Flowflex™ COVID-19 Antigen Rapid Test Package Insert

REF L031-13015	English

A rapid test for the detection of SARS-CoV-2 nucleocapsid antigens in anterior nasal swab specimens.
For professional in vitro diagnostic use only. For the most up to date information on COVID-19, please visit: www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection.html

INTENDED USE

The Flow*flex* COVID-19 Antigen Rapid Test is a rapid, lateral flow chromatographic immunoassay intended for the qualitative detection of the SARS-CoV-2 nucleocapsid protein antigen from anterior nasal swab specimens directly from individuals within 7 days of symptom onset or without symptoms or other epidemiological reasons to suspect COVID-19. The test is intended for serial testing of symptomatic individuals for use at least twice with 48 hours between tests, or for serial testing of asymptomatic individuals for use at least three times with 48 hours between tests.
Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. This antigen is generally detectable in anterior nasal swabs 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 are 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 an individual'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. Laboratories may be required to report results to the appropriate public health authorities.
The Flow*flex* COVID-19 Antigen Rapid Test is intended for use by healthcare professionals or operators who are proficient in performing tests in point of care settings.

SUMMARY AND EXPLANATION

The novel coronaviruses 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. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

PRINCIPLE

The Flow*flex* COVID-19 Antigen Rapid Test is a qualitative membrane based chromatographic immunoassay for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in human anterior nasal swab specimens.
When specimens are processed and added to the test cassette, SARS-CoV-2 antigens, if present in the specimen, will react with the colored anti-SARS-CoV-2 antibody-coated particles, which have been pre-coated on the test strip. The antigen-antibody complex then migrates toward the membrane by capillary action. This complex is then captured by anti-SARS-CoV-2 monoclonal antibodies immobilized at the test line region, and a colored line appears on the membrane. Test results are interpreted visually at 15-30 minutes based on the presence or absence of visually colored lines.
To serve as a procedure control, a red or pink line will always appear in the control line region after proper volume of specimen has been added, and membrane wicking has occurred.

REAGENTS

The test cassette contains anti-SARS-CoV-2 antibodies.

PRECAUTIONS

- Read the Flow*flex* COVID-19 Antigen Rapid Test Package Insert carefully before performing a test. Follow directions for use. Failure to follow directions may produce inaccurate test results.
- 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.
- This product has been designed only for the detection of SARS-CoV-2 antigen, not for any other viruses or pathogens.
- False negative test results may occur if a specimen is incorrectly collected or handled.
- To obtain accurate results, the test must be performed as indicated in this Instructions for Use.
- **INVALID RESULTS**, indicated by no Control Line, can occur when an insufficient volume of sample solution is added to the test cassette's sample well. Four (4) drops of solution must be dispensed into the sample well of test cassette, by gently squeezing the tube.
- Swabs in the kit are approved for use with Flow*flex* COVID-19 Antigen Rapid Test. Do not use other swabs.
- Do not use on anyone under two years of age. Keep test kit and materials out of the reach of children and pets, before and after use.
- Leave the test cassette sealed in its pouch until just before use. Once opened, the test cassette should be used within 60 minutes.
- Do not use the test after the expiration date shown on the test cassette pouch.
- Do not use if any of the test kit contents or packaging is damaged or open.
- Test components are single use. Do not re-use. Do not use with multiple specimens.
- Make sure there is sufficient light when reading and interpreting test results.
- Do not use nasal sprays for at least 30 minutes before collecting a nasal sample.
- Remove any piercings from the nose before starting the test. Do not use on anyone who is prone to nosebleeds or has had facial injuries or head injuries/surgery in the past six months.
- Inadequate or improper nasal swab sample collection may result in false negative test results.
- Do not touch the swab tip when handling the swab.
- Test samples immediately after collection, and no more than one hour after the swab is added to the reagent solution, if stored at room temperature.
- The test is intended to be read at 15 minutes. If the test is read before 15 minutes or after 30 minutes, false negative or false positive results may occur, and the test should be repeated with a new test cassette.
- Avoid exposure of your skin, eyes, nose, or mouth to the solution in the extraction tube.
- Do not ingest any kit components
- Cross-reactivity with human coronavirus HKU1 cannot be completely ruled out.
- The control swabs should be used within 60 minutes
- The reagent solution in the tube contains a harmful chemical (see table below). If the solution contacts the skin or eye, flush with plenty of water.

Hazardous Ingredients for the Reagent Solution		
Chemical Name/ Concentration	Harms (GHS) code for each ingredient	Concentration
TX-100	Acute toxicity, Oral (Category 4), H302 Skin irritation (Category 2), H315 Serious eye damage (Category 1), H318 Short-term (acute) aquatic hazard (Category 1), H400 Long-term (chronic) aquatic hazard (Category 1), H410	1%
Sodium Azide	Acute toxicity, Oral (Category 2), H300 Acute toxicity, Dermal (Category 1), H310 Specific target organ toxicity - repeated exposure, Oral (Category 2), Brain, H373 Short-term (acute) aquatic hazard (Category 1), H400 Long-term (chronic) aquatic hazard (Category 1), H410	0.02%

If INHALED: Move to fresh air. If not breathing, give artificial respiration. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. Immediate medical attention is required.
If Contact with SKIN: Immediately remove all contaminated clothing. Flush the skin with plenty of water for at least 15 minutes. Immediate medical attention is required.
If Contact with EYES: Immediately flush the eyes with plenty of water for at least 15 minutes. Ensure adequate flushing by separating the eyelids with fingers. Immediate medical attention is required.
If INGESTED: Rinse the mouth with water. Do not induce vomiting. Risk of aspiration! Keep airways free. Pulmonary failure possible after aspiration of vomit. Immediate contact a physician or Poison Control Center.

STORAGE AND STABILITY

- The kit can be stored at temperatures between 36-86°F (2-30°C).
- The test is stable until the expiration date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- DO NOT FREEZE.
- Do not use after the expiration date.

MATERIALS

Materials Provided

- Test Cassettes
- Positive Control Swab
- Disposable Nasal Swabs
- Tube holder
- Extraction Buffer Tubes
- Negative Control Swab
- Package Insert

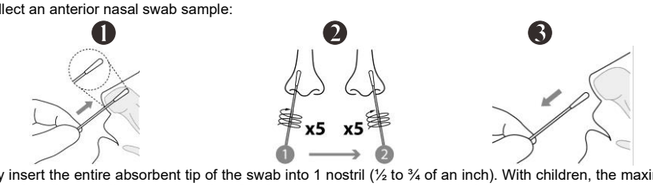
Note: *This test comes in a 25-test quantity.*

Materials Required But Not Provided

- Timer

SPECIMEN COLLECTION AND PREPARATION

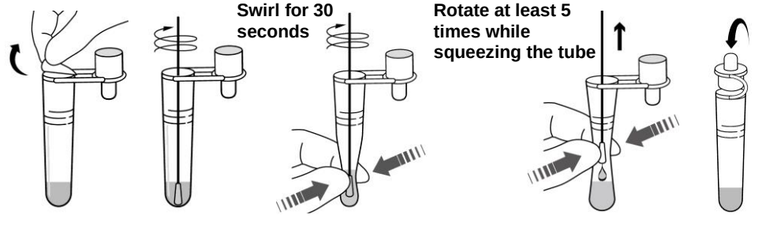
- The Flow*flex* COVID-19 Antigen Rapid Test is performed using anterior nasal swab specimens.
- Wash or sanitize your hands. Make sure they are dry before starting the test.
- Open the test cassette pouch and lay the cassette on a clean, flat surface.
- To collect an anterior nasal swab sample:

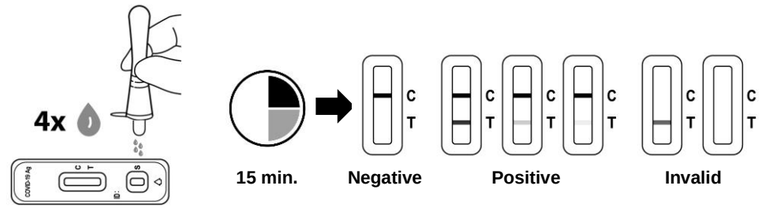


1. Gently insert the entire absorbent tip of the swab into 1 nostril (½ to ¾ of an inch). With children, the maximum depth of insertion into the nostril may be less than ¾ of an inch and you may need to have a second person to hold the child's head while swabbing.
   Note: A false negative result may occur if the nasal swab specimen is not properly collected.
2. Firmly rub the swab in a circular motion around the inside wall of the nostril 5 times. Take approximately 15 seconds to collect the specimen. Be sure to collect any nasal drainage that may be present onto the swab. Repeat this in the other nostril using the same swab.
3. Remove the swab from the nostril and place into the extraction buffer tube.

DIRECTIONS FOR USE

Allow the test and extraction buffer to reach room temperature (15-30 °C) prior to testing.
1. Remove the foil from the top of the extraction buffer tube. Place the tube in the tube holder.
2. Immediately place the swab into the tube and swirl for 30 seconds.
3. Rotate the swab 5 times **while squeezing the tube**.
4. Remove the swab **while squeezing the tube** to extract as much liquid as possible. Dispose the swab in the trash.
5. Attach the dropper tip firmly onto the tube. Mix thoroughly by swirling or flicking the bottom of the tube.
   Note: A false negative result may occur if the swab was not swirled at least 30 seconds or rotated 5 times.
6. Place the cassette on a clean, flat surface. Gently squeeze the tube and dispense **4 drops** of solution into the Sample Well. Dispose the tube in the trash.
   Note: A false negative or invalid result may occur if less than 4 drops of fluid are added to the Sample Well.
7. Set the timer for 15 minutes. Result should be read at 15 minutes. Do not read after 30 minutes. Dispose the test cassette in the trash.
   Note: A false negative or false positive result may occur if the test result is read before 15 minutes or after 30 minutes.





INTERPRETATION OF RESULTS

(Please refer to the illustration above)
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
Without Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	Positive	Positive for COVID-19
	Negative	Negative	Negative	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.
NEGATIVE: Only one red or pink control line appears in the control line region (C). No apparent red or pink line appears in the test line region (T). This means that no SARS-CoV-2 antigen was detected. The amount of antigen in a sample may decrease as the duration of illness increases.
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.
- Test 2 more times at least 48 hours apart if the individual does not have 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.
POSITIVE:* Two distinct red or pink lines appear. One red or pink line in the control line region (C) and the other red or pink line in the test line region (T). This means that the presence of SARS-CoV-2 antigen was detected, and the patient is very likely to be infected with the virus and presumed to be contagious. Repeat testing does not need to be performed if the patient has a positive result at any time. Test results should always be considered in the context of clinical observations and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations) in making a final diagnosis and patient management decisions.
* **NOTE:** The intensity of the red or pink color in the test line (T) may vary depending on the level of the SARS-CoV-2 antigen present in the specimen. Therefore, any shade of red or pink in the test line region (T) should be considered positive.
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect operation are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, call (800) 838-9502 for assistance.

QUALITY CONTROL

Internal procedural controls are included in the test. A red or pink line appearing in the control line region (C) is an internal procedural control. The appearance of the procedural control line indicates that proper volume of specimen has been added and capillary flow occurred. If the procedural control line does not develop in 15 minutes, the test result is considered invalid, and retesting with a new cassette is recommended.
Positive and Negative control swabs are supplied with each kit. These control swabs should be used to ensure that the test cassette and that the test procedure is performed correctly. Follow the "DIRECTIONS FOR USE" section to perform the control test.
The control swabs can be tested under any of the following circumstances:
1. When new lot of tests are used and/or when a new operator performs the test.
2. At periodic intervals as dictated by local requirements, and/or by the user's Quality Control procedures.

LIMITATIONS

1. The Flow*flex* COVID-19 Antigen Rapid Test is for *in vitro* diagnostic use only. The test should be used for the detection of SARS-CoV-2 antigens in anterior nasal swab specimens only. The intensity of the test line does not necessarily correlate to SARS-CoV-2 viral titer in the specimen.
2. Specimens should be tested as quickly as possible after specimen collection.
3. Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
4. A false-negative result may occur if the level of antigen in a sample is below the detection limit of the test.
5. False negative results may occur if the sample was collected or handled incorrectly.
6. A false negative result may occur if the swab is not swirled at least 30 seconds or rotated five times
7. A false negative or invalid result may occur if less than 4 drops of fluid are added to the Sample Well.
8. A false negative or false positive result may occur if the test result is read before 15 minutes or after 30 minutes
9. 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.
10. The Flow*flex* COVID-19 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.
11. Test results should be correlated with other clinical data available to the physician.
12. A positive or negative test result does not rule out co-infections with other pathogens such as other viral or bacterial infections.
13. Negative results are presumptive, do not rule out COVID-19 and it may be necessary to obtain additional testing with a molecular assay, if needed for patient management.
14. A negative result is not intended to rule out other viral or bacterial infections.

15. If the differentiation of specific SARS viruses and strains is needed, additional testing is required, in consultation with state or local public health departments, is required. The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between March and May 2021. 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. 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.
16. The performance of this device has not been assessed in a population vaccinated against COVID-19.

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 and/or at least three times over five days with at least 48 hours between tests for asymptomatic individuals. A self-test may be used for this additional testing
3. For Point of Care antigen tests, a negative result should also be followed up with repeat, or serial testing; a self-test may be used for this additional testing.
4. The performance of this test was not clinically validated for serial testing in patients with or without symptoms consistent with COVID-19. 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 FDA.
5. 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.

PERFORMANCE CHARACTERISTICS

Clinical Sensitivity, Specificity and Accuracy

The performance of Flow*flex* COVID-19 Antigen Rapid Test was established in an all-comers clinical study conducted between March 2021 and May 2021 with 172 nasal swabs self-collected or pair-collected by another study participant from 108 individual symptomatic patients (within 7 days of onset) suspected of COVID-19 and 64 asymptomatic patients. All subjects were screened for the presence or absence of COVID-19 symptoms within two weeks of study enrollment. The study was conducted in a simulated home setting environment at two study sites in U.S. All study participants performed the test unassisted and interpreted the result, using only the product labeling. The Flow*flex* COVID-19 Antigen Rapid Test results were compared to an FDA EUA RT-PCR COVID-19 assay to determine test performance in the tables below:

Table 1. Performance of Flow*flex* COVID-19 Antigen Rapid Test in ALL subjects

Flow*flex* COVID-19 Antigen Rapid Test	RT-PCR method		
	Positive	Negative	Total
Positive	39	0	39
Negative	3	130	133
Total	42	130	172
Positive Percent Agreement (PPA)	93% (95%CI: 81% - 99%)		
Negative Percent Agreement (NPA)	100% (95%CI: 97% - 100%)		

Table 2. Performance of the Flow*flex* COVID-19 Antigen Rapid Test in Symptomatic subjects

Flowflex COVID-19 Antigen Rapid Test	RT-PCR method		
	Positive	Negative	Total
Positive	28	0	28
Negative	2	78	80
Total	30	78	108
Positive Percent Agreement (PPA)	93% (95%CI: 78% - 99%)		
Negative Percent Agreement (NPA)	100% (95%CI: 95% - 100%)		

Table 3. Performance of the Flow*flex* COVID-19 Antigen Rapid Test in Asymptomatic subjects

Flow*flex* COVID-19 Antigen Rapid Test	RT-PCR method		
	Positive	Negative	Total
Positive	11	0	11
Negative	1	52	53
Total	12	52	64
Positive Percent Agreement (PPA)	92% (95%CI: 62% - 100%)		
Negative Percent Agreement (NPA)	100% (95%CI: 93% - 100%)		

Table 4. Cumulative PPA results by days since symptom onset

Days Since Symptom Onset	# Specimens Tested	# Cumulative Positive Flow*flex* COVID-19 Antigen Rapid Test	Cumulative Positive RT-PCR	Cumulative PPA
0 to 1 day	29	6	7	86%
0 to 2 days	64	15	16	94%
0 to 3 days	90	20	21	95%
0 to 4 days	96	21	22	95%
0 to 5 days	100	23	24	96%
0 to 6 days	106	26	28	93%
0 to 7 days	108	28	30	93%
Asymptomatic	64	11	12	92%

Patient Demographics: A total of 172 patients participated in the clinical study. Ages of patients ranged from 2 years to 93 years. Age distribution and the positive results broken down by age of the patient are shown below.

Table 5. Age distribution of patients and specimen positivity

Age Group	Flow*flex* COVID-19 Antigen Rapid Test (N=175)		
	Total	Total Positive	Prevalence
2-13 years	18	5	28%
14- 24 years	25	4	16%
25- 64 years	94	21	23%
≥ 65 years	35	9	27%
Total	172	39	23%

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 of Health (NIH) and the University of Massachusetts Chan Medical School in collaboration with the US FDA.

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 the table below.

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%)	N/A	4/9 (44.4%)	3/7 (42.9%)	N/A

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 Sensitivity: Limit of Detection (LoD)

The Limit of Detection (LoD) of the Flow*flex* COVID-19 Antigen Rapid Test was determined using limiting dilutions of the heat-inactivated SARS-CoV-2 virus (USA-WA1/2020). Nasal swabs from healthy donors were collected and eluted with PBS. The swab eluates were combined and mixed thoroughly to create a negative clinical matrix pool to be used as the diluent. Inactivated SARS-CoV-2 virus was diluted in this negative clinical matrix pool to generate virus dilutions for testing.

The contrived nasal swab samples were prepared by absorbing 50 µL of each virus dilution onto the swab. The contrived swab samples were processed and tested according to the package insert.

SARS-CoV-2 Concentration in nasal matrix	Number of Positives/Total	% Detected
2.5 x 10^3 $TCID_{50}$/mL	60/60	100%

LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time.
Based on this testing, the LoD in nasal matrix was confirmed to be 2.5 x 10^3 $TCID_{50}$/mL

Analytical Specificity: Cross-Reactivity and Microbial Interference

Cross-reactivity was evaluated by testing a panel of related pathogens and microorganisms that are likely to be present in the nasal cavity. Each organism and virus were tested in the absence or presence of heat-inactivated SARS-CoV-2 virus (USA-WA1/2020) at a low concentration.

No cross-reactivity or interference was observed with the following organisms when tested at the concentration presented in the table below.

Potential Cross Reactant		Test Concentration	Cross-Reactivity Results	Interference Results
Virus	Adenovirus	1.14 x 10^6 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Enterovirus	9.50 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Human coronavirus 229E	1.04 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Human coronavirus OC43	2.63 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Human Metapneumovirus	1.25 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	MERS-coronavirus	7.90 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Influenza A	1.04 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Influenza B	1.04 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Parainfluenza virus 1	1.25 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Parainfluenza virus 2	3.78 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Parainfluenza virus 3	1.0 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Parainfluenza virus 4	2.88 x 10^6 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Respiratory syncytial virus	3.15 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
	Rhinovirus	3.15 x 10^5 $TCID_{50}$/mL	No cross-reactivity	No Interference
Bacteria	*Bordetella pertussis*	2.83 x 10^9 CFU/mL	No cross-reactivity	No Interference
	Chlamydia pneumonia	3.5 x 10^7 IFU/mL	No cross-reactivity	No Interference
	Chlamydia trachomatis	3.13 x 10^8 CFU/mL	No cross-reactivity	No Interference
	Haemophilus influenzae	1.36 x 10^8 CFU/mL	No cross-reactivity	No Interference
	Legionella pneumophila	4.08 x 10^9 CFU/mL	No cross-reactivity	No Interference
	Mycobacterium tuberculosis	1.72 x 10^7 CFU/mL	No cross-reactivity	No Interference
	Mycoplasma pneumoniae	7.90 x 10^7 CFU/mL	No cross-reactivity	No Interference
	Staphylococcus aureus	1.38 x 10^7 CFU/mL	No cross-reactivity	No Interference
	Staphylococcus epidermidis	2.32 x 10^9 CFU/mL	No cross-reactivity	No Interference
	Streptococcus pneumoniae	1.04 x 10^8 CFU/mL	No cross-reactivity	No Interference
	Streptococcus pyogenes	4.10 x 10^6 CFU/mL	No cross-reactivity	No Interference
	Pneumocystis jirovecii-S. cerevisiae	8.63 x 10^7 CFU/mL	No cross-reactivity	No Interference
	Pseudomonas aeruginosa	1.87 x 10^8 CFU/mL	No cross-reactivity	No Interference
Yeast	*Candida albicans*	1.57 x 10^8 CFU/mL	No cross-reactivity	No Interference

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, in-silico analysis was used to assess the degree of protein sequence homology. The comparison between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 revealed a low homology of 36.7% across 82.8% of the SARS-CoV-2 nucleocapsid sequence. The result suggests that cross-reactivity with human coronavirus HKU1 cannot be completely ruled out.

Compared the sequence homology between the SARS-CoV-2 nucleocapsid protein and the structural proteins of SARS coronavirus (SARS-CoV) and with given the substantial homology rate (91.5%), there is high probability of cross-reactivity between the nucleocapsid proteins of SARS-CoV-2 and SARS-CoV. The Flow*flex* COVID-19 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

Endogenous Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated. In addition to the materials that are found in the nasal cavity, substances that are commonly found on the hands were also tested. Each substance was tested in the absence or presence of SARS-CoV-2 virus (USA-WA1/2020) at a low concentration. The performance of Flow*flex* COVID-19 Antigen Rapid Test was not affected by any of the potentially interfering substances listed in the table below at the concentrations tested.

Interfering Substance	Source/Item	Test Concentration	Cross-Reactivity Results	Interference Results
Biotin	Sigma/ B4501	3500 ng/mL	No cross-reactivity	No interference
Chloraseptic Throat Lozenge (Menthol/Benzocaine)	Chloraseptic	1.5 mg/mL	No cross-reactivity	No interference
Cough Lozenge (Menthol)	Ricola	1.5 mg/mL	No cross-reactivity	No interference
Dyclonine Hydrochloride	Sigma/PHR1849	1.5mg/mL	No cross-reactivity	No interference
Fluticasone propionate	Flonase	5% v/v	No cross-reactivity	No interference
Mucin	Sigma/M3895	0.5% w/v	No cross-reactivity	No interference
Mupirocin	Sigma/M7694	10 mg/mL	No cross-reactivity	No interference
Nasal Drops (Phenylephrine)	Equate (Walmart)	15% v/v	No cross-reactivity	No interference
Nasal Spray (Cromolyn)	NasalCrom	15% v/v	No cross-reactivity	No interference
Nasal Spray (Homeopathic)	ALKALOL	1:10 Dilution	No cross-reactivity	No interference
Nasal Spray (Oxymetazoline HCl)	Afrin	15% v/v	No cross-reactivity	No interference
Naso GEL (NeilMed)	NeilMed	5% v/v	No cross-reactivity	No interference
Sore Throat Phenol Spray	Equate (Walmart)	15% v/v	No cross-reactivity	No interference
Tamiflu (Oseltamivir Phosphate)	Tamiflu	5 mg/mL	No cross-reactivity	No interference
Tobramycin	Sigma/LRAC4285	4 µg/mL	No cross-reactivity	No interference
Whole Blood	In-house	4% v/v	No cross-reactivity	No interference
Zicam	Zicam	5% v/v	No cross-reactivity	No interference

Potential Interfering Household Items	Source /Item	Test Concentration	Cross-Reactivity Results	Interference Results
Body & Hand Lotion	Aveeno	0.5% w/v	No cross-reactivity	No interference
Body Lotion, with 1.2% dimethicone	Aveeno	0.5% w/v	No cross-reactivity	No interference
Hand Lotion	Bath & Body	5% w/v	No cross-reactivity	No interference
Hand Sanitizer with Aloe, 62% ethyl alcohol	Hand in Hand	5% v/v	No cross-reactivity	No interference
Hand Sanitizer cream lotion	Dove	15% v/v	No cross-reactivity	No interference
Hand Sanitizer, 80% ethanol, fast drying	Allied Photo Chemical	15% w/v	No cross-reactivity	No interference
Hand soap liquid gel	SoftSoap	10% w/v	No cross-reactivity	No interference

High Dose Hook Effect

No high dose hook effect was observed when tested with up to a concentration of 1.0 x 10^6 $TCID_{50}$/mL of heat-inactivated SARS-CoV-2 virus (USA-WA1/2020) with the Flow*flex* COVID-19 Antigen Rapid Test.

BIBLIOGRAPHY

1. Shuo Su, Gary Wong, Weifeng Shi, et al. Epidemiology, Genetic recombination, and pathogenesis of coronaviruses. Trends in Microbiology, June 2016, vol. 24, No. 6: 490-502
2. Susan R. Weiss, Julian L. Leibowitz, Coronavirus Pathogenesis, Advances in Virus Research, Volume 81: 85-164

Index of Symbols

	Manufacturer		Date of manufacture
Σ	Contains sufficient for <*n*> tests	REF	Catalogue number
IVD	*In vitro* diagnostic medical device		Use-by date
	Consult instructions for use	LOT	Batch code
	Temperature limit		Do not reuse

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