ViraDx™ SARS-CoV-2/Flu A+B Rapid Antigen Test

Rapid Immunoassay for Direct Detection and Differential Diagnosis of SARS-CoV-2, Influenza Type A and Influenza Type B Antigens

For In Vitro Diagnostic Use Only

INTENDED USE

ViraDx™ SARS-CoV-2/Flu A+B Rapid Antigen Test is a lateral flow immunoassay intended for the in vitro rapid, simultaneous qualitative detection and differentiation of nucleocapsid antigen from SARS-CoV-2, influenza A and influenza B directly from anterior nasal or nasopharyngeal swab specimens obtained from individuals, who are suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider, within the first five days of onset of symptoms. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. This test is authorized for laboratory use and for use at the Point of Care (POC).

Results are for the simultaneous identification of nucleocapsid antigens of SARS-CoV-2, influenza A and influenza B, but does not differentiate between SARS-CoV and SARS-CoV-2 viruses and is not intended to detect influenza C antigens. These viral antigens are generally detectable in anterior nasal or nasopharyngeal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but the 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 the disease. Laboratories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative SARS-CoV-2 results should be treated as presumptive and confirmed with a molecular assay, if necessary, for patient management. 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. 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.

Negative influenza A and B test results should be treated as presumptive. It is recommended these results be confirmed by viral culture or Health Canada licensed influenza A and B molecular assay. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.

Performance characteristics for influenza A and B were established during the 2007-2009 and the 2014-2016 influenza seasons when influenza A/H1N1, A/H1N1 pandemic, A/H3N2, influenza B/Victoria lineage and B/Yamagata lineage were the predominant influenza viruses in circulation according to the Flu Activity & Surveillance reports from the CDC. When other influenza viruses are emerging, performance characteristics may vary.

The performance of this test for SARS-CoV-2 was established based on the evaluation of a limited number of clinical specimens collected between September 2020 and April 2021. The clinical performance has not been established in 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.

If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. A viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is intended for use by medical professionals and laboratory personnel trained to perform the test.

SUMMARY AND EXPLANATION

Influenza is a highly contagious acute viral infection of the respiratory tract. It is a communicable disease easily transmitted from person to person through aerosol droplets excreted when sneezing and coughing. Common symptoms include high fever, chills, headache, cough, sore throat and malaise. The type A influenza virus is more prevalent and is the primary pathogen associated with serious epidemics. The type B virus causes a disease that is generally not as severe as that caused by the type A virus.

An accurate diagnosis of influenza based on clinical symptoms is difficult because the initial symptoms of influenza are similar to those of numerous other illnesses. Therefore, it can be confirmed only by laboratory diagnostic testing.[1] Early differential diagnosis of influenza type A or type B can allow for proper treatment with appropriate antiviral therapy while reducing the incidence of inappropriate treatment with antibiotics. Early diagnosis and treatment are of particular value in a clinical setting where an accurate diagnosis can assist the healthcare professional with the management of influenza patients who are at risk for complications.[2]

In December 2019, a cluster of atypical pneumonia patients epidemiologically linked to a wet market in Wuhan (Hubei province, China) was detected. Initially, the novel coronavirus was named 2019-nCoV. Later it was named the SARS-CoV-2 virus, as it is very similar to the one that caused the outbreak of severe acute respiratory disease (SARS) in 2003. At the end of January 2020, the World Health Organization (WHO) declared the new infectious disease COVID-19 a global emergency. On March 11, 2020, the WHO recognized the new infectious disease as a pandemic. COVID-19 has demonstrated the capability of spreading rapidly, leading to significant impacts on the healthcare system and causing societal disruption. The ongoing COVID-19 pandemic has infected millions of people worldwide. To respond effectively to the COVID-19 outbreak, rapid detection of cases, stringent performance assessment and increase in the current diagnostic capacity are still urgently needed. The symptoms of COVID-19 are similar to those of other viral respiratory disease and include fever or chills, cough, shortness of breath or difficulty of breathing, fatigue, muscle or body aches, headache, the new loss of taste or smell, sore throat, congested or runny nose, nausea or vomiting or diarrhea, etc. As the early symptoms of COVID-19 are similar to those of seasonal influenza A or B, a rapid detection test to specifically diagnose symptomatic patients is urgently needed.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. 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.

PRINCIPLE OF PROCEDURE

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is a modification of the Health Canada licensed device, *Status* Flu A&B. The modification of the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test device consists of the addition of a test line of monoclonal antibody and a pad containing monoclonal antibody-dye conjugate for the detection of SARS-CoV-2 antigen from anterior nasal or nasopharyngeal swab patient specimen. ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is intended to aid in the rapid differential diagnosis of influenza A, B and SARS-CoV-2 viral infection.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is a lateral flow immuno-chromatographic assay which utilizes the chemical extraction of viral antigens followed by solid-phase immunoassay technology. ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is designed to detect antigens from SARS-CoV-2, influenza A and /or influenza B in anterior nasal or nasopharyngeal swab specimens from individuals with signs and symptoms of respiratory infection, suspected of COVID-19 or flu by their healthcare provider, within the first five days of onset of symptoms. It is intended to aid in the rapid differential diagnosis of SARS-CoV-2, influenza A and /or influenza B viral infections. ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is validated for use with direct specimens without transport media.

In the test procedure, an anterior or nasopharyngeal swab specimen is collected and placed into an Extraction Tube filled with Extraction Reagent for one minute. During this time the antigen is extracted from disrupted virus particles. The Extraction Tube is then inverted and the solution is applied to the test device. The solution of extracted specimen flows onto the test strip and migrates through the pads and membrane of the test strip. The pads contain detector antibodies conjugated to gold dye and the membrane contains immobilized capture antibodies. If SARS-CoV-2, influenza A and/or influenza B antigens are present in the specimen, they will react with anti-SARS-CoV-2 antibody coupled to gold dye particles and/or anti-influenza antibody coupled to gold dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized capture antibody line(s) on the membrane, and generate a colored line in the specific test line position. The rest of the sample and unbound/bound dye complexes continue to migrate

to the Control line position (C), where immobilized antibodies to the anti-SARS-CoV-2 and anti-influenza antibodies capture the dye complexes and form the Control line. Formation of the Control line serves as an internal control to demonstrate that test reagents are functional, antibody-dye conjugates in the dye pad have been hydrated and released and that sufficient sample has been applied to allow for migration through the Test and Control lines. If the Control line does not appear within the designated incubation time, the result is invalid and the test should be repeated using a new test device and specimen.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test has three Test lines, one for SARS-CoV-2, one for influenza A and one for influenza B. The three Test lines allow for the separate and differential identification of SARS-CoV-2, influenza A and/or B from a single specimen. If any Test line appears in the test result window, together with the Control line, the test result is positive for SARS-CoV-2 and/or influenza. The test detects, but does not differentiate, between the SARS-CoV and SARS-CoV-2 viruses.

REAGENTS

Each ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kit contains enough reagents and materials for 25 tests. The following components are included in a kit.

Materials Provided

25 ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test devices. The test strip in each device contains mouse monoclonal antibodies to nucleocapsid protein of influenza A, influenza B and SARS-CoV-2. The device is individually pouched.
25 Extraction Reagent Capsules. For use with swab specimens; 300 µL of Phosphate buffer with detergents and preservative.
25 Extraction Reagent Tubes. For preparing specimen.
25 Sterile Swabs. For swab specimen collection.
1 Positive Control Swab. Influenza A, B and SARS-CoV-2 antigen (non-infective recombinant nucleocapsid).
1 Negative Control Swab. Inactivated Group B Streptococcus antigen (non-infective).
1 Package Insert.
1 Quick Reference Instructions.

Materials Required, But Not Provided

Timer

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use only.
2. This product has been authorized only for the detection of proteins from SARS-CoV-2, influenza A and influenza B, not for any other viruses or pathogens.
3. Do not use after the expiration date printed on the outside of the box.
4. ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is only intended for use with direct anterior nasal or nasopharyngeal swab specimens and is not validated or authorized for use with viral transport media.
5. Do not reuse used test devices, swabs, extraction tubes or control swabs.
6. Inadequate or inappropriate sample collection, storage and transport may yield false test results.
7. To obtain accurate results, the Package Insert instructions must be followed.
8. Dispose of containers and unused contents in accordance with federal, state and local regulatory requirements.
9. Use only the swabs provided for collecting specimens. Other swabs may not work properly.
10. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

11. Extraction Reagent contains 0.09% sodium azide. Avoid contact with eyes, sensitive mucous membranes, cuts, abrasions, etc. If the reagent comes in contact with skin or eyes, flush with a large volume of water.
12. Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
13. All specimens should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens and test devices.
14. The ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test device should remain in its original sealed pouch until ready for use. Do not use the test if the seal is broken or the pouch is damaged.
15. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, the specimen should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

STORAGE AND STABILITY

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test may be stored at 2-30°C (35-86°F) in the original sealed pouch, away from direct sunlight. Kit contents are stable until the expiration date printed on the pouch or box.

SPECIMEN COLLECTION AND PREPARATION

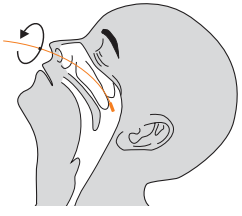
- Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative test results. Training in specimen collection is highly recommended because of the importance of specimen quality.
- To collect anterior nasal or nasopharyngeal swab specimens, only the swab provided in the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kit should be used.
- Use fresh samples for best performance. Freshly collected specimens should be tested immediately. If necessary, swab samples can be stored for up to 4 hours at room temperature or up to 8 hours at 2-8°C.
- Transport media should not be used. This test has not been validated or authorized using viral transport media.

Specimen Collection Procedure

Good sample collection is the most important first step for an accurate test result. Therefore, carefully follow the instructions below for collection of anterior nasal or nasopharyngeal swab specimens to obtain as much secretion as possible.

To Collect Nasopharyngeal Swab Specimen

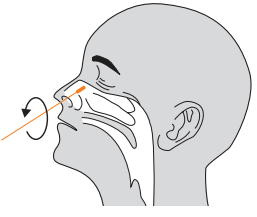
Use a flocked swab provided in the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kit only. Tilt patient's head back 70 degrees. Gently and slowly insert a minitip swab with a flexible shaft through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockages create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.



Nasopharyngeal Swab

To Collect Anterior Nasal Swab Specimen

Use a flocked swab provided in the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kit only. Insert the entire soft end of the swab into the patient's nostril no more than ¾ of an inch (1.5 cm) into the patient's nose. Slowly rotate the swab, gently pressing against the inside of the patient's nostril at least 4 times for a total of 15 seconds. Get as much secretion as possible on the soft end of the swab. Gently remove the swab. Using the same swab, repeat in the second nostril with the same end of the swab.

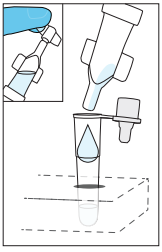


Anterior Nasal Swab

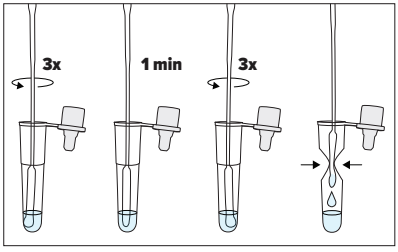
TEST PROCEDURE

Procedural Notes

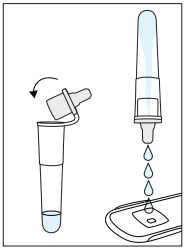
- The test procedure below must be followed to obtain accurate and reproducible results.
- Reagents, specimens and devices must be at room temperature (18-30°C) for testing.
- Do not open the foil pouch until you are ready to perform the test.
- Label the device with the patient identification or control to be tested.
- Place test device on a level surface.



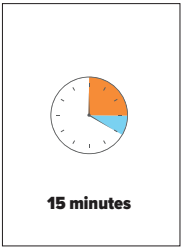
1. Tear the tab off the Extraction Reagent capsule and squeeze it to dispense all of the solution into the Extraction Tube.



2. Insert the specimen swab into the Extraction Tube and rotate it 3 times to mix the specimen. Let stand for 1 minute with the swab in Extraction Tube. Rotate swab 3 times again to mix the specimen. Squeeze swab against the Extraction Tube to retain as much of the liquid as possible, then remove and discard the swab.



3. Place cap on Extraction Tube, invert and empty the contents of the Extraction tube onto the sample well of the test device.



4. Start timing for 15 minutes. **Read results at 15 minutes.** Results should not be read after 20 minutes.

Note: To ensure proper test performance, it is important to read results at 15 minutes. False positive or false negative results can occur if the test is not read between 15 and 20 minutes.

INTERPRETATION OF RESULTS

Positive: Determination of a positive result is made at fifteen (15) minutes. A reddish purple Control line (C position) and a reddish purple Test line (influenza A, influenza B or S position) indicate that influenza A, B and/or SARS-CoV-2 antigen has been detected. Lines at the A and C positions indicate the presence of influenza type A viral antigen, lines at the B and C positions indicate the presence of influenza type B viral antigen and lines at the S and C positions indicate the presence of SARS-CoV-2 viral antigen in the specimen. A positive result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.

Note: The Test line (reddish purple 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. Even a light or faint Test line must be interpreted as a positive result.

Negative: A reddish purple Control line (C position) only, with no Test line at the A, B, S positions, indicates that influenza A, B antigen or SARS-CoV-2 antigen has not been detected. A negative result does not exclude influenza viral or SARS-CoV-2 viral infection. **Determination of negative results should not be made before 15 minutes.**

Negative results are presumptive and may need to be confirmed with a molecular assay.

Invalid: A reddish purple line should always appear at the Control line position (C position). If a line does not form at the Control line position in 15 minutes, the test result is invalid and the test should be repeated with a new ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test device.

Note: Co-infection with influenza A, B and/or SARS-CoV-2 is rare. If results are positive for more than one antigen, i.e., influenza A, B and/or COVID-19, the patient specimens should be re-tested.

A reddish purple S, A and/or B line(s) with C line is POSITIVE.	C line only **NEGATIVE (-)**	No C line **INVALID**
 **A line:** Influenza type A  **B line:** Influenza type B  **S line:** COVID-19  **A & S lines:** Influenza type A & COVID-19*  **B & S lines:** Influenza type B & COVID-19*  **A & B lines:** Influenza type A & B*  **A, B & S lines:** Influenza type A, B & COVID-19* ***Note:** Co-infection with influenza A, B and/or SARS-CoV-2 is rare. If results are positive for more than one antigen, i.e., influenza A, B and/or COVID-19, the patient specimens should be re-tested.	 **Negative results are presumptive and may need to be confirmed with a molecular assay.**	  **Repeat with new sample and device.**

LIMITATIONS

- A negative test result does not exclude infection with SARS-CoV-2, influenza A or B. **Negative test results are presumptive and may need to be confirmed with a molecular test.** Therefore, the results obtained with ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test should be used in conjunction with clinical findings to make an accurate diagnosis. Additional testing is required to confirm the absence of infection, in consultation with state or local public health departments.
- This test detects both viable (live) and non-viable SARS-CoV-2, influenza A and B. Test performance depends on the amount of virus (antigen) in the specimen and may or may not correlate with viral culture or molecular assay results performed on the same specimen.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- Positive test results do not rule out co-infections with other pathogens.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- Positive test results do not identify specific influenza A virus subtypes.
- If differentiation of specific SARS or influenza A subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test uses highly targeted epitope specific monoclonal antibodies. As in most immunoassays, it may fail to detect, or detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.
- Performance of the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test has not been established for monitoring antiviral treatment of influenza and SARS-CoV-2.
- Performance of ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test has not been established for novel variants of SARS-CoV-2.
- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses emerge, performance characteristics may vary.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The performance of this test has not been evaluated for specimen types other than those specified in the Intended Use.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between September 2020 and April 2021. The clinical performance has not been established in 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.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The performance of ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test was not evaluated for SARS-CoV-2 detection with samples collected in viral transport media and should not be used with this test.
- Children tend to shed influenza virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults will result in lower sensitivity than testing specimens from children.
- Positive and negative predictive values are highly dependent on prevalence. 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 activity when prevalence is moderate to low.
- Individuals who received nasally administered influenza A vaccine may produce positive test results for up to three days after vaccination.
- ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test can distinguish among influenza A, B and SARS-CoV-2 viruses, but it cannot differentiate influenza subtypes.
- 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.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.

USER QUALITY CONTROL

Internal Quality Control

Each ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test device has built-in controls. The Control line at the C position can be considered as an internal positive procedural control; i.e., a proper amount of sample was used, sample was properly added to the Extraction Well, sample migrated properly and the reagent system worked properly. A distinct reddish-purple Control line should always appear if the test has been performed correctly. If the Control line does not appear, the test result is invalid and a new test should be performed. If the problem persists, contact Lumos Diagnostics Technical Support at 1.855.LumosDx or 1.855.568.6739 for technical assistance. A clear background in the Test Result Window is considered an internal negative procedural control. If the test is performed correctly and the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test device is working properly, the background in the Test Result Window will be clear, providing a distinct result.

External Quality Control

Good laboratory practice includes the use of external controls to ensure proper kit performance. It is recommended that external control testing be performed with each new operator and before using a new lot or shipment of ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kits to confirm the expected Q.C. results, using the external controls provided in the kit. The frequency of additional Q.C. tests should be determined according to your laboratory's standard Q.C. procedures and local, state and federal regulations or accreditation requirements. Upon confirmation of the expected results, the kit is ready for use with patient specimens. If external controls do not perform as expected, do not use the test results. Repeat the tests or contact Lumos Diagnostics Technical Support. The built-in reddish purple Control line indicates only the integrity of the test device and proper fluid flow.

The ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test kit contains two external Control Swabs. Test the Control Swabs in the same manner as patient specimens. When the positive control is tested, reddish purple lines appear at the C as well as A, B and S positions. When the Negative Control Swab is tested, a reddish purple line appears at the C position only.

If the controls do not perform as expected, do not report patient results.

The use of positive and negative controls from other commercial kits has not been established with ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test.

EXPECTED VALUES

The rate of positives in COVID-19 testing varies depending on many factors, including the specimen collection method, the disease prevalence, and the geographic location. The prevalence of influenza varies every year and the rate of positives in influenza testing varies depending on many factors, including the specimen collection method, the test method used, the disease prevalence and the geographic location. The expected values based on previous *Status* Flu A&B results are 30.3% for influenza A and 13.8% for influenza B during the 2007-2009 prospective clinical study, and were 33.6% for influenza A and 9.8% for influenza B during the 2014-2016 prospective clinical study.

PERFORMANCE CHARACTERISTICS

Clinical Performance – Nasopharyngeal Swab Specimen

A prospective study was performed in which two hundred eighteen (218) direct nasopharyngeal swabs were sequentially enrolled (between September 2020 and April 2021) and tested fresh. The samples were collected from symptomatic patients suspected of infection with COVID-19, at five Point of Care (POC) CLIA waived clinical sites. To be enrolled in the study, patients had to present at the participating study site with signs and symptoms of respiratory infection generally observed from SARS-CoV-2, influenza A and/or influenza B, during the study period. Patients presenting within five (5) days of symptom onset were included in the study. Two nasopharyngeal swab specimens were collected from each patient; one swab specimen to be tested using a comparator method for the detection of SARS-CoV-2, an FDA Emergency Use Authorized RT-PCR assay for the detection of SARS-CoV-2 and influenza A and B, and the other swab specimen to be tested at the study site.

SARS-CoV-2 Performance (ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test)

Patient Demographics

Patient demographics (age, the elapsed time from date of symptom onset) are available for the 218 patients participating in this study. COVID-19 Positive results are broken down by age and days post symptom onset in the following tables.

Patient Demographics (COVID-19 *positive* = 54)

Age	ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test		
	Total	# COVID-19 Positive	Prevalence
≤ 5 years	16	1	6.3%
6 to 21 years[a]	68	10	14.7%
22 to 59 years[b]	109	37	33.9%
≥ 60 years[c]	24	6	25.0%
Unknown[d]	1	0	N/A

a. One patient was ViraDx negative and positive by reference extracted RT-PCR.
b. One patient was ViraDx negative and positive by reference extracted RT-PCR.
c. Two patients were ViraDx negative and positive by reference extracted RT-PCR.
d. One patient did not provide age information.

Specimen Positivity Breakdown Based On Days Post Onset (COVID-19 positive = 54)

Days Post Symptom Onset	ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test		
	Total # Tested	# COVID-19 Positive	% Positive
0[e]	38	8	21.1%
1	71	5	7.0%
2	59	16	27.1%
3	25	13	52.0%
4	12	6	50.0%
5[f]	13	6	46.2%

e. Three specimens were ViraDx negative and positive by reference extracted RT-PCR.
f. One specimen was ViraDx negative and positive by reference extracted RT-PCR.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: COVID-19 (SARS-CoV-2)

		Reference Extracted RT-PCR: SARS-CoV-2			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	SARS-CoV-2 Positive	54	0	54	Sensitivity: 93.1% 95% CI: 83.6% to 97.3%
	SARS-CoV-2 Negative	4	160	164	Specificity: 100% 95% CI: 97.7% to 100.0%
Total		58	160	218	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: Influenza A

		Reference Extracted RT-PCR: Influenza A			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza A Positive	0	0	0	NA
	Influenza A Negative	0	218	218	NPA: 100% 95% CI: 98.3% to 100.0%
Total		0	218	218	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: Influenza B

		Reference Extracted RT-PCR: Influenza B			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza B Positive	0	0	0	NA
	Influenza B Negative	0	218	218	NPA: 100% 95% CI: 98.3% to 100.0%
Total		0	218	218	

Clinical Performance – Anterior Nasal Swab Specimen

A prospective study was performed in which one hundred ninety three (193) direct anterior nasal swab specimens were sequentially enrolled (between September 2020 and April 2021) and tested fresh. The samples were collected from symptomatic patients suspected of infection with COVID-19, at five Point of Care (POC) CLIA waived clinical sites. To be enrolled in the study, patients had to present at the participating study site with signs and symptoms of respiratory infection generally observed from SARS-CoV-2, influenza A and/or influenza B, during the study period. Patients presenting within five (5) days of symptom onset were included in the study. One nasopharyngeal swab specimen and one anterior nasal swab specimen were collected from each patient; one nasopharyngeal swab to be tested using a comparator method for the detection of SARS-CoV-2, an FDA Emergency Use Authorized RT-PCR assay for the detection of SARS-CoV-2 and influenza A and B, and one anterior nasal swab specimen to be tested at the study site.

SARS-CoV-2 Performance (ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test)

Patient Demographics

Patient demographics (age, the elapsed time from date of symptom onset) are available for the 193 patients participating in this study. COVID-19 Positive results are broken down by age and days post symptom onset in the tables below.

Patient Demographics (COVID-19 *positive* = 45)

Age	**ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test**		
	Total	# COVID-19 Positive	Prevalence
≤ 5 years	16	1	6.3%
6 to 21 years[a]	65	9	13.8%
22 to 59 years[b]	94	31	33.0%
≥60 years[c]	18	4	22.2%

a. One patient was ViraDx negative and positive by reference extracted RT-PCR.
b. One patient was ViraDx negative and positive by reference extracted RT-PCR.
c. Two patients were ViraDx negative and positive by reference extracted RT-PCR.

Specimen Positivity Breakdown Based On Days Post Onset (COVID-19 positive = 45)

Days Post Symptom Onset	**ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test**		
	Total # Tested	# COVID-19 Positive	% Positive
0[d]	32	5	15.6%
1	70	4	5.7%
2	53	15	28.3%
3	21	11	52.4%
4	7	4	57.1%
5	10	6	60.0%

d. Three specimens were ViraDx negative and positive by reference extracted RT-PCR.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: COVID-19 (SARS-CoV-2)

		Reference Extracted RT-PCR: SARS-CoV-2			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	SARS-CoV-2 Positive	45	0	45	Sensitivity: 93.8% 95% CI: 83.2% to 97.9%
	SARS-CoV-2 Negative	3	145	148	Specificity: 100% 95% CI: 97.4% to 100.0%
Total		48	145	193	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: Influenza A

		Reference Extracted RT-PCR: Influenza A			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza A Positive	0	0	0	NA
	Influenza A Negative	0	193	193	NPA: 100% 95% CI: 98.1% to 100.0%
Total		0	193	193	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to reference PCR: Influenza B

		Reference Extracted RT-PCR: Influenza B			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza B Positive	0	0	0	NA
	Influenza B Negative	0	193	193	NPA: 100% 95% CI: 98.1% to 100.0%
Total		0	193	193	

In the absence of fresh clinical influenza A or B positive specimens, twenty one (21) archived nasopharyngeal specimens in VTM (Remel M6 media) were confirmed positive using Cepheid Xpert. The samples were tested to confirm comparable performance between the ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test and the *Status* Flu A&B test. Results from the testing of ten (10) influenza A positive samples and eleven (11) influenza B positive samples were combined and analyzed.

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to *Status* Flu A&B: Influenza A

		Status Flu A&B: Influenza A			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza A Positive	10	0	10	PPA: 100.0% 95% CI: 72.3% to 100.0%
	Influenza A Negative	0	11	11	NPA: 100.0% 95% CI: 74.1% to 100.0%
Total		10	11	21	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test performance compared to *Status* Flu A&B: Influenza B

		Status Flu A&B: Influenza B			Performance (95% CI)
		Positive	Negative	Total	
ViraDx SARS-CoV-2/ Flu A+B Rapid Antigen Test	Influenza B Positive	11	0	11	PPA: 100.0% 95% CI: 74.1% to 100.0%
	Influenza B Negative	0	10	10	NPA: 100.0% 95% CI: 72.3% to 100.0%
Total		11	10	21	

ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test is a lateral flow immunoassay intended to aid in the rapid differential diagnosis of influenza A, B and COVID-19 viral infections. It is a modification of the test device used in the FDA-cleared *Status* Flu A&B and BioSign Flu A+B (K182157) to include monoclonal antibodies for the detection of SARS-CoV-2. Data for the detection of influenza A and B by the *Status* Flu A&B test are presented below.

***Status* Flu A&B**

Prospective Clinical Study from 2007 to 2009

A prospective clinical study was conducted from January 2007 to March 2008 and during March and April 2009 to determine the performance of *Status* Flu A&B for nasopharyngeal swab specimens.

The samples were collected at 5 sites in the USA from patients who visited physicians' offices and clinics with signs and symptoms of respiratory infection during the study period. All collected samples were tested with *Status* Flu A&B, and were cultured. The culture was initially used as the comparator method. The samples that produced discrepant results between *Status* Flu A&B and viral culture were further analyzed with an FDA-cleared real time RT-PCR Flu A and B assay (PCR comparator assay hereafter).

The total number of patients tested was 862, of which 30% were 5 and younger, 38% were 6-21 years old, and the rest were older than 21. Forty-eight (48) percent were male and 52% were female. A total of 253 nasopharyngeal aspirate specimens and 609 anterior nasal ornasopharyngeal swab specimens were included in the performance analyses below.

Nasopharyngeal/Anterior Nasal Swab Samples (combined): Comparison with Viral Culture

	Virus Culture Results			
Status Flu A&B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	59	131*	190	Sensitivity: 90.8% 95% CI: 81.3-95.7%
Flu A Negative	6**	413	419	Specificity: 75.9% 95% CI: 72.2-79.3%
Total	65	544	609	

*Of 131 discrepant results, 107 were positive by both *Status* and the PCR comparator assay.

**Of 6 discrepant results, 1 was negative by both *Status* and the PCR comparator assay.

	Virus Culture Results			
Status Flu A&B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	47	55*	102	Sensitivity: 85.5% 95% CI: 73.8-92.4%
Flu B Negative	8**	499	507	Specificity: 90.1% 95% CI: 87.3-92.3%
Total	65	554	609	

*Of 55 discrepant results, 27 were positive by both *Status* and the PCR comparator assay.

**Of 8 discrepant results, 3 were negative by both *Status* and the PCR comparator assay.

Subsequently all available remnant nasopharyngeal swab and anterior nasal swabs samples that produced concordant results between *Status* Flu A&B and viral culture (a subset of the concordant nasopharyngeal/anterior nasal swab samples) were also further analyzed with the PCR comparator assay to supplement the PCR testing performed on discordant specimens. This subset of concordant samples between *Status* Flu A&B and viral culture includes 46% of all concordant positive samples and 33% of all concordant negative samples for the Flu A analyte, and 23% of all concordant positive samples and 31% of all concordant negative samples for the Flu B analyte.

Performance of the *Status* Flu A&B against the PCR comparator assay for all nasopharyngeal and anterior nasal swab samples are presented in the tables below.

Nasopharyngeal and Anterior Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
Status Flu A&B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	165	25	190	Sensitivity: 92.2% 95% CI: 87.3-95.3%
Flu A Negative	14	405	419	Specificity: 94.2% 95% CI: 91.6-96.0%
Total	179	430	609	

	PCR Results			
Status Flu A&B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	72	30	102	Sensitivity: 90.0% 95% CI: 81.5-94.8%
Flu B Negative	8	499	507	Specificity: 94.3% 95% CI: 92.0-96.0%
Total	80	529	609	

Prospective Clinical Study from 2014 to 2016

An additional prospective clinical study was conducted from December 2014 to May 2016 to evaluate the performance of *Status* Flu A&B for nasopharyngeal and anterior nasal swab specimens when used by operators at CLIA-waived sites. The nasopharyngeal and anterior nasal swab specimens were collected at 7 CLIA waived sites in the USA from patients with signs and symptoms of respiratory infection during the study period. All collected samples were tested with both the *Status* Flu A&B and the PCR comparator assay. The total number of patients tested prospectively in this clinical study was 307, of which 37% were 5 and younger, 50% were 6-21 years old, and the rest were older than 21. Forty-nine (49) percent were male and 51% were female.

The data showing the performance of the *Status* Flu A&B assay against the PCR comparator assay for all the prospectively collected and tested swab samples from 2014 to 2016 are presented in the tables below.

Nasopharyngeal and Anterior Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
Status Flu A&B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	101	2	103	Sensitivity: 90.2% 95% CI: 83.3-94.4%
Flu A Negative	11	193	204	Specificity: 99.0% 95% CI: 96.3-99.7%
Total	112	195	307	

	PCR Results			
Status Flu A&B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	27	3	30	Sensitivity: 81.8% 95% CI: 65.6-91.4%
Flu B Negative	6	271	277	Specificity: 98.9% 95% CI: 96.8-99.6%
Total	33	274	307	

Prospective Clinical Study from 2007 to 2009 and from 2014 to 2016

Combined prospective clinical data from the 2007 to 2009 study and the 2014 to 2016 CLIA waiver study against the PCR comparator assay are presented in the tables below.

Nasopharyngeal and Anterior Nasal Swab Samples (combined): Comparison with PCR

	PCR Results			
Status Flu A&B	Flu A Positive	Flu A Negative	Total	Performance
Flu A Positive	266	27	293	Sensitivity: 91.4% 95% CI: 87.6-94.1%
Flu A Negative	25	598	623	Specificity: 95.7% 95% CI: 93.8-97.0%
Total	291	625	916	

	PCR Results			
Status Flu A&B	Flu B Positive	Flu B Negative	Total	Performance
Flu B Positive	99	33	132	Sensitivity: 87.6% 95% CI: 80.3-92.5%
Flu B Negative	14	770	784	Specificity: 95.9% 95% CI: 94.3-97.1%
Total	113	803	916	

ANALYTICAL PERFORMANCE

Limit of Detection (LOD)

Limit of detection (LOD) for SARS-CoV-2 and influenza A and B in ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test was determined by evaluating different concentrations of heat inactivated viruses. Natural nasopharyngeal swab specimens were obtained from healthy donors and confirmed negative for COVID-19 and influenza A and B using ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test. Negative natural nasopharyngeal swab specimens were eluted in PBS. Swab elutes were combined and mixed thoroughly to create a negative clinical matrix pool to be used as the diluent. The viruses were diluted in this natural nasopharyngeal swab matrix pool to generate virus dilutions for testing. Nasopharyngeal swab samples were prepared by adding 50µL of each virus dilution onto the sterile swab. The swab samples were tested according to the test procedure in package insert.

Virus Strains	Sources	LoD	#Positive/#Total	% Positive
SARS-COV-2 USA-WA1/2020	ATCC® Number, VR-1986HK™	2.7 x 10^3 $TCID_{50}$/mL	20/20	100
Influenza A Victoria/361/11(H3N2)	Zeptometrix, Cat# 0810240CF	4.51 x 10^1 $TCID_{50}$/mL	20/20	100
Influenza A A/California/07/2009(H1N1)	ATCC, Cat# VR-1894	2.08 x 10^5 $CEID_{50}$/mL	20/20	100
Influenza B Victoria/504/00	Zeptometrix, Cat# 0810571CF	5.64 x 10^2 $TCID_{50}$/mL	20/20	100
Influenza B Yamagata/16/88	Zeptometrix, Cat# 0810518CF	2.72 x 10^2 $TCID_{50}$/mL	20/20	100

Analytical Reactivity/Inclusivity

The analytical reactivity of the monoclonal antibodies targeting SARS-CoV-2 in ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test were evaluated with the currently available SARS-CoV-2 strains.

2019-nCoV Strain/ Isolate	Source/Type	Analytical Reactivity
USA-WA1/2020	Zeptometrix Cat# 0810587CFHI/NR-52281	3.68 x10^3 $TCID_{50}$/mL
Hong Kong/VM20001061/2020	Zeptometrix Cat# 0810590CFHI/NR-52282	3.68 x10^3 $TCID_{50}$/mL
Italy-INMI1	Zeptometrix Cat# 0810589CFHI/NR-52284	6.52 x10^3 $TCID_{50}$/mL

The 2021 CDC Human Influenza Panel was tested with ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test and *Status* Flu A&B tests. The panel was tested as per the swab protocol recommended by the CDC. Briefly, a series of 5-fold dilutions were prepared with each panel. These dilutions were tested in five replicates until two consecutive dilutions were negative. Test results are tabulated below.

CDC human influenza virus A panel (VP2021) test result (swab sample)

Influenza Virus (Type/Subtype)	Virus Strain Name	Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (no. of positives /5 replicates)									
A(H3N2)	A/Perth/16/2009	Concentration	5	$2x10^{7.3}$	$4x10^{6.3}$	$8x10^{5.3}$	$1.6x10^{5.3}$	$3.2x10^{4.3}$	$6.4x10^{3.3}$	$1.28x10^{3.3}$	$2.56x10^{2.3}$
		Status Flu A&B	n/a	5/5	5/5	5/5	5/5	5/5	4/5	0/5	0/5
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	5/5	5/5	4/5	0/5	0/5
A(H3N2)	A/Tasmania/503/2020	Concentration	$10^{8.5}$	$2x10^{7.5}$	$4x10^{6.5}$	$8x10^{5.5}$	$1.6x10^{5.5}$	$3.2x10^{4.5}$	$6.4x10^{3.5}$	$1.28x10^{3.5}$	$2.56x10^{2.5}$
		Status Flu A&B	n/a	5/5	3/5	0/5	0/5	5/5	3/5	0/5	0/5
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	3/5	0/5	0/5	5/5	4/5	0/5	0/5
A(H1N1) pdm09	A/Christ Church /16/2010	Concentration	$10^{9.2}$	$2x10^{8.2}$	$4x10^{7.2}$	$8x10^{6.2}$	$1.6x10^{6.2}$	$3.2x10^{5.2}$	$6.4x10^{4.2}$	n/a	n/a
		Status Flu A&B	n/a	5/5	5/5	5/5	4/5	0/5	0/5	n/a	n/a
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	4/5	0/5	0/5	n/a	n/a
A(H1N1) pdm09	A/ Victoria/2570 /2019	Concentration	$10^{8.2}$	$2x10^{7.2}$	$4x10^{6.2}$	$8x10^{5.2}$	$1.6x10^{5.2}$	$3.2x10^{4.2}$	$6.4x10^{3.2}$	n/a	n/a
		Status Flu A&B	n/a	5/5	5/5	5/5	3/5	0/5	0/5	n/a	n/a
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	3/5	0/5	0/5	n/a	n/a

CDC human influenza virus B panel (VP2021) test result (swab sample)

Influenza Virus (Type/Subtype)	Virus Strain Name	Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (no. of positives /5 replicates)								
B (Victoria lineage)	B/Michigan/09/2011	Concentration	$10^{6.9}$	$2x10^{5.9}$	$4x10^{4.9}$	$8x10^{3.9}$	$1.6x10^{3.9}$	$3.2x10^{2.9}$	$6.4x10^{1.9}$	n/a
		Status Flu A&B	n/a	5/5	5/5	5/5	3/5	0/5	0/5	n/a
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	3/5	0/5	0/5	n/a
B (Victoria lineage)	B/Washington/02/2019	Concentration	$10^{9.3}$	$2x10^{8.3}$	$4x10^{7.3}$	$8x10^{6.3}$	$1.6x10^{6.3}$	$3.2x10^{5.3}$	$6.4x10^{4.3}$	n/a
		Status Flu A&B	n/a	5/5	5/5	5/5	4/5	0/5	0/5	n/a
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	4/5	0/5	0/5	n/a
B (Yamagata lineage)	B/Texas/81/2016	Concentration	$10^{8.1}$	$2x10^{7.1}$	$4x10^{6.1}$	$8x10^{5.1}$	$1.6x10^{5.1}$	$3.2x10^{4.1}$	$6.4x10^{3.1}$	n/a
		Status Flu A&B	n/a	5/5	5/5	5/5	5/5	0/5	0/5	n/a
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	5/5	5/5	0/5	0/5	n/a
B (Yamagata lineage)	B/Phuket/3073/2013	Concentration	$10^{9.9}$	$2x10^{8.9}$	$4x10^{7.9}$	$8x10^{6.9}$	$1.6x10^{6.9}$	$3.2x10^{5.9}$	$6.4x10^{4.9}$	$1.28x10^{4.9}$
		Status Flu A&B	n/a	5/5	5/5	3/5	5/5	1/5	0/5	0/5
		ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test	n/a	5/5	5/5	3/5	5/5	2/5	0/5	0/5

The analytical inclusivity for influenza A and B was demonstrated with *Status* Flu A&B using a total of 49 influenza strains: 34 strains of influenza A type and 15 strains of influenza B type. Additional information detailing this testing can be found in *Status* Flu A&B package insert.

Analytical Specificity (Cross-reactivity)

Cross-reactivity of ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test was evaluated by testing a panel of related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in clinical specimens and could potentially cross-react with ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test including ten (10) bacteria, eighteen (18) viruses and one (1) negative matrix. Each organism and virus were tested in the absence or presence of heat inactivated SARS-CoV-2, influenza A, and B at 3 x LoD. No cross-reactivity was seen with the listed microorganisms when tested at the concentration presented in the table below.

Cross-Reactivity SARS-CoV-2

Potential Cross-Reactant	Concentration Tested
Human coronavirus 229E	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL
Adenovirus C1	1.0 x 10^5 $TCID_{50}$/mL
Human Metapneumovirus(hMPV)	3.89 x 10^4 $TCID_{50}$/mL
Parainfluenza virus 1, C35	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 2, Greer	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 3, C243	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 4, CH19503	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/California/2/2014(H3N2)	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/Hong Kong/8/68(H3N2)	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/California/07/2009(H1N1)	1.0 x 10^5 $TCID_{50}$/mL
Influenza B B/Russia/69	1.0 x 10^5 $TCID_{50}$/mL
Influenza B B/Florida/02/06	1.0 x 10^5 $TCID_{50}$/mL

Human enterovirus 71Strain: H	1.0 x 10^5 $TCID_{50}$/mL
Human respiratory syncytial virus, A2	1.0 x 10^5 PFU/mL
Rhinovirus 2060	1.0 x 10^5 PFU/mL
Haemophilus influenza	4 x 10^4 cfu/mL
Streptococcus pneumoniae	2.0 x 10^4 cfu/mL
Streptococcus pyogenes, Bruno	4.0 x 10^6 cfu/mL
Candida albicans	1.0 x 10^6 cfu/mL
Bordetella pertussis, 18323	1.0 x 10^6 cfu/mL
Mycoplasma pneumoniae	1.0 x 10^6 cfu/mL
Chlamydia pneumoniae TW-183	1.0 x 10^6 IFU/mL
Legionella pneumophila	1.0 x 10^6 cfu/mL
Pneumocystis jirovecii	1.0 x 10^6 cfu/mL
Staphylococcus epidermidis	1.0 x 10^6 cfu/mL
Staphylococcus aureus	11.0 x 10^6 cfu/mL
Pooled human nasal wash	NA

Cross-Reactivity Influenza A

Potential Cross-Reactant	**Concentration Tested**
Human coronavirus 229E	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL
Adenovirus C1	1.0 x 10^5 $TCID_{50}$/mL
Human Metapneumovirus(hMPV)	3.89 x 10^4 $TCID_{50}$/mL
Parainfluenza virus 1, C35	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 2, Greer	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 3, C243	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 4, CH19503	1.0 x 10^5 $TCID_{50}$/mL
Influenza B B/Russia/69	1.0 x 10^5 $CEID_{50}$/mL
Influenza B B/Florida/02/06	1.0 x 10^5 $TCID_{50}$/mL
Human enterovirus 71Strain: H	1.0 x 10^5 $TCID_{50}$/mL
Human respiratory syncytial virus, A2	1.0 x 10^5 PFU/mL
Rhinovirus 2060	1.0 x 10^5 PFU/mL
Haemophilus influenza	4 x 10^4 cfu/mL
Streptococcus pneumoniae	2.0 x 10^4 cfu/mL
Streptococcus pyogenes, Bruno	4.0 x 10^6 cfu/mL
Candida albicans	1.0 x 10^6 cfu/mL
Bordetella pertussis, 18323	1.0 x 10^6 cfu/mL
Mycoplasma pneumoniae	1.0 x 10^6 cfu/mL
Chlamydia pneumoniae TW-183	1.0 x 10^6 IFU/mL
Legionella pneumophila	1.0 x 10^6 cfu/mL
Pneumocystis jirovecii	1.0 x 10^6 cfu/mL
Staphylococcus epidermidis	1.0 x 10^6 cfu/mL
Staphylococcus aureus	11.0 x 10^6 cfu/mL
Pooled human nasal wash	NA

Cross-Reactivity Influenza B

Potential Cross-Reactant	Concentration Tested
Human coronavirus 229E	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL
Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL
Adenovirus C1	1.0 x 10^5 $TCID_{50}$/mL
Human Metapneumovirus(hMPV)	3.89 x 10^4 $TCID_{50}$/mL
Parainfluenza virus 1, C35	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 2, Greer	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 3, C243	1.0 x 10^5 $TCID_{50}$/mL
Parainfluenza virus 4, CH19503	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/California/2/2014(H3N2)	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/Hong Kong/8/68(H3N2)	1.0 x 10^5 $TCID_{50}$/mL
Influenza A A/California/07/2009(H1N1)	1.0 x 10^5 $CEID_{50}$/mL
Human enterovirus 71Strain: H	1.0 x 10^5 $TCID_{50}$/mL
Human respiratory syncytial virus, A2	1.0 x 10^5 PFU/mL
Rhinovirus 2060	1.0 x 10^5 PFU/mL
Haemophilus influenza	4 x 10^4 cfu/mL
Streptococcus pneumoniae	2.0 x 10^4 cfu/mL
Streptococcus pyogenes, Bruno	4.0 x 10^6 cfu/mL
Candida albicans	1.0 x 10^6 cfu/mL
Bordetella pertussis, 18323	1.0 x 10^6 cfu/mL
Mycoplasma pneumoniae	1.0 x 10^6 cfu/mL
Chlamydia pneumoniae TW-183	1.0 x 10^6 IFU/mL
Legionella pneumophila	1.0 x 10^6 cfu/mL
Pneumocystis jirovecii	1.0 x 10^6 cfu/mL
Staphylococcus epidermidis	1.0 x 10^6 cfu/mL
Staphylococcus aureus	11.0 x 10^6 cfu/mL
Pooled human nasal wash	NA

To estimate the likelihood of cross-reactivity with SARS-CoV-2, influenza A or B virus in the presence of organisms that were not available for wet testing, due to unavailability of BSL-3 access, *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.

- The comparison between SARS-CoV-2 nucleocapsid protein, MERS-CoV and *human coronavirus* HKU1 revealed that cross-reactivity cannot be ruled out. Homology for HKU1 and MERS-CoV is relatively low, at 48.5% across 91% of sequence and 36.7% across 82% of the sequence, respectively.
- Wet testing with SARS-coronavirus was not conducted. However, in silico analysis indicated that cross-reactivity is likely.
- No significant similarity found between *Mycobacterium tuberculosis*, and SARS-CoV-2, or between *Mycobacterium tuberculosis* and influenza A or B, however, cross-reactivity cannot be ruled out.
- No significant similarity found between *SARS-Coronavirus* and influenza A or B, however, cross-reactivity cannot be ruled out.
- No significant similarity found between *MERS-coronavirus* and influenza A or B, however, cross-reactivity cannot be ruled out.
- No significant similarity found between *Human coronavirus HKU* and influenza A or B, however, cross-reactivity cannot be ruled out.

Endogenous Interfering Substances

The potential interference of endogenous substances with the antibodies used for the detection of COVID-19, influenza A and B was examined by testing nineteen (19) substances in a negative clinical matrix, in the absence or presence of each virus; at 3 x LOD concentrations for SARS-CoV-2, influenza A and influenza B. The interference study was conducted using medically relevant concentrations of the potentially interfering substances listed below to assess the potential interference of the substances on the performance of ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test. No interference was seen with the listed substances when tested at the concentration presented in the table below.

Interfering Substance	Active Ingredient	Concentration
Mucin	Mucin	5.0 mg/mL
Whole blood (human)	Blood	5%
Halls Cough Suppressant/ Oral Anesthetic Drops	Menthol	1.5 mg/mL
Nasacort Allergy 24H	Triamcinolone acetonide	5%
Rhinocort Allergy Spray	Budesonide (Glucocorticoid)	5%
ZICAM Cold Remedy + Multi-Symptom Relief	Galphimia glauca, luffa operculata, sabadilla	5%
Afrin Nasal Spray	Oxymetazoline HCL	15%
Cepacol Extra Strength	Benzocaine, Menthol	1.5 mg/mL
Flonase Allergy Relief	Fluticasone Propionate (Glucocorticoid)	5%
Oseltamivir	Oseltamivir	5 mg/mL
Saline nasal spray	Saline	15%
NasoGEL(NeilMed)	Sodium Chloride, Sodium Bicarbonate, Sodium Hyaluronate	5%
Tobramycine	Tobramycin	10 µg/mL
Zanamivir	Zanamivir	282.0 ng/mL
CVS Sinus Relief Nasal spray	Phenylephrine hydrochloride	15%
NasalCrom Nasal spray	Cromolyn sodium	15%
Sore throat phenol spray	Phenol	15%
Homeopathic (Alkalol)	Galphima glauca 6X, Luffa operculata 6X, Sabadila 6X	1:10 dilution
Mupirocin	Mupirocin	10 mg/mL

High-Dose Hook Effect

A high-dose hook effect was not detected in ViraDx SARS-CoV-2/Flu A+B Rapid Antigen Test for the SARS-CoV-2, influenza A and B viral strains at the concentration listed below.

Virus Type	Viral Strain	Concentration tested
SARS-CoV-2	USA-WA1/2020	1.15×10^7 TCID50/mL
Influenza A (H3N2)	A/California/2/2014	5.8×10^5 $TCID_{50}$/mL
Influenza A (H3N2)	A/Hong Kong/8/68	1.26×10^6 $TCID_{50}$/mL
Influenza A (H3N2)	Victoria/361/11	1.41×10^5 $TCID_{50}$/mL
Influenza A (H1N1)	A/California/07/2009	5.2×10^7 $CEID_{50}$/mL
Influenza B	B/Russia/69	1.5×10^6 $CEID_{50}$/mL
Influenza B	B/Florida/02/06	1.05×10^6 $TCID_{50}$/mL
Influenza B	B/Victoria/504/00	1.41×10^5 $TCID_{50}$/mL
Influenza B	B/Yamagata/16/88	1.70×10^5 $TCID_{50}$/mL

Co-Infection (Competitive Interference)

For co-infection, SARS-CoV-2 at levels near LOD was tested in the presence of high levels of influenza A or influenza B and influenza A and influenza B at levels near LOD were tested in the presence of high levels of SARS-CoV-2. No competitive interference was seen between SARS-CoV-2 and influenza A and B in this testing at the concentration listed in the tables below.

Cross-Reactivity Influenza A

Competitive virus	Concentration	Competitive target virus	Concentration	Competitive Target Percent Positivity
Influenza A (H3N2) A/California/2/2014	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza A A/Hong Kong/8/68(H3N2)	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza A (H3N2) Victoria/361/11	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza A (H1N1) A/California/07/2009	1.0 x 10^5 $CEID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
SARS-CoV-2 USA-WA1/2020	1.0 x 10^5 $TCID_{50}$/mL	Influenza A (H3N2) Victoria/361/11	6.5 x 10^1 $TCID_{50}$/mL	100%
SARS-CoV-2 USA-WA1/2020	1.0 x 10^5 $TCID_{50}$/mL	Influenza A (H1N1) A/California/07/2009	3.0 x 10^5 $CEID_{50}$/mL	100%

Cross-Reactivity Influenza B

Competitive virus	Concentration	Competitive target virus	Concentration	Competitive Target Percent Positivity
Influenza B B/Russia/69	1.0 x 10^5 $CEID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza B B/Florida/02/06	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza B Victoria/504/00	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
Influenza B Yamagata/16/88	1.0 x 10^5 $TCID_{50}$/mL	SARS-CoV-2 USA-WA1/2020	4.0 x 10^3 $TCID_{50}$/mL	100%
SARS-CoV-2 USA-WA1/2020	1.0 x 10^5 $TCID_{50}$/mL	Influenza B Victoria/504/00	8.0 x 10^2 $TCID_{50}$/mL	100%
SARS-CoV-2 USA-WA1/2020	1.0 x 10^5 $TCID_{50}$/mL	Influenza B Yamagata/16/88	4.0 x 10^2 $TCID_{50}$/mL	100%

ASSISTANCE

If you have any questions regarding the use of this product, please contact Lumos Diagnostics Technical Support at 1.855.LumosDx or technical.support@lumosdiagnostics.com.

REFERENCES

1. Shaw MW, Arden NH, Massab HF. New aspects of influenza viruses. Clin Microbiol Rev 1992;5(1):74-92.
2. WHO recommendations on the use of rapid testing for influenza diagnosis, July 2005.
3. Design Considerations for Pivotal Clinical Investigations for Medical Devices: Guidance for Industry, Clinical Investigators, Institutional Review Boards and Food and Drug Administration Staff, November 7, 2013 (Page 45).

GLOSSARY OF SYMBOLS

Consult instructions for use	Do not use if package is damaged	Catalog number
2°C – 30°C Temperature limit	Do not re-use	Positive Control
Use-by date	in vitro diagnostic medical device	Negative Control
Contains sufficient for 1 test	Batch code	Distributor
Contains sufficient for 25 tests		Manufacturer

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