Ag Rapid Test Result		
	Positive	Negative	Total
Positive	155	15	170
Negative	2	523	525
Total	157	538	695

Relative Sensitivity: 91.2% (95% CI: 85.9-95.0%); Relative Specificity: 99.6% (95% CI: 98.6-100%); Overall Agreement: 97.6% (95% CI: 96.1-98.6%)

2. Analytical Performance

2.1 Analytical Sensitivity (Limit of Detection, LoD)

The LoD of the *OnSite* COVID-19 Ag Rapid Test was determined by evaluating a serial dilution of Gamma-Irradiated SARS-CoV-2 virus lysate (BEI Resources, NR-52287). Multiple negative nasopharyngeal or nasal swab specimens were eluted in buffer and were combined and mixed thoroughly to create clinical negative matrix pools for each matrix, to be used as the diluent. Inactivated SARS-CoV-2 virus lysate was diluted in each of these matrices to generate virus dilutions for testing. Each NP or nasal swab was spiked with 50 µL of each virus dilution, extracted with extraction buffer and tested according to the product IFU. The assay LoD was determined for both NP and nasal swab specimens as the lowest concentration that was detected ≥ 95% of the time in the respective specimen matrix.

The LoD of the *OnSite* COVID-19 Ag Rapid Test in both nasopharyngeal and nasal swab matrices was determined to be 140 $TCID_{50}$/mL. The *OnSite* COVID-19 Ag Rapid Test can detect the Alpha (U.K.), Beta (South Africa), Gamma (Brazil), Delta (India), Eta (Nigeria), Iota (USA), Kappa (India), Lambda (Peru), P.2 (Brazil), B.1.620, and Omicron (South Africa) variants at similar levels as the original SARS-CoV-2 strain.[5]

2.2 Analytical Specificity (Cross-Reactivity and Microbial Interference)

The analytical specificity of the *OnSite* COVID-19 Ag Rapid Test was evaluated by testing commensal and pathogenic microorganisms that may be present in the nasal cavity. Each of the organisms was tested in triplicate in the absence or presence of 2-3X LoD recombinant SARS-CoV-2 NP antigen. No cross-reactivity (except SARS-coronavirus) or interference were seen with the following microorganisms when tested at the concentration presented in the table below:

Potential Cross-Reactant	Concentration	Cross-Reactivity (Yes/No)	Microbial Interference (Yes/No)
SARS-coronavirus NP antigen	25 µg/mL	Yes (3/3 positive)	No (3/3 positive)
MERS-coronavirus NP antigen	25 µg/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus HKU1 NP antigen	66 µg/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus 229E	1.77×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus OC43	0.53×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Human coronavirus NL63	0.51×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Adenovirus	7×10^8 NIU/mL	No (3/3 negative)	No (3/3 positive)
Human Metapneumovirus (hMPV)	0.76×10^4 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 1	5.01×10^4 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 2	1.6×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 3	1.6×10^6 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Parainfluenza virus 4	1.15×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Influenza A NP antigen	180 µg/mL	No (3/3 negative)	No (3/3 positive)
Influenza B NP antigen	200 µg/mL	No (3/3 negative)	No (3/3 positive)
Enterovirus	2.8×10^5 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Respiratory syncytial virus	2.8×10^4 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Rhinovirus	2.2×10^5 PFU/mL	No (3/3 negative)	No (3/3 positive)
Haemophilus influenzae	5.2×10^5 CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pneumoniae	$>2\times10^3$ CFU/mL	No (3/3 negative)	No (3/3 positive)
Streptococcus pyogenes	3.6×10^5 CFU/mL	No (3/3 negative)	No (3/3 positive)
Candida albicans	4.5×10^6 $TCID_{50}$/mL	No (3/3 negative)	No (3/3 positive)
Pooled human nasal wash – *representative of normal respiratory microbial flora*	N/A	No (3/3 negative)	No (3/3 positive)
Bordetella pertussis	3.9×10^7 CFU/mL	No (3/3 negative)	No (3/3 positive)
Mycoplasma pneumoniae	4.4×10^5 CFU/mL	No (3/3 negative)	No (3/3 positive)
Chlamydophila pneumoniae	1.4×10^7 IFU/mL	No (3/3 negative)	No (3/3 positive)
Legionella pneumophila	7.8×10^5 CFU/mL	No (3/3 negative)	No (3/3 positive)
Mycobacterium tuberculosis	$>2\times10^3$ CFU/mL	No (3/3 negative)	No (3/3 positive)
Pneumocystis jirovecii (PJP)	3.45×10^6 CFU/mL	No (3/3 negative)	No (3/3 positive)

3. Interfering Substances

The following potentially interfering substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with the OnSite COVID-19 Ag Rapid Test at the concentrations listed in the following table and were found not to affect test performance for detection of both positive and negative specimens:

Interfering Substance	Concentration	Interference (Yes/No)	Interfering Substance	Concentration	Interference (Yes/No)
Mucin	0.5%	No (6/6 correct)	Ribavirin	1 mg/mL	No (6/6 correct)
Whole Blood	4%	No (6/6 correct)	Peramivir	1 mg/ml	No (6/6 correct)
Phenylephrine	15% v/v	No (6/6 correct)	Tobramycin	4 µg/mL	No (6/6 correct)
Fluconazole	5% w/v	No (6/6 correct)	Diphenhydramine	0.08 mg/dL	No (6/6 correct)
Budesonide	5% w/v	No (6/6 correct)	Dextromethorphan	1.56 µg/dL	No (6/6 correct)
Nasal Gel	2% v/v	No (6/6 correct)	Acetaminophen	199 uM	No (6/6 correct)
Menthol	1.5 mg/mL	No (6/6 correct)	Acetylsalicylic Acid	3 mg/dL	No (6/6 correct)
Benzocaine	1.5 mg/mL	No (6/6 correct)	Mupirocin	10 mg/mL	No (6/6 correct)
Lopinavir	5 mg/mL	No (6/6 correct)	HAMA	4 ng/mL	No (6/6 correct)
Zanamivir	5 mg/mL	No (6/6 correct)	Biotin	100 ug/mL	No (6/6 correct)
Oseltamivir	5 mg/mL	No (6/6 correct)			

4. Hook Effect

No high dose hook effect was observed when tested with up to a concentration of 3×10^8 pg/mL of recombinant SARS-CoV-2 NP antigen with the *OnSite* COVID-19 Ag Rapid Test.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing for the presence of SARS-CoV-2 antigen 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 use only by healthcare professionals or personnel trained in rapid test procedure. For *in vitro* diagnostic use only.
3. The *OnSite* COVID-19 Ag Rapid Test is limited to the qualitative detection of SARS-CoV-2 antigen. The intensity of the test line does not have linear correlation with virus titer in the specimen.
4. 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.
5. A negative or non-reactive result for an individual subject indicates absence of detectable of SARS-CoV-2 antigen. However, a negative or non-reactive result does not preclude the possibility of SARS-CoV-2 virus infection.
6. A negative or non-reactive result can occur if the quantity of the SARS-CoV-2 virus (antigen) present in the specimen is below the detection limit of the assay, or if the virus detected was 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 *OnSite* COVID-19 Ag Rapid Test detects both viable and non-viable SARS-CoV and 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.
8. Performance of the *OnSite* COVID-19 Ag Rapid Test has been validated in specimens stored in multiple viral transport media (VTM). However, specimens stored in PBS or saline solutions should not be tested on the *OnSite* COVID-19 Ag Rapid Test.
9. Performance of the test has not been established for monitoring antiviral treatment of SARS-CoV-2 infection.

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Index of Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number		Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive,
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

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English version

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