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Sona Nanotech COVID-19 Lateral Flow Assay

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■ Intended Use

The **Sona Nanotech COVID-19 Lateral Flow Assay** is an immunochromatographic assay for the qualitative detection of the spike protein antigen from SARS-CoV-2 in nasopharyngeal (NP) swab specimens from individuals who are suspected of COVID-19 by their healthcare provider. The assay is intended for professional and laboratory use.

The Sona Nanotech COVID-19 LFA does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 spike protein antigen. Antigen is generally detectable in upper respiratory specimens 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. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. If you are located outside of the USA, consult with your public health authority regarding reporting requirements.

Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary for patient management. Negative results do not rule out COVID-19 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.

The Sona Nanotech COVID-19 LFA is intended for use by trained clinical laboratory personnel and individuals trained in point of care settings.

■ Summary and Explanation

Coronavirus Disease 2019 (COVID-19) is a severe respiratory illness caused by the SARS-CoV-2 Coronavirus. The first documented cases of COVID-19 were reported in Wuhan City, China in December 2019. The WHO declared that COVID-19 was a pandemic on March 11, 2020, since then human infection has spread globally, with millions of confirmed infections and over 700,000 deaths[1].

The incubation period for COVID-19, which is the time between exposure to the virus (becoming infected) and symptom onset, is on average 5-6 days, however, can be up to 14 days[2]. The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough, shortness of breath and loss of taste or smell.

■ Principles of the Test

The **Sona Nanotech COVID-19 Lateral Flow Assay** employs lateral flow technology in a sandwich design to detect spike protein antigen from SARS-CoV and SARS-CoV2. The test allows for the detection of SARS-CoV and SARS-CoV-2. The test detects, but does not differentiate, between the two viruses.

The patient nasopharyngeal swab is placed in a microfuge tube containing reagent solution which extracts the sample from the swab tip. After extraction, the sample is applied to the test cartridge using a Dual Bulb Fixed Volume Pipette where SARS-CoV-2 spike protein antigens in the specimen will react with the reagents in the test cartridge.

If the specimen contains SARS-CoV-2 spike protein antigens, a grey-to-blue Test Line along with a blue procedural Control Line will appear on the test cartridge indicating a positive result. If SARS-CoV-2 spike protein antigens are not present, or are present at very low levels, only the blue procedural Control Line will appear.

■ Reagents and Materials Supplied (Table 1)

Test Kit	REF	Quantity
Sona Nanotech COVID-19 LFA	CV2-25	1
Reagents and Components	**REF**	**Quantity**
COVID-19 LFA Test Cartridge	TC-CV2	25
1.5ml Microfuge Tube	MT-CV2	25
Dual Bulb Fixed Volume Pipette (100µL)	TP-CV2	25
Nasopharyngeal Swab	NS-CV2	25
COVID-19 Reagent Solution Dropper Bottle (White Cap)	RS-CV2	2 x 15ml
Instructions for use	IFU-CV2	1
Procedure Card	PC-CV2	1

■ Reagents and Materials Required but not Supplied

- Timer or clock
- 1.5mL Microfuge tube rack
- PPE (Personal Protective Equipment)
- COVID-19 External Positive Control Dropper Bottle (Green Cap) – 3mL (Ref: EPC-CV2) *
- COVID-19 External Negative Control Dropper Bottle (Red Cap) – 3mL (Ref: ENC-CV2) *

*External Control Dropper Bottles may be purchased separately by contacting Sona Nanotech Customer Support Services. Refer to the Assistance section in this document for contact details.

■ Warnings and Precautions

- For in vitro diagnostic use only.
- Seek specific training or guidance if you are not experienced with specimen collection and handling procedures.
- To obtain accurate results, you must follow these Instructions for Use.
- Do not use the test kit beyond the stated expiration date marked on the pouch and package label.
- Do not use the test kit if the pouch is damaged or the seal is broken.
- Do not mix components from different lot numbers because each lot is quality control tested as a standard batch unit.
- Do not reuse the test components.
- All patient samples should be handled as being potentially infectious. Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Decontaminate and dispose of all samples, reaction kits and potentially contaminated materials safely in accordance with your biohazard waste disposal regulation.
- The test cartridge is sensitive to humidity as well as heat. Perform the test immediately after removing the test cartridge from the foil pouch.
- Do not touch the membrane in the result window of the test cartridge.
- When collecting a nasopharyngeal swab sample, use the nasopharyngeal swab supplied within this kit or a suitable alternative i.e. a nasopharyngeal swab with a polyester flocked mini-tip and plastic shaft.
- Do not use nasopharyngeal swabs that have been treated with calcium alginate.
- Prior to collecting the nasopharyngeal sample swab, the patient should be instructed to blow their nose.
- Inadequate or inappropriate specimen collection may yield false negative test results.
- Results must not be interpreted past 30 minutes from adding the sample to the test cassette.
- Nasopharyngeal swab samples stored or transported in Viral Transport Media (VTM) **must not be used** with this device.
- Nasopharyngeal swab samples should be tested immediately after collection.

■ Kit Storage and Stability

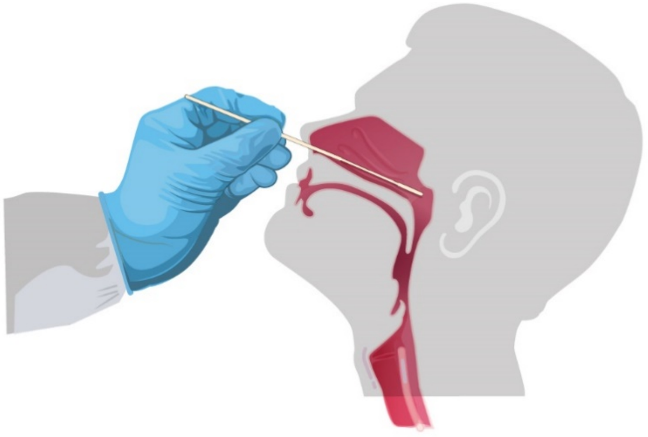
- Store the test kit at 2 - 30°C (35-86°F). DO NOT FREEZE.
- Do not store the test kit in direct sunlight.
- The test kit is stable within the expiration date marked on the pouch and package label.

■ Specimen Collection and Handling

Proper specimen collection is critical to the performance of this Test. Specimens should be tested immediately after collection.

Specimen Collection Nasopharyngeal Swab Sample:
Prior to collecting the nasopharyngeal sample swab, **the patient should be instructed to blow their nose.**

To collect a nasopharyngeal swab sample, carefully insert the sterile nasopharyngeal swab into the nostril that presents the least nasal secretions under visual inspection. Insert the swab through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. The swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab 3 – 5 times in a clockwise direction. Leave swab in place for several seconds to absorb secretions.



■ Quality Control - Refer to Procedure Card for illustrations

Built-in Control Features

The Sona Nanotech COVID-19 Lateral Flow Assay contains built-in procedural control features. The manufacturer's recommendation for daily control is to document these built-in procedural controls for the first sample tested each day.

Each Sona Nanotech COVID-19 Lateral Flow Assay test device has built-in controls. The Control line can be considered as an internal positive procedural control, i.e., a proper amount of sample was used, sample was properly added to the sample port, sample migrated properly, and the reagent system worked properly. A distinct blue Control line should always appear if the test has been performed correctly. If the blue Control line does not develop at 15 minutes, the test result is invalid, and a new test should be performed.

A built-in negative control is provided by the clearing of blue background color, verifying that the test has been performed correctly. Within 15 minutes, the result area should be white to light blue and allow the clear interpretation of the test result. If background color appears and interferes with interpretation of the test result, the result is considered invalid. Should this occur, review the procedure and repeat the test with a new Device.

External Quality Control

External controls may also be used to demonstrate that the reagents and assay procedure perform properly.

Sona Nanotech recommends that positive and negative controls be 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.

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

External Positive and Negative Control Dropper Bottles should be tested using the External Control Test Procedure provided in this Package Insert.

Test Procedure

Procedural Notes

- The test procedure below must be followed to obtain accurate and reproducible results. Expiration date: Check expiration on each individual test package or outer box before using. Do not use any test past the expiration date on the label.
- Reagents, specimens, and test cartridges must be at 15-30°C for testing.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time. Use only one patient sample for each test cartridge.
- Label the test cartridge with the patient identification or control to be tested.
- Place test cartridge on a level surface.

Direct Nasopharyngeal Swab Procedure

1.

- **a)** Unscrew the WHITE cap from the Reagent Solution Dropper Bottle.
- **b)** Add **1mL** of the Reagent Solution to the Microfuge Tube supplied, add dropwise to the **1mL line** indicated on the **Microfuge Tube**. To obtain accurate results it is important to ensure that the Reagent Solution is as close as possible to the 1mL line. If too much is added a new Microfuge Tube should be used.

2.

- **a)** Immerse the nasopharyngeal sample swab into the Reagent Solution, roll the swab at least 3 times while pressing the head against the bottom and side of the Microfuge Tube. Leave the swab in the Microfuge Tube for 1 minute.
- **b)** Roll the swab head against the inside of the Microfuge Tube at least 3 times as you remove it.
- **c)** Dispose of the used swab in accordance with your biohazard waste disposal protocol.

3.

- **a)** Fill the provided small, clear fixed volume pipette (100µL) with the patient sample from the microfuge tube.
- **b)** To fill the fixed volume pipette with the patient sample FIRMLY squeeze the top bulb.
- **c)** Still squeezing, place the fixed volume pipette tip into the patient sample until the tip is approximately halfway into the microfuge tube. Avoid the collection of mucoid substances when filling the pipette.
- **d)** With the fixed volume pipette tip still in the patient sample, slowly release pressure on bulb to fill the pipette.

4.

- **a)** Squeeze the upper bulb of the fixed volume pipette again to dispense 100µL of the sample into the test cartridge Sample Port.
- **b)** Read test results at 15 minutes.

Refer to the Procedure Card A – Direct Nasopharyngeal Swab Procedure for a visual representation of the procedure.

External Control Dropper Bottle Procedure

1.

- **a)** Note: The External Positive Control Dropper Bottle has a GREEN Cap, the External Negative Control Dropper Bottle has a RED cap.
- **b)** Remove the cap from the External Control Dropper Bottle.

2.

- **a)** Holding the Dropper Bottle directly above the cassette sample port squeeze the bottle gently to dispense **4 drops** of the control solution.
- **b)** Read test results at 15 minutes.

Refer to the Procedure Card B – External Control Dropper Bottle Procedure for a visual representation of the procedure.

Interpretation of the Result

Positive*: At 15 minutes the appearance of ANY shade of a grey-to-blue Test Line AND the appearance of a blue procedural Control Line indicates a positive result for the presence of SARS-CoV-2 viral antigen.

Note: The Test line may vary in shade and intensity (light or dark, weak or strong) depending on the concentration of antigen detected. The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result.

*A positive result does not rule out co-infections with other pathogens.

Negative Result:**

At 15 minutes, the appearance of ONLY the blue procedural Control Line indicates SARS-CoV-2 viral antigen was not detected. A negative result should be reported as a presumptive negative for the presence of SARS-CoV-2 antigen.

**A negative result does not exclude SARS-CoV-2 viral infection. Negative results should be confirmed by a confirmatory test.

Invalid Result:

If at 15 minutes, the blue procedural Control Line does not appear, even if any shade of a grey-to-blue Test Line appears, the result is considered invalid. If at 15 minutes, the background color does not clear and it interferes with the reading of the test, the result is considered invalid. If the test is invalid, a new test should be performed with a new patient sample and a new Device.

Warning: Results must not be interpreted past 30 minutes

Limitations

- The contents of this kit are to be used for the qualitative detection of SARS-CoV-2 antigen from nasopharyngeal swabs.
- This test detects both viable (live) and non-viable, SARS-CoV, and SARS-CoV-2.
- Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
- Failure to follow the Test Procedure and Interpretations of Test Results may adversely affect test performance and/or invalidate the Test Result.
- Test Results must be evaluated in conjunction with other clinical data available to the physician.
- Negative test results do not rule-out possible other non-SARS-CoV-2 viral infections.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- Positive test results do not rule out co-infections with other pathogens.
- Positive and negative predictive values are highly dependent on prevalence.
- Users should test specimens immediately after specimen collection.
- False negative test results are more likely during peak activity when prevalence of disease is high.
- False positive test results are more likely during periods of low SARS-CoV-2 activity when prevalence is moderate to low.

Summary of Performance Characteristics

Analytical Performance – Limit of Detection (LoD)

The confirmed LoD using live SARS-CoV-2 virus diluted in simulated nasal matrix (SNM) was 4.5 × 10^3 $TCID_{50}$/ml. The LoD of sample diluted in Reagent Solution is 2.14 × 10^2 $TCID_{50}$/mL

Analytical Performance – Inclusivity/Cross reactivity

A total of 28 viruses, bacteria, and fungi, and pooled nasal wash were tested for exclusivity and microbial interference.

Only SARS-CoV resulted in a positive test result. None of the organisms tested resulted in a false-negative result in SARS-CoV-2 spiked samples.

Analytical Performance – Hook Effect

No hook effect was seen with SARS-CoV-2 isolate USA_WA1/2020 at 6.81 x 10^4 $TCID_{50}$/ml. or Hong Kong/VM20001061/2020 at 1.95 x 10^5 $TCID_{50}$/ml.

Analytical Performance - Interfering Substances

False positive results were not observed at 2.5% v/v with Zicam and NeilMed Naso GEL.

False negative results were not observed with Oxymetazoline nasal spray at 7.5% v/v and 3.75% v/v nasal gel.

Clinical Performance

The confirmed PPA (Sensitivity) is 85%, (CI95% - 72.6 - 96.6), NPA (specificity) is 90% (CI95% -82.1 - 97.6) utilising 99 patient samples from symptomatic (59) and asymptomatic (40) patients.

Analytical Performance Characteristics

The Sona Nanotech COVID-19 LFA Assay validation was performed using Puritan 25-3316-H Nasopharyngeal Swabs.

■ Analytical Performance – Limit of Detection (LoD)

A preliminary LoD was determined by first testing serial ten-fold dilutions (1/10, 1/100, and 1/1000) of SARS-CoV-2 stock diluted in simulated nasal matrix (SNM – phosphate buffered saline with 15% glycerol, 2.5% porcine mucin, and 1% human blood). A 50 µL sample of SARS-CoV-2 diluted in SNM was added to a tube, then a dry nasopharyngeal swab added to the tube followed by 1000 µL of Reagent Solution (1:20 dilution). The swab was used to mix the sample, then 100 µL of diluted sample loaded onto triplicate Test Cartridges. The 1/10 dilution tested positive on all three replicates, and the 1/100 and 1/1000 dilutions tested negative on all three replicates. To further define the preliminary LoD, the 1/10 dilution was serially diluted two-fold in SNM to prepare 1/20, 1/40, and 1/80 dilutions of SARS-CoV-2. The 1/20 dilution tested positive on all three replicates, and the 1/40 and 1/80 dilutions tested negative.

The 1/20 dilution was selected as the preliminary LoD of 3.41 ×10^3TCID$_{50}$/ml. The results of the preliminary LoD testing are summarized in Table 2.

Table 2 – Preliminary LoD determination

SAMPLE	TCID50/mL (in SNM)	TEST RESULTS					
		REPLICATE 1		REPLICATE 2		REPLICATE 3	
		+	-	+	-	+	-
Positive control	-	+	+	+	+	+*	+*
Negative control	-	-	+	-	+	-	+
SNM (unspiked)	-	-	+	-	+	-	+
1/10 SARS-CoV-2	6.81×10^3	+	+	+	+	+	+
1/100 SARS-CoV-2	6.81×10^2	-	+	-	+	-	+
1/1000 SARS-CoV-2	6.81×10^1	-	+	-	+	-	+
1/20 SARS-CoV-2	3.41×10^3	+	+	+	+	+	+
1/40 SARS-CoV-2	1.70×10^3	-	+	-	+	-	+
1/80 SARS-CoV-2	8.51×10^2	-	+	-	+	-	+

T = Test Line C = Control Line.
* Not enough positive control reagent to perform test.

An additional twenty (20) replicates at the presumptive LoD of 3.41 × 10^3 TCID^{50}/mL were prepared and tested for confirmation, resulting in only 15/20 positive results. Since the LoD is defined as the virus concentration in which ≥ 19/20 test replicates are positive, a 1/15 dilution of SARS-CoV-2 in SNM (4.50x10^3 TCID50/mL) was prepared. All twenty replicates of the 1/15 dilution tested positive, resulting in a confirmed LoD (1X LoD) of 4.50 × 10^3 TCID^{50}/mL. The LoD was challenged by testing twenty replicates at a 0.1X LoD concentration of 4.50 × 10^2 TCID^{50}/mL. All twenty 0.1X LoD replicates tested negative (data not shown). The results of the LoD confirmation testing are summarized in Table 3 and table 4.

Table 3- LoD Confirmation

SAMPLE	TCID50/mL (in SNM)	TEST RESULTS	
		T	C
1/20 SARS-CoV-2	3.41×10^3	-	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	+	+
1/20 SARS-CoV-2	3.41×10^3	-	+
1/20 SARS-CoV-2	3.41×10^3	-	+
1/20 SARS-CoV-2	3.41×10^3	-	+
1/20 SARS-CoV-2	3.41×10^3	-	+

T = Test Line. C = Control Line.

Table 4 – LoD Confirmation

SAMPLE	TCID50/mL (in SNM)	TEST RESULTS	
		T	C
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+
1/15 SARS-CoV-2	4.50×10^3	+	+

T = Test Line. C = Control Line.

The LoD for the Sona Nanotech COVID-19 Lateral Flow Assay was determined to be 4.50 × 10^3 TCID^{50}/mL using live SARS-CoV-2 virus diluted in SNM. When the 1:20 sample dilution factor is considered (50 µL of SARS-CoV-2 spiked SNM added to 1000 µL of Reagent Solution), the LoD for the Sona Nanotech COVID-19 Lateral Flow Assay as loaded onto the Test Cassette is 2.14 × 10^2 TCID^{50}/mL.

■ Exclusivity and Microbial interference

To determine if other respiratory pathogens that could be present in a nasopharyngeal (NP) swab sample could cause a false-positive test result, a panel of sixteen (16) viruses, eight (8) bacteria, three (3) fungi, and pooled human nasal wash were tested by spiking 1:20 SNM:Reagent Solution with high concentrations of organism stocks. Target organism concentrations were ≥ 10^5 TCID^{50}/mL, PFU/mL, or CEID^{50}/mL for viruses, and ≥ 10^6 cfu/mL for bacteria and fungi. When the target concentration was not achievable due to the titer of the stock culture, a 1/10 dilution was tested. A 1/10 dilution in SNM:Reagent Solution was used for testing pooled nasal wash.

The organisms utilized for exclusivity and microbial interference testing are listed in Table 5. Exclusivity test results are summarized in Table 9. False-positive results were seen only with SARS-CoV. The cross-reactivity with SARS-CoV was not surprising due to similarities in amino acid sequence between SARS-CoV-2 and SARS-CoV (76% homology with multiple regions of identical amino acid sequence).

Table 5. Exclusivity and Microbial Interference Organisms

ID	Organism	Source/Strain/ID#	Lot#/Harvest Date	Stock Conc.	Test Conc.	Units
229E	Human coronavirus 229E	ATCC VR-740	70033323	1.6×10^7	1.6×10^5	TCID50/mL
OC43	Human coronavirus OC43	Zeptometrix 0810024CF	323852	1.51×10^6	1.5×10^5	TCID50/mL
NL63	Human coronavirus NL63	MRI	N/A	4.4×10^5	4.4×10^4	TCID50/mL
SARS	SARS-coronavirus	MRI Urbani	WS#1 3/24/20	1.58×10^4	1.58×10^4	TCID50/mL
MERS	MERS-coronavirus	MRI EMC/2012	WS#1 3/25/20	5.0×10^4	5.0×10^3	TCID50/mL
AV1	Adenovirus 1	ATCC VR-1	70007874	2.2×10^7	2.2×10^5	TCID50/mL
hMPV	hMPV	BEI NR-22227	355	2.8×10^6	2.8×10^5	TCID50/mL
P1	Parainfluenza virus 1	ATCC VR-94	70016021	1.6×10^7	1.6×10^5	TCID50/mL
P2	Parainfluenza virus 2	ATCC VR-92	70004593	5.9×10^6	3.0×10^5	TCID50/mL
P3	Parainfluenza virus 3	ATCC VR-93	70027437	1.6×10^8	1.6×10^5	TCID50/mL
P4	Parainfluenza virus 4a	ATCC VR-1377	64398052	1.6×10^5	1.6×10^4	TCID50/mL
FluA	Influenza A	ATCC VR-1894	70014833	5.2×1^{07}	5.2×10^5	CIED50/mL
FluB	Influenza B	ATCC VR-1931	70020870	7.8×10^6	3.9×10^5	TCID50/mL

ID	Organism	Source/Strain/ID#	Lot#/Harvest Date	Stock Conc.	Test Conc.	Units
EV68	Enterovirus 68	ATCC VR-1826	70019851	1.6×10^{7}	1.6×10^{5}	TCID50/mL

Table 5. Continued - Exclusivity and Microbial Interference Organisms

ID	Organism	Source/Strain/ID#	Lot#/Harvest Date	Stock Conc.	Test Conc.	Units
RSV	Respiratory syncytial virus	ATCC VR-26	70024483	8.0×10^{6}	8.0×10^{5}	PFU/ML
RV	Rhinovirus	ATCC VR-1601	57866034	8.89×10^{5}	8.89×10^{4}	TCID50/mL
HI	*Haemophilus influenzae*	ATCC 49247	70023140	5.4×10^{8}	5.4×10^{6}	cfu/mL
SPN	*Streptococcus pneumoniae*	ATCC 49619	70027361	8.95×10^{5}	8.95×10^{4}	cfu/mL
SPY	*Streptococcus pyogenes*	ATCC 19615	70016309	1.39×10^{7}	1.39×10^{6}	cfu/mL
CA	*Candida albicans*	ATCC 14503	08/24/18	1.7×10^{9}	1.7×10^{7}	cfu/mL
BP	*Bordetella pertussis*	ATCC 9797	09/04/18	2.4×10^{9}	2.4×10^{7}	cfu/mL
MP	*Mycoplasma pneumonia*	ATCC 15531-TTR	70022921	1.0×10^{9}	1.0×10^{7}	cfu/mL
CP	*Chlamydia pneumoniae*	ATCC VR-1356	70019109	4.0×10^{7}	4.0×10^{6}	Ifu/mL
LP	*Legionella pneumophila*	Zeptometrix 801645	323903	1.88×10^{10}	1.88×10^{8}	cfu/mL
MT	*Mycobacterium tuberculosis*	Zeptometrix 801660	323674	6.86×10^{7}	6.86×10^{6}	cfu/mL
PC	*Pneumocystis carinii*	ATCC PRA-159	62297170	1.0×10^{8}	1.0×10^{6}	nuclei/mL
PJ	*P. jiroveci-S. cerevisiae* recombinant	Zeptometrix 801698	322301	1.56×10^{8}	1.56×10^{6}	cfu/mL
PNJ	Pooled Human Nasal Wash	Lee Biosolutions 991-26	20-03-511	N/A	N/A	N/A

Table 6. Exclusivity Test Results

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External Positive Control	+	+	+	+	+	+	Pass
External Negative Control	-	+	-	+	-	+	Pass
229E	-	+	-	+	-	+	Pass
OC43	-	+	-	+	-	+	Pass
NL63	-	+	-	+	-	+	Pass
SARS	+	+	+	+	+	+	Fail
MERS	-	+	-	+	-	+	Pass
AV1	-	+	-	+	-	+	Pass
hMPV	-	+	-	+	-	+	Pass
P1	-	+	-	+	-	+	Pass
P2	-	+	-	+	-	+	Pass
P3	-	+	-	+	-	+	Pass
P4	-	+	-	+	-	+	Pass
FluA	-	+	-	+	-	+	Pass
FluB	-	+	-	+	-	+	Pass
EV68	-	+	-	+	-	+	Pass
RSV	-	+	-	+	-	+	Pass
RV	-	+	-	+	-	+	Pass
HI	-	+	-	+	-	+	Pass
SPN	-	+	-	+	-	+	Pass
SPY	-	+	-	+	-	+	Pass
CA	-	+	-	+	-	+	Pass
BP	-	+	-	+	-	+	Pass
MP	-	+	-	+	-	+	Pass
CP	-	+	-	+	-	+	Pass
LP	-	+	-	+	-	+	Pass
MT	-	+	-	+	-	+	Pass
PC	-	+	-	+	-	+	Pass
PJ	-	+	-	+	-	+	Pass
PNJ	-	+	-	+	-	+	Pass

To determine if any of the respiratory pathogens in the test panel could interfere with the detection of a true positive test result, 1:20 SNM:Reagent Solution was spiked with both high concentration of potential interfering organism stock and a low level of SARS-CoV-2 (3X LoD). Results from Microbial Interference testing are summarized in Table 7. None of the organisms tested caused a false-negative test result in diluted SNM spiked with both organism and 3X LoD SARS-CoV-2.

Table 7. Microbial interference test results

Sample ID	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
	Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External Positive Control	+	+	+	+	+	+	Pass
External Negative Control	-	+	-	+	-	+	Pass
229E	-	+	-	+	-	+	Pass
OC43	-	+	-	+	-	+	Pass
NL63	-	+	-	+	-	+	Pass
SARS	+	+	+	+	+	+	Fail
MERS	-	+	-	+	-	+	Pass
AV1	-	+	-	+	-	+	Pass
hMPV	-	+	-	+	-	+	Pass
P1	-	+	-	+	-	+	Pass
P2	-	+	-	+	-	+	Pass
P3	-	+	-	+	-	+	Pass
P4	-	+	-	+	-	+	Pass
FluA	-	+	-	+	-	+	Pass
FluB	-	+	-	+	-	+	Pass
EV68	-	+	-	+	-	+	Pass
RSV	-	+	-	+	-	+	Pass
RV	-	+	-	+	-	+	Pass
HI	-	+	-	+	-	+	Pass
SPN	-	+	-	+	-	+	Pass
SPY	-	+	-	+	-	+	Pass
CA	-	+	-	+	-	+	Pass
BP	-	+	-	+	-	+	Pass
MP	-	+	-	+	-	+	Pass
CP	-	+	-	+	-	+	Pass
LP	-	+	-	+	-	+	Pass
MT	-	+	-	+	-	+	Pass
PC	-	+	-	+	-	+	Pass
PJ	-	+	-	+	-	+	Pass
PNJ	-	+	-	+	-	+	Pass

Pneumocystis jirovecii, was included in the exclusivity and microbial interference test panel as it is the causative agent of *P. jirovecii* pneumonia (PJP). However, *P. jirovecii* is difficult to culture. As a surrogate for *P. jirovecii*, a *P. jiroveci-S. cerevisiae* recombinant that was utilized for exclusivity and microbial interference testing in a predicate FDA EUA cleared COVID-19 lateral flow immunoassay was tested. Additionally, *Pneumocystis carinii* strain M167-6 was included in the test panel. *Pneumocystis jirovecii* was previously known as *Pneumocystis carinii*. In 2002 the name for the causative agent of *Pneumocystis carinii* pneumonia (PCP) in humans[3] was changed from *Pneumocystis carinii* to *Pneumocystis jirovecii*.

Human coronavirus HKU1 was not included in the wet testing panel, as it is difficult to culture and a source for this virus could not be found. Attempts to source clinical samples positive for HKU1 were also unsuccessful. Due to the inability to wet test HKU1 for exclusivity and interference, an *in silico* analysis was performed in which the spike protein amino acid sequences of HKU1 and SARS-CoV-2 were compared. The results of the *in silico* analysis are attached as Appendix A. In summary, one potential cross-reacting linear epitope was identified, and no potential cross-reacting conformational epitopes were identified. If one of the monoclonal antibodies used in the Sona Nanotech COVID-19 Lateral Flow Assay targets this common linear epitope, then cross-reactivity or interference could occur in a patient sample containing HKU1 coronavirus.

■ Interfering substances

To determine if endogenous or exogenous substances that might be present in a nasopharyngeal (NP) swab sample could cause a false-positive test result, a panel of eighteen (18) substances were tested by spiking 1:20 SNM:Reagent Solution at the concentrations recommended by the FDA[4].

The substances and concentrations tested are listed in Table 8, and test results summarized in Table 9. No false- positive results were seen with Naso GEL (NeilMed) and Zicam at 2.5% v/v.

Table 8 – interfering substances

Substance	Source/Item#	Lot No.	Exp. Date
Human Whole Blood (EDTA tube)	BIOIVT	HMN421040	7/29/2020
Mucin (bovine submaxillary type I-S)	Sigma M1778	SLCD6129	6/9/20211
Ricola (Menthol)	Ricola	N280101	0/31/2022
Sucrets (Dyclonin/Menthol)	Sucrets	026320T11	3/1/2022
Chloraseptic (Menthol/Benzocaine)	Chloraseptic Max	00989M11	8/1/2021
Naso GEL (NeilMed)	NeilMed	NG-214	2/1/2023
Nasal Drops (Phenylephrine)	CVS health	OAK0862	10/1/2022
Nasal Spray (Oxymetazoline)	CVS Health	72008	1/1/2022
Nasal Spray (Cromolyn)	Nasal Crom	294521	6/1/2021
Nasal Gel (Oxymetazoline)	Afrin	TN000AM	9/1/2020
Zicam	Zicam	B73765	11/1/2021
Homeopathic (Alkalol)	Alkalol	P8A004	1/1/2020
Fisherman's Friend	Lofthouse of Fleetwood	BL00136	9/3/2022
Sore Throat Phenol Spray	Chloraseptic	8161	10/1/2022
Tobramycin	Sigma TL1014	SLCB7407	6/9/2021
Mupirocin	N/A	N/A	N/A

Substance	Source/Item#	Lot No.	Exp. Date
Tamiflu (Oseltamivir Phosphate)	Sigma SML-1606	0000097332	N/A
Fluticasone Propionate	CVS Health	RL7205	9/1/2021

Table 9 – Interfering substances results

Sample ID	Test Conc.	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External positive control	N/A	+	+	+	+	+	+	Pass
External negative control	N/A	-	+	-	+	-	+	Pass
Human Whole Blood (EDTA tube)	4% v/v	-	+	-	+	-	+	Pass
Mucin (bovine submaxillary type I-S)	0.5%	-	+	-	+	-	+	Pass
Ricola (Menthol)	1.5mg/mL	-	+	-	+	-	+	Pass
Sucrets (Dyclonin/Menthol)	1.5mg/mL	-	+	-	+	-	+	Pass
Chloraseptic (Menthol/Benzocaine)	1.5mg/mL	-	+	-	+	-	+	Pass
Naso GEL (NeilMed)	2.5% v/v	-	+	-	+	-	+	Pass
Nasal Drops (Phenylephrine)	15% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Oxymetazoline)	15% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Oxymetazoline)	7.5% v/v	-	+	-	+	-	+	Pass
Nasal Spray (Cromolyn)	15% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	10% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	7.5% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	3.75% v/v	-	+	-	+	-	+	Pass
Zicam	2.5% v/v	-	+	-	+	-	+	Pass
Homeopathic (Alkalol)	10% v/v	-	+	-	+	-	+	Pass
Fisherman's Friend	1.5mg/mL	-	+	-	+	-	+	Pass
Sore Throat Phenol Spray	15% v/v	-	+	-	+	-	+	Pass
Tobramycin	1.5ug/mL	-	+	-	+	-	+	Pass
Mupirocin	10mg/mL	-	+	-	+	-	+	Pass
Tamiflu (Oseltamivir Phosphate)	5mg/mL	-	+	-	+	-	+	Pass
Fluticasone Propionate	%5 v/v	-	+	-	+	-	+	Pass

To determine if any of the potential interfering substances in the test panel could interfere with the detection of a true positive test result, 1:20 SNM:Reagent Solution was spiked with both potential interfering substance and a low level of SARS-CoV-2 (3X LoD). The test results are summarized in Table 10.

At the initial recommended test concentration, false-negative results were observed with Nasal Spray (Oxymetazoline) at 15% v/v and Nasal Gel (Oxymetazoline) at 10% v/v. The false-negative results dissipated when lower concentrations of substance were tested (Nasal Spray (Oxymetazoline) at 7.5% v/v and Nasal Gel (Oxymetazoline) at 3.75% v/v).

Table 10 - Interfering Substances Spiked with 3X LoD SARS-CoV-2 Test Results

Sample ID	Test Conc.	Replicate 1		Replicate 2		Replicate 3		Pass/Fail
		Test Line	Control Line	Test Line	Control Line	Test Line	Control Line	
External positive control	N/A	+	+	+	+	+	+	Pass
External negative control	N/A	-	+	-	+	-	+	Pass
Human Whole Blood (EDTA tube)	4% v/v	+	+	+	+	+	+	Pass
Mucin (bovine submaxillary type I-S)	0.5%	+	+	+	+	+	+	Pass
Ricola (Menthol)	1.5mg/mL	+	+	+	+	+	+	Pass
Sucrets (Dyclonin/Menthol)	1.5mg/mL	+	+	+	+	+	+	Pass
Chloraseptic (Menthol/Benzocaine)	1.5mg/mL	+	+	+	+	+	+	Pass
Naso GEL (NeilMed)	2.5% v/v	+	+	+	+	+	+	Pass
Nasal Drops (Phenylephrine)	15% v/v	+	+	+	+	+	+	Pass
Nasal Spray (Oxymetazoline)	15% v/v	-	+	-	+	-	+	Fail
Nasal Spray (Oxymetazoline)	7.5% v/v	+	+	+	+	+	+	Pass
Nasal Spray (Cromolyn)	15% v/v	-	+	+	+	+	+	Fail
Nasal Gel (Oxymetazoline)	10% v/v	-	+	-	+	-	+	Fail
Nasal Gel (Oxymetazoline)	7.5% v/v	-	+	-	+	-	+	Pass
Nasal Gel (Oxymetazoline)	3.75% v/v	+	+	+	+	+	+	Pass
Zicam	2.5% v/v	+	+	+	+	+	+	Pass
Homeopathic (Alkalol)	10% v/v	+	+	+	+	+	+	Pass
Fisherman's Friend	1.5mg/mL	+	+	+	+	+	+	Pass
Sore Throat Phenol Spray	15% v/v	+	+	+	+	+	+	Pass
Tobramycin	1.5ug/mL	+	+	+	+	+	+	Pass
Mupirocin	10mg/mL	+	+	+	+	+	+	Pass
Tamiflu (Oseltamivir Phosphate)	5mg/mL	+	+	+	+	+	+	Pass
Fluticasone Propionate	%5 v/v	+	+	+	+	+	+	Pass

■ Inclusivity and Hook Effect

No hook effect was seen with SARS-CoV-2 isolate USA_WA1/2020 at 6.81 x 104 TCID50/ml. or Hong Kong/VM20001061/2020 at 1.95 x 105 TCID50/ml.

Clinical Performance Characteristics

■ Clinical Performance

Clinical performance characteristics of the Sona COVID19 antigen test was evaluated in a prospective study at two different sites, where 99 patients were enrolled into the study and tested. Patients were a mix of symptomatic patients suspected of COVID19 (59) and asymptomatic (40) patients.

All samples were collected by qualified personnel using the nasopharyngeal swab method. Swabs were collected and tested on the Sona COVID19 antigen test according to product instructions, in a blinded fashion. The performance of the Sona COVID19 antigen test were compared to results of nasopharyngeal swabs, collected, stored in 3ml viral transport medium (VTM) and tested with an Emergency Use Authorised (EUA) molecular (RT-PCR) test for detection of SARS-CoV-2.

External control testing, using Sona COVID19 positive and negative controls were performed prior to sample testing each day, at all study sites.

Table 11 – Summary of performance of Sona COVID19 antigen test for the rapid detection of SARS-CoV-2 compared to RT-PCR

Sona COVID19 antigen test	**RT-PCR comparator method**		
	Positive	**Negative**	**Total**
Positive	33	6	39
Negative	6	54	60
Total	39	60	99
Positive Predictive agreement (PPA) – Sensitivity = 84.6% (CI 95% - 72.6 - 96.6)			
Negative Predictive Agreement (NPA) – Specificity = 90.0% (CI 95% - 82.1 - 97.6)			
Correlation to comparator (OPA) = 87.9%			

Infection within 0-8 days of symptom onset and/or low Ct counts have both been associated with an increased risk of infectiousness of COVID-19. Symptoms of infection and viral loads are present in patients in the early stages of the disease, with viral loads at levels detectable by rapid antigen test during this phase. As viral loads decrease, sensitivity of tests starts to diminish.

In this subgroup, The Sona LFA identified 17/18 RT-PCR positive patient samples that presented symptoms within 0-8 days (94% Sensitivity – CI95% - 76.0 – 100). As days from symptom onset increases, sensitivity levels decrease.

Table 12 - Positive Results Stratified by Days Since Symptom Onset (Sensitivity)

Days since symptom onset	Cumulative RT-PCR Positive (+)	Cumulative Sona COVID19 LFA (+)	PPA (Sensitivity)	95% Confidence Interval	Average Ct value
1 to 2	1	1	100%	68.8 - 100	17.2
3 to 4	3	3	100%	70.6 - 100	27.5
5 to 6	7	7	100%	73.9 - 100	28.9
7 to 8	18	17	94%	76.0 - 100	27.1
9 to 10	30	26	87%	76.4 - 97.6	30.0
11 to 12	31	27	87%	77.2. - 96.8	33.0
13 to 14	35	30	86%	78.7 - 93.3	30.0
15 to 16	37	32	86%	80.3 - 91.7	34.1
17 to 18	39	33	85%	80.2 - 89.8	31.8

■ Assistance

If you have any questions regarding the use of this product, please call Sona Nanotech's Technical Support Number +1-902-209-2232, Monday through Friday, from 9:00 a.m. to 5:00 p.m., Atlantic Time. If outside Canada, contact your local distributor or info@sonanano.com

■ References

[1] World Health Organization Coronavirus disease 2019 (COVID-19) Situation Report – 209; 16 Aug 2020.

[2] World Health Organization Coronavirus disease 2019 (COVID-19) Situation Report – 73; 2 April 2020.

[3] Stringer JR, Beard CB, Miller RF, Wakefield AE. 2002. A new name (Pneumocystis jiroveci) for Pneumocystis from humans. Emerg Infect Dis. Sep;8(9):891-6

[4] U.S. Food & Drug Administration. Antigen Template for Manufacturers. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) - Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff. Version May 11, 2020. Retrieved from: https://www.fda.gov/media/137907/download.

■ Glossary of symbols

🏭 **Sona Nanotech**
Dartmouth, NS B2Y 4M9 Canada
Sonanano.com

LOT
Batch code

🏭
Manufacturer

iu
Intended use

i
Consult instructions for use

Σ 25
Contains sufficient for 25 determinations

CONTROL +
Positive control

CONTROL –
Negative control

⌛
Use by

🌡
Temperature limitation

IVD
For *In Vitro* diagnostic use

EC REP
OBELIS S.A
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CE
CE mark