For *in vitro* Diagnostic Use

INTENDED USE

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen directly from SARS-CoV-2 in mid-turbinate nasal swabs from individuals who are suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset when tested at least twice over three days with 48 hours between tests.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in mid-turbinate during the acute phase of infection. 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 bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results should be treated as presumptive, and do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control 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, and confirmed with a molecular assay, if necessary, for patient management.

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is intended for use by healthcare professionals in Point of Care (POC) setting.

SUMMARY AND EXPLANATION

The novel coronaviruses (2019-nCoV) 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. The median incubation time is estimated to be approximately 5 days with symptoms estimated to be present within 12 days of infection.[3] The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough, shortness of breath.

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is a rapid lateral flow immunoassay for the qualitative detection and diagnosis of SARS-CoV-2 directly from nasal swabs, without viral transport media. The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) kit contains all components required to carry out an assay for SARS-CoV-2.

PRINCIPLE OF THE PROCEDURE

The COVID-19 Antigen Test is a sandwich immunochromatographic assay that uses antibodies to detect SARS-CoV-2 nucleocapsid antigen extracted from nasal swab specimen.

When the extracted specimen is added on the sample well, the specimen is absorbed into the device and migrates along the test strip by capillary action, mixes with the SARS-CoV-2 nucleocapsid protein antibody conjugated colloidal gold and flows across the the pre-coated membrane. When the COVID-19 antigen level in the specimen is at or above the target cutoff (the detection limit of the test), the antigen bound to the nucleocapsid protein antibody-colloidal gold conjugate is captured by the mouse SARS-CoV-2 nucleocapsid protein monoclonal IgG antibody immobilized in the Test Region (T) of the device, and this produces a colored test band that indicates a positive result. When the SARS-CoV-2 nucleocapsid protein antigen level in the specimen is zero or below the target cutoff, there is not a visible colored band in the Test Region (T) of the device. This indicates a negative result.

To serve as a procedure control, a red line will appear at the Control Region (C), if the SARS-CoV-2 monoclonal antibody colloidal gold conjugate binding to the goat anti-mouse antibody immobilized on the membrane and indicating that sufficient sample volume had been added to the device and that the test has worked properly. The device does not use biotin-Streptavidin/avidin chemistry in any of the steps for coupling reagents.

The device is not intended to be used with viral transport media.

MATERIALS

Materials Provided

- 20 Individually sealed pouches, each pouch contains:
  - 1 Test Cassette
  - 1 Desiccant sachet
- 20 Sample Extraction Tubes and 20 Drippers
- 20 Sterile Nasal Swabs
- Extraction buffer (2*6 mL)
- 1 Positive Control Swab (1): swab is coated with non-infectious recombinant SARS-CoV-2 antigen
- 1 Negative Control Swab (1): swab soaked with sample extraction buffer, then dried (buffer with less than 0.1% sodium azide)
- Test Tube Rack (1)
- Product Insert (1)
- Quick Reference Guide (1)

Materials Required but not Provided

- Timer or watch
- Personal protective equipment, such a protective gloves, medical mask, goggles, and lab coat.
- Appropriate biohazard waste container and disinfectants.

PRECAUTIONS

- For *in vitro* diagnostic use
- This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Do not reuse the used test cassette, single use only.
- Do not mix components from different kit lots.
- Wear suitable protective clothing, gloves (nitrile or latex), and eye/face protection when handling patient samples or used kit components.
- Do not store specimens in viral transport media for specimen storage.
- Do not touch the reaction area of test cassette.
- Avoid excessively high temperature in the experiment environment. Test cassettes and extraction buffer stored at low temperature need to be returned to room temperature before opening to avoid moisture absorption.
- If the solution contacts the skin or eye, flush with copious amounts of water.
- Do not reuse the used test cassettes, extraction tubes, extraction buffers, or control swabs.
- The test cassette must remain sealed in the protective foil pouch until use. The user should never open the foil pouch of the test cassette exposing it to the ambient environment until the test cassette is ready for immediate use
- The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) must only be used with the extraction buffer provided in the kit.
- When collecting a nasal swab sample, use the nasal swab provided in the kit.
- Inadequate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- To obtain accurate results, you must follow the product's instructions for use.
- Individuals with color-impaired vision may not be able to adequately interpret test results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wash hands thoroughly after handling.

- For additional information on safety, handling, and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at wondfousa.com.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- The performance of the device has not been assessed on specimens from individuals who have been infected with emerging variants of SARS-CoV-2 of public health concern.

STORAGE AND STABILITY

Store the test kit at 2～30℃, away from direct sunlight, moisture, and heat. Test kit contents are stable until the expiration date printed on the package. Do not freeze.

QUALITY CONTROL

Built-in Control

The appearance of a red procedural Control Line provides positive control by demonstrating sufficient flow has occurred and the functional integrity of the Test Strip was maintained. **If a red procedural Control Line does not develop, then the test result is invalid.**

A built-in negative control is provided by the clearing of red background color, verifying that the test has been performed correctly. Within 15 minutes, the result area should be white to light pink and allow the clear interpretation of the test result. **If background color remains and interferes with interpretation of the test result, then the test result is invalid.** Should this occur, review the procedure and repeat the test with a new patient sample and a new test cassette. If it is necessary to collect another patient specimen. patient swabs or reagents cannot be reused.

External Quality Control

External controls supplied in the test kit, one negative swab and one positive swab, may also be used to monitor the performance of the test.

The external controls are to be tested using the nasal swab test procedure provided in the Instructions for Use or in the Quick Reference Guide.

It is recommended that the positive and negative controls are run once for each untrained operator, once for each new shipment of kits – provided that each different lot received in the shipment is tested – and as deemed additionally necessary by your internal quality control procedures, and in accordance with Local, State, and Federal regulations or accreditation requirements.

Positive Control should present two pink-to-red bands at both Test Line (T) and Control Line (C).

Negative Control should present a single pink-to-red bank at the Control Line (C) only.

If the controls do not perform as expected, repeat the test or contact Technical Support before testing patient specimens.

SPECIMEN COLLECTION AND PREPARATION

Wear appropriate personal protection equipment and gloves prior running each test and handling patient specimens. Change gloves between handling of specimens suspected of COVID-19.

Open the test kit, remove a sample extraction tube and a bottle of extraction buffer. Place the sample extraction tube in the test tube rack. Uncap the extraction buffer bottle and add 10 drops of extraction buffer into the extraction tube.

DOs and DON'Ts of Sample Collection

- Do collect sample as soon as possible after onset of symptoms.
- Do test sample immediately.
- Use only swabs provided with the kit.
- Refer to: Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from persons for COVID-19 at https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Nasal Specimen Collection Procedure:

Note: It is important to obtain as much secretion as possible.

For optimal test performance with a nasal swab specimen, use the swabs supplied in the kit.

1. Tilt the patient's head back 70 degrees.
2. While gently rotating the swab, insert swab less than one inch (about 2 cm) into nostril parallel to the palate (not upwards) until resistance is met at turbinates.
3. Rotate the swab against the nasal wall for 5 times or more and repeat in other nostril using the same swab.
4. Withdraw the swab from the nostril.
5. The swab should be placed in the sample extraction tube immediately after being collected. Do not return the swab to its original paper packaging.

Sample Transport and Storage

Samples should be tested as soon as possible after collection. Based on data generated with the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method), nasal swabs are stable for up to 2 hours at room temperature in a clean, dry transport tube.

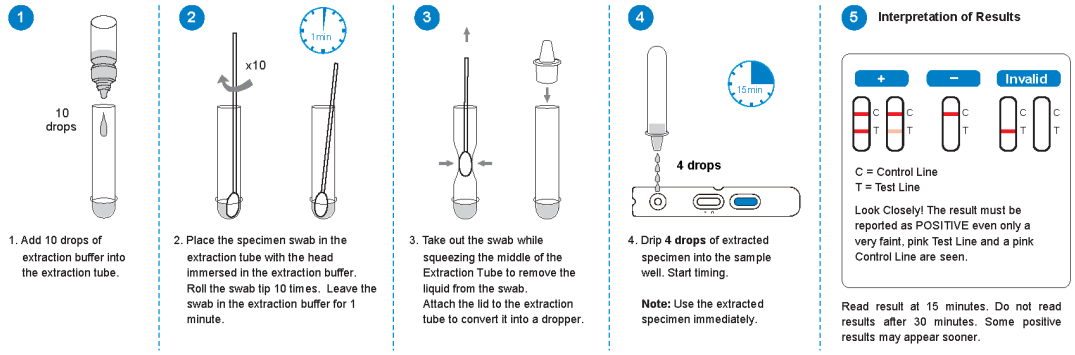
TEST PROCEDURE

Test Notes

- Use fresh nasal swab specimens for best performance. Freshly collected specimens should be tested immediately.
- Ensure the test kit, including the cassette and extraction buffer, equilibrates to room temperature prior to use. Perform the test at room temperature (15-30 ºC).
- All clinical specimens must be at room temperature prior to testing.
- Do not open the pouch until you are ready to perform the test.
- Remove the test device from its foil pouch and place it on a clean, flat surface.
- Label the test cassette with the patient identification or control to be tested.
- Less than 4 drops of extracted specimen added to the sample well could lead to invalid results.

Test Procedure for Patient Specimens

1. Put the Sample Extraction Tube on the test tube rack. Add **10 drops** of extraction buffer into the extraction tube.
2. Place the swab sample in the extraction tube. Make sure the swab head is immersed in the extraction buffer. Roll the swab tip 10 times against the bottom and sides of the extraction tube to release the specimen from the swab tip. Return the test tube to the test tube rack and leave the swab in the extraction buffer for one minute.
3. Take out the swab while squeezing the middle of the extraction tube to remove the liquid from the swab. Discard the used swab in accordance with the bio-hazard waste disposal protocol. Firmly attach the lid provided to the top of the extraction tube to convert it into a dropper.
4. The extracted sample must be used immediately. Invert the extraction tube and lid (now converted into a dropper); hold it vertically as shown in step 4 in Figure 1 below and add four drops of extracted specimen slowly into the sample well of the test cassette. Start the timer.
5. Read result at 15 minutes. Do not read results after 30 minutes.



NOTE

Less than 4 drops of extracted specimen added to the sample well could lead to invalid results.

Figure 1 - Patient Specimen Nasal Swab Test Procedure

Test Procedure for Swab Controls

1. Put the Sample Extraction Tube on the test tube rack. Add **12 drops** of extraction buffer into the extraction tube.
2. Remove the swab control from the pouch. Follow Step 2-5 of the Test Procedure for Patient Specimens. NOTE: Swab controls need 12 drops of extraction buffer instead of the ten drops required for the patient specimen.

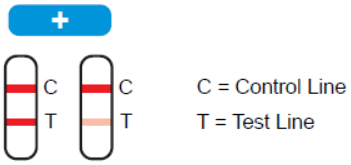
SERIAL TESTING

Individuals with symptoms tested within 48 hours after the first negative test, should be tested for a total of at least two tests.

RESULT INTERPRETATION

Positive Result

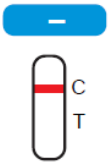
Colored bands appear at both test line (T) and control line (C). It indicates a positive result for the 2019-nCoV antigen in the specimen. Repeat testing does not need to be performed if the patient has a positive result at any time.



Note: Look closely! The result must be reported as POSITIVE even only a very faint, pink Test Line and a pink Control Line are seen.

Negative Result

Colored band appears at control line (C) only. It indicates that the concentration of the 2019-nCoV antigen is zero or below the detection limit of the test.



If you receive a negative test, follow the instructions below:

- If you have COVID-19 symptoms, test again 48 hours after the first negative test, for a total of at least two tests.
- If any of the repeat tests are positive, you most likely have COVID-19 and should follow current Public Health measures.

To increase the chance that the negative result for COVID-19 is accurate, you should: Test again in 48 hours if the individual

has symptoms on the first day of testing.

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider.

All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. 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, including infection control decisions.

Repeat Testing Result

Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results

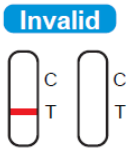
Status on First Day of Testing	First Result Day 1	Second Result Day 3	Third Result Day 5	Interpretation
With Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	N/A	Negative for COVID-19

Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

If all repeat tests are negative and you are concerned you have COVID-19, you may choose to test again using an antigen test or consult with your health care provider regarding molecular testing.

Invalid Result

No visible colored band appears at control line after performing the test. The directions may have not been followed correctly or the test may have deteriorated. It is recommended that the specimen should be re-tested by either (1) using the leftover extraction buffer in the dropper and a new cassette or (2) repeating with a new cassette and a new sample.



LIMITATIONS OF PROCEDURE

1. The detection of viral antigen is dependent upon proper specimen collection, handling, transportation, storage, and preparation, including extraction. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
2. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
3. This device is a qualitative test and does not provide information on the viral load present in the specimen.
4. This test cannot rule out diseases caused by other bacterial or viral pathogens.
5. Cross-reactivity with other respiratory tract organisms may lead to erroneous results.
6. The performance of this device has not been evaluated for immunocompromised individuals.
7. The prevalence of infection will affect the test's predictive value.
8. Positive test results do not rule out co-infections with other pathogens.
9. Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.

10. Negative results should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.
11. If the differentiation of specific SARS virus and strains is needed, additional testing, in consultation with state or local public health departments, is required.
12. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation.
13. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Serial Testing (Repeat Testing) Information and Limitations

1. Serial testing (i.e., testing every other day) is more likely to detect COVID-19, both when you do or do not have any symptoms.
2. A negative result should be followed up with repeat, or serial testing at least twice over three days with at least 48 hours between tests for symptomatic individuals. A self-test may be used for this additional testing.
3. The performance of this test was not clinically validated for serial testing. Serial testing recommendations are supported by the study conducted by the National Institutes for Health (NIH) and the University of Massachusetts Chan Medical School in collaboration with the US FDA.
4. All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary. If you continue to have symptoms of COVID-19, and both your first and second tests are negative, you may not have COVID-19, however you should follow-up with a healthcare provider.

CLINICAL PERFORMANCE

Clinical performance characteristics of the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) was evaluated in a multisite prospective study in the U.S in which patients were enrolled and tested. A total of three (3) investigational sites throughout the U.S. participated in the study. Testing was performed by operators with no laboratory experience. In this study testing was conducted by eight (8) intended users. No training on the use of the test was provided to the operators.

To be enrolled in the study, patients had to be presenting at the participating study centers with suspected COVID-19. Patients who presented within 7 days of symptom onset were included in the study. Two nasal swabs were collected from patients and tested using the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) at all study sites. An FDA Emergency Use Authorized real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2 was utilized as the comparator method for this study.

At all sites, one nasal swab was tested directly with the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) according to product instructions and the other swab was tested using the reference comparator method. Swabs were assigned to testing with the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) or RT-PCR testing and were tested by minimally trained operators who were blinded to the RT-PCR test result. All sites shipped the RT-PCR sample to a testing laboratory.

This clinical performance data reflects the accuracy of the test when testing once. This test was not clinically validated for serial testing. The serial testing recommendations are supported by the study conducted by the National Institutes for Health (NIH) and the University of Massachusetts Chan Medical School in collaboration with the US FDA.

The performance of Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) was established with 101 nasal swabs collected from individual symptomatic patients (within 7 days of onset) who were suspected of COVID-19.

Table 1 - Summary of the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) Compared to RT-PCR for Nasal Swabs for Detection of SARS-CoV-2

Wondfo 2019-nCoV Antigen	Reference RT-PCR Results

Test Results for Detection of SARS-CoV-2	Positive	Negative	Total
Positive	47	0	47
Negative	8	46	54
Total	55	46	101

Positive Percent Agreement = 85.5% (95% CI: 73.8% ~ 92.4%)

Negative Percent Agreement = 100% (95% CI: 92.3% ~ 100%)

Overall Percent Agreement = 92.1% (95% CI: 85.1% ~ 95.9%)

NOTE: A total of 102 samples was collected; however, one sample was excluded from analysis since it was considered as an invalid result.

Table 2 indicates positive and negative results broken down by days since symptom onset demonstrating similar performance of the assay through seven days post symptoms onset.

Table 2 - Stratified Performance Based on the Days from the Onset of the Symptoms

Day Range	TP	FN	FP	TN	Total	Sensitivity	Specificity
0	2	0	0	1	3	100%	100%
0 - 1	8	1	0	6	15	88.9%	100%
0 - 2	13	2	0	13	28	86.7%	100%
0 - 3	18	2	0	20	40	90.0%	100%
0 - 4	26	2	0	27	55	92.9%	100%
0 - 5	33	4	0	35	72	89.2%	100%
0 - 6	42	4	0	42	88	91.3%	100%
0 - 7	47	8	0	46	101	85.5%	100%

Serial Testing clinical performance

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RT-PCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing.

Performance of the antigen test with serial testing in individuals is described in Table 3.

Table 3 - Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	ASYMPTOMATIC ON FIRST DAY OF TESTING			SYMPTOMATIC ON FIRST DAY OF TESTING		
	Ag Positive/PCR Positive (Antigen Test Performance % PPA)					
	1 Test	2 Tests	3 Tests	1 Test	2 Tests	3 Tests
0	9/97 (9.3%)	35/89 (39.3%)	44/78 (56.4%)	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	17/34 (50.0%)	23/34 (67.6%)	25/32 (78.1%)	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	16/21 (76.2%)	15/20 (75.0%)	13/15 (86.7%)	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	20/28 (71.4%)	21/27 (77.8%)	16/18 (88.9%)	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	13/23 (56.5%)	13/22 (59.1%)	4/11 (36.4%)	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	5/9 (55.6%)	5/8 (62.5%)		4/9 (44.4%)	3/7 (42.9%)	

1 Test= one (1) test performed on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.
2 Tests= two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.
3 Tests= three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

ANALYTICAL PERFORMANCE

Limit of Detection (Analytical Sensitivity)

The LoD for the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) was established using limiting dilutions of a viral sample inactivated by gamma irradiation. The highest concentration was $1x10^6$ $TCID_{50}$/ mL. In this study, designed to estimate the LoD of the assay when using a direct nasal swab, the starting material was spiked into a volume of pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2. An initial range finding study was performed testing devices in triplicate using a 10-fold dilution series. At each dilution, 50 µL samples were added to swabs and then tested in the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) using the product's instructions for use in a blinded manner. The test results were read by another laboratory personnel, confirmed and recorded by a third person. A concentration was chosen between the last dilution to give 3 positive results (3/3) and the first to give one negative results (2/3). Using this concentration, the LoD was further refined with a 2-fold dilution series. The last dilution demonstrating 100% positivity was then tested in an additional 20 replicates tested per lot in the same way (a total of three lots).

Based on the results above, the final LoD was confirmed as $5x10^3$ $TCID_{50}$/mL.

Cross-Reactivity (Analytical Specificity) and Microbial Interference

Cross-Reactivity and Microbial Interference studies were conducted to determine if other respiratory pathogens that could be present in a nasal sample could cause a false-positive test result, or interfere with a true positive result. A panel of seventeen (17) viruses, nine (9) bacteria and one (1) fungi, and pooled human nasal wash was evaluated in this study. Each sample was tested in

triplicate. No cross-reactivity or interference was seen with the following microorganisms when tested at the concentration presented in Table 4 below.

Table 4 - Summarized Result of Cross-Reactivity Study

Microorganism Name	Test Concentration
Human coronavirus NL63	$1x10^5$ $TCID_{50}$/mL
MERS-coronavirus	$1x10^5$ $TCID_{50}$/mL
Human coronavirus 229E	$1x10^5$ $TCID_{50}$/mL
Human coronavirus OC43	$1x10^5$ $TCID_{50}$/mL
Human Adenovirus 1	$1x10^6$ $TCID_{50}$/mL
Human Metapneumovirus 3 (hMPV-3) Type B1	$1x10^5$ $TCID_{50}$/mL
Parainfluenza virus Type 1	$1x10^7$ $TCID_{50}$/mL
Parainfluenza virus Type 2	$1x10^5$ $TCID_{50}$/mL
Parainfluenza virus Type 3	$1x10^7$ $TCID_{50}$/mL
Parainfluenza virus Type 4A	$1x10^5$ $TCID_{50}$/mL
Influenza A/Perth/16/09 (H3N2)	$1x10^5$ $TCID_{50}$/mL
Influenza A/California/07/09 (H1N1)	$1x10^5$ $TCID_{50}$/mL
Influenza B/Brisbane/60/08 (Victoria lineage)	$1x10^5$ $TCID_{50}$/mL
Influenza B/Wisconsin/01/10 (Yamagata lineage)	$1x10^5$ $TCID_{50}$/mL
Enterovirus B111 2015 isolate	$1x10^5$ $TCID_{50}$/mL
Respiratory syncytial virus	$1x10^5$ $TCID_{50}$/mL
Rhinovirus Type 1A	$1x10^5$ $TCID_{50}$/mL
Haemophilus influenzae type b (Eagan)	$1x10^7$ CFU/mL
Streptococcus pneumoniae Z022	$1x10^7$ CFU/mL
Streptococcus pyogenes Z018	$1x10^7$ CFU/mL
Candida albicans Z006	$1x10^7$ CFU/mL
Pooled human nasal wash	NA
Bordetella pertussis A639	$1x10^7$ CFU/mL
Mycoplasma pneumoniae M129	$1x10^7$ CCU/mL
Chlamydia pneumoniae	$1x10^7$ IFU/mL
Legionella pneumophila Philadelphia	$1x10^7$ CFU/mL
Staphylococcus aureus MRSA; COL	$1x10^7$ CFU/mL
Staphylococcus epidermidis MRSE; PR62A	$1x10^7$ CFU/mL

To estimate the likelihood of cross-reactivity with SARS-CoV-2 virus in the presence of organisms that were not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology.

- Homology exists between the SARS-CoV-2 nucleocapsid protein and the Human Coronavirus HKU1. The homology is relatively low, but the possibility of cross-reactivity cannot be ruled out.
- High homology exists between the SARS-CoV-2 nucleocapsid protein and hCoV-SARS. This presents a high likelihood of cross-reactivity.
- No significant similarities were found between the SARS-CoV-2 nucleocapsid protein and the pneumocystis jirovecii (PJP). This presents a low likelihood of cross-reactivity.
- No significant similarities were found between the SARS-CoV-2 nucleocapsid protein and the mycobacterium tuberculosis. This presents a low likelihood of cross-reactivity.

Hook Effect

No high dose hook effect was observed when tested up to a concentration of $4.57x10^6$ $TCID_{50}$/ mL of UV-Inactivated SARS-CoV-2 virus with the Wondfo Antigen test card.

Endogenous Interference Substances Studies

A study was conducted to determine if any substances, naturally present in respiratory specimens or that may be artificially introduced

into the nasal cavity listed in the table interfere in the performance of the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method). Test performance was evaluated in the absence and presence of SARS-CoV-2 (3x LoD). None of the substances listed in the Table 4 below interfered with the performance of the Wondfo 2019-nCoV Antigen Test (Lateral Flow Method).

Table 5 - List of potential endogenous interference substances

Potentially Interfering Substance	Original Substance Form	Working Concentration
Whole Blood	Liquid	4%
Mucin	Powder (20mg in 0.4mL 0.9% saline)	0.5%
Chloraseptic (Menthol/Benzocaine)	Tablet (Crush, 15mg in 1mL 0.9% saline)	1.5 mg/mL
Naso GEL (NeilMed)	Liquid	5% v/v
CVS Nasal Drops (Phenylephrine)	Liquid	15% v/v
Afrin (Oxymetazoline)	Liquid	15% v/v
CVS Nasal Spray (Cromolyn)	Liquid	15% v/v
Zicam	Liquid	5% v/v
Homeopathic (Alkalol)	Liquid	1:10 dilution
Sore Throat Phenol Spray	Liquid	15% v/v
Tobramycin	Powder (10mg in 1mL 0.9% saline)	4 µg/mL
Mupirocin	Powder (10mg in 100µL DMSO)	10 mg/mL
Fluticasone Propionate	Liquid	5% v/v
Tamiflu (Oseltamivir Phosphate)	Powder (75mg in 1.5mL 0.9% saline)	5 mg/mL

ASSISTANCE

If you have any questions regarding the use of this product, please call Wondfo's Technical Support Number (+1) 630-468-2199 from 7:00 a.m. to 6:00 p.m., Central Time.

REFERENCES

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2. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html Accessed March 30, 2020.
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5. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Azman AS, Reich NG, Lessler J. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020 May 5;172(9):577-582.

INDEX OF SYMBOLS

Tests per kit	See Instruction for Use	Expiry Date
LOT Batch Number	Manufacturing Date	Keep Dry
Manufacturer	REF Catalog #	Keep away from Sunlight
Store between 2~30℃	Do not reuse	IVD For *In vitro* Diagnostic Use

CONTROL + Positive control

CONTROL − Negative control

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