



aptitude[®]

Metrix[®] COVID/Flu Test

Instructions for Use For Healthcare Providers

For *in vitro* diagnostic use.

For use under Emergency Use Authorization (EUA) only.

For use with the Metrix Reader (Gen 2).

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1. Intended Use

The Metrix® COVID/Flu Test is a reverse transcription and loop-mediated isothermal amplification (RT-LAMP) test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B nucleic acid. This test is authorized for non-prescription home use with anterior nasal (AN) swab specimens from individuals aged 14 years or older (self-collected) or individuals aged 2 years or older (collected by an adult) with signs and symptoms of respiratory infection consistent with COVID-19. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

The Metrix COVID/Flu Test is intended for use in the differential detection of SARS-CoV-2, influenza A, and influenza B nucleic acid in clinical specimens and is not intended to detect influenza C. SARS-CoV-2, influenza A, and influenza B nucleic acid is generally detectable in anterior nasal swab specimens during the acute phase of infection.

Positive results indicate the presence of viral nucleic acid, but clinical correlation with past medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent detected may not be the definitive cause of disease. Individuals who test positive with the Metrix COVID/Flu Test should self-isolate and seek follow up care with their physician or healthcare provider as additional testing may be necessary.

Negative results do not rule out SARS-CoV-2, influenza A, and/or influenza B infection and should not be used as the sole basis for treatment or other management decisions, including infection control decisions. Negative results should be considered in the context of current prevalence of infection, an individual's recent exposures, history and the presence of clinical signs and symptoms consistent with respiratory infection.

Individuals who test negative and continue to experience symptoms of fever, cough and/or shortness of breath may still have a respiratory infection and should seek follow up care with their healthcare provider.

The Metrix COVID/Flu Test is only for use under the Food and Drug Administration's Emergency Authorization.

2. Summary and Explanation of the Test

The Metrix COVID/Flu Test is a molecular *in vitro* diagnostic test for the qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B viral RNA in anterior nasal swab specimens from individuals with symptoms of respiratory infection. The Metrix COVID/Flu Test requires the use of the Metrix Reader (Gen 2), which is available separately.

3. Principles of the Procedure

The Metrix COVID/Flu Test is a compact nucleic acid amplification test (NAAT) that detects the genetic material of SARS-CoV-2, influenza A, and influenza B in unprocessed anterior nasal swab specimens using an isothermal molecular amplification reaction that is an equivalent alternative to polymerase chain reaction (PCR). The Metrix COVID/Flu Test utilizes a multi-gene amplification method utilizing primers that target both the nucleocapsid (N) and open reading frame (ORF-1) genes of SARS-CoV-2, Segment 7 of Influenza A, and Segment 8 of Influenza B. The Metrix COVID/Flu Test also contains an internal control that monitors for sample lysis, proper assay execution, sample inhibition, amplification, and assay reagent function.

The consumable Metrix COVID/Flu Test kit consists of four components: Collector, Cap, Sensor, and a Nasal Swab. The Metrix COVID/Flu Test kit is

used with the reusable Metrix Reader (Gen 2), which is available separately. To prepare for a test, a self-collected anterior nasal swab sample is first placed in the Collector. Then, the Cap is attached to the Collector to release neutralization buffer (NB) from the Cap to mix with the sample. The NB is designed facilitate sample lysis and release nucleic acids for downstream detection. The Collector is then connected to the Sensor to allow the sample to mix with reagents stored in the Sensor. Finally, the Sensor is inserted into the Metrix Reader (Gen 2) to initiate the reaction and detect the presence of SARS-CoV-2, influenza A, and influenza B RNA.

The system utilizes an electrochemical reporting technology to monitor the amplified double-stranded cDNA concentration in real-time in respective reaction chambers. The amplification creates a characteristic electrochemical signal analogous to the fluorescence signal of a laboratory PCR reaction. The electrochemical signal is analyzed automatically by the Metrix Reader (Gen 2), and the test result is reported as a combination of colors and positions of LED lights on the Metrix Reader (Gen 2). Once a Sensor is inserted into the Metrix Reader (Gen 2), a result is obtained within 20 minutes.

A positive result occurs when one or more target channels (SARS-CoV-2, Flu A, or Flu B) pass the detection threshold. A negative result occurs when only the internal control channel passes the detection threshold. An invalid result occurs when all channels (target and internal control) fail to pass the detection threshold.

4. Materials

4.1 Materials Provided

Each Metrix COVID/Flu Test comes with sufficient material to perform 1 or 25 tests (as specified on the outer packaging). Each of the following components is for single use only:

- Cap
- Collector
- Swab
- Sensor
- Test Instructions

4.2 Materials Required but Not Provided in Test Kit

Each Metrix COVID/Flu Test requires:

- Metrix Reader (Gen 2), which is reusable

5. Warnings and Precautions

5.1 General

- For *in vitro* diagnostic use.
- Do not use if kit is visibly damaged.
- For non-prescription home use.
- The Metrix COVID/Flu Test and Metrix Reader (Gen 2) are for use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA.

- This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A, and influenza B, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Follow all instructions carefully. Correct use is required for accuracy.
- Only use the test components provided. Do not re-use any components for another test. Only the Metrix Reader (Gen 2) may be re-used multiple times.
- Positive results are indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B RNA.

5.2 Storage and Handling

- Store all components at 59 °F to 86 °F (15 °C to 30 °C).
- Do not open kit components until you are ready to perform testing.
- Samples should be tested as quickly as possible after collection.
- Do not use the Metrix COVID/Flu Test past the Use By date.
- Do not use components that are visibly damaged.
- All components other than the Metrix Reader (Gen 2) are single-use and should be disposed of after use.
- Treat all biological specimens, including used test components, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions. Guidelines for specimen handling are available from the U.S.

Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.¹⁻³

- Do not disassemble the Metrix COVID/Flu Test Cap, Collector, Sensor, or any assemblies.
- Touch only the handle of the Swab with your hands to avoid contaminating the soft tip.
- Do not ingest any contents of this kit. Keep out of reach of children.
- Avoid contact with skin and eyes.

6. Operating Conditions

- The test should be used between 59 °F and 86 °F (15 °C and 30 °C).
- The test is best used in a room with adequate lighting away from glare.
- The Metrix Reader (Gen 2) should be used on a level surface without movement.
- If a power failure occurs or if the Metrix Reader (Gen 2) is unplugged while the Sensor is inserted, the test result is invalid and the patient should be retested using a fresh sample.

7. Procedure

7.1 Start Here

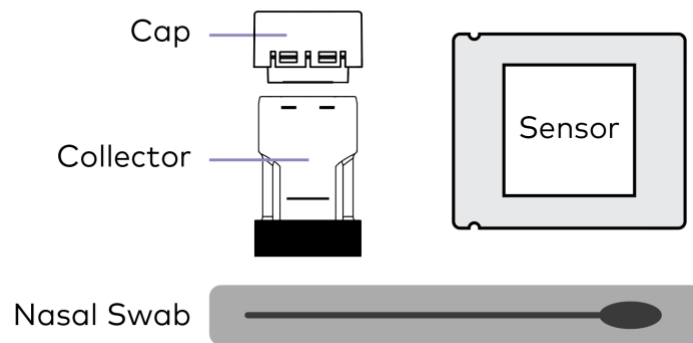
- Carefully read all instructions before beginning the test.

Scan the QR
code with the
camera on your
mobile device



- Complete the entire procedure without delay between steps.
- Contents:

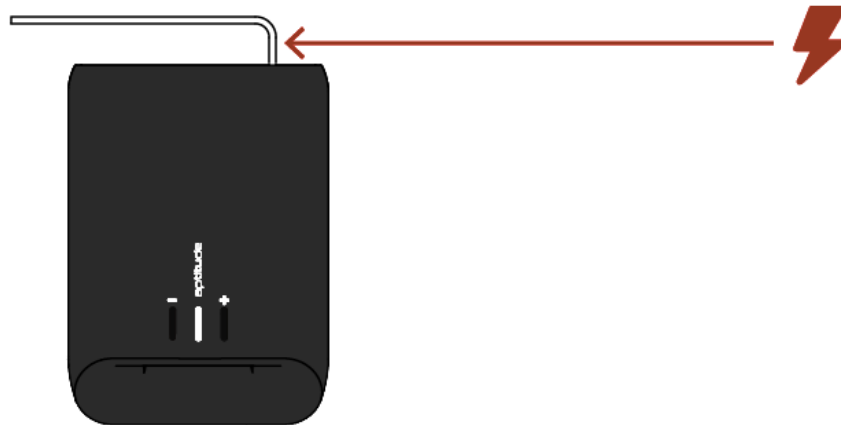
Materials required to run one test:



- Metrix Reader (Gen 2) required. Available separately.

7.2 STEP 1: Power Up the Metrix Reader (Gen 2)

- Connect the Metrix Reader (Gen 2) to the power supply. The center light will turn **solid white** (not flashing) when ready.



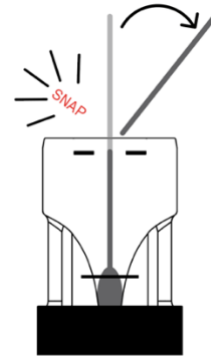
7.3 STEP 2: Collect Your Sample

- Insert the nasal swab into your nostril until the tip is fully inside. Stop when you meet resistance (about 1 inch for adults, ½ inch for children).
- Roll the swab against the inside of your nostril 5 times.



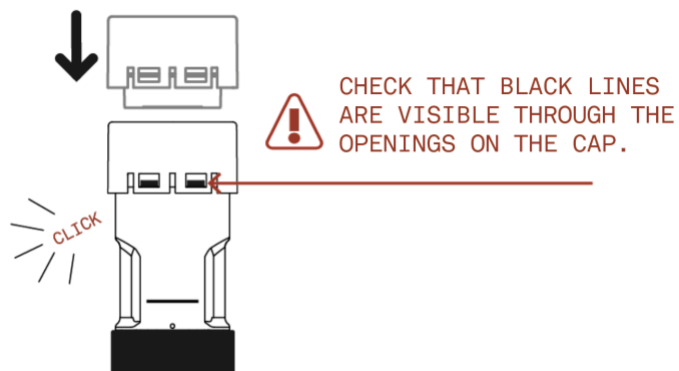
REPEAT WITH OTHER NOSTRIL

- Firmly insert the swab into collector until it cannot go any further.
- Snap off and discard the swab handle.



7.4 STEP 3: Cap Your Sample

- Put the cap onto the collector and press down **firmly** until the cap clicks into place.



7.5 STEP 4: Shake to Mix the Sample

- Shake the collector **very hard** for 20 seconds to mix.

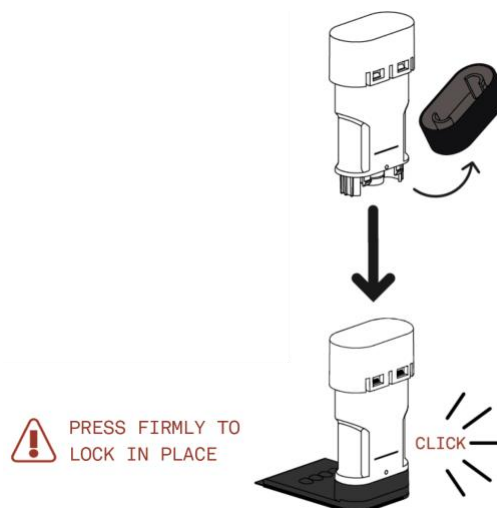


7.6 STEP 5: Attach Collector to the Sensor

- Open the sensor pouch and place the sensor on a flat surface.



- Remove the **black plastic cover** from the bottom of the collector. **Firmly** insert the collector into the sensor until it **clicks** and locks in place.



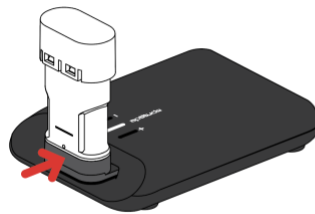
7.7 STEP 6: Run the Test

- Check the reader status.



CONFIRM THAT THE READER IS READY.
THE CENTER LIGHT WILL BE SOLID
(NOT FLASHING).

- Insert the sensor into reader until it cannot go further. The test will begin automatically and will take 20 minutes to complete.



Flashing White



Test in progress

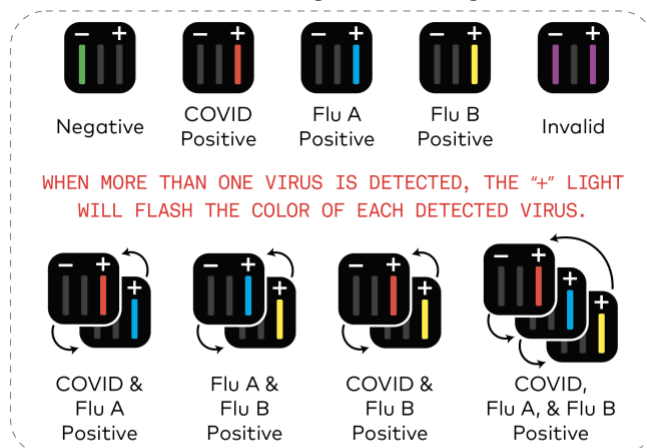
Flashing Red



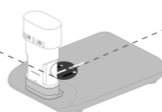
Test error, see troubleshooting section

7.8 STEP 7: Read Your Results

- Use the following visual signifiers to note the results of the test:

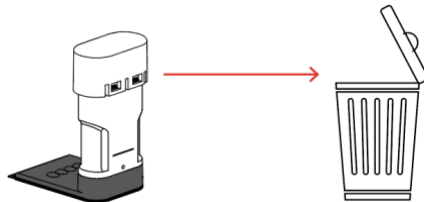


FOR PROOF OF TEST, TAKE PHOTO(S) OR VIDEO OF YOUR RESULT. RESULTS WILL DISPLAY UNTIL THE SENSOR IS REMOVED.



7.9 STEP 8: Discard Your Sensor

- Pull the sensor out of the reader. Discard the sensor. **Do not disassemble.**
- The Metrix Reader (Gen 2) is now ready to begin another test.



7.10 After Your Test

- Please seek follow up care from a healthcare physician if your symptoms persist or if you are concerned about your health.
- To report your Metrix COVID/Flu Test results to public health agencies, please visit: aptitudemetrix.com/publichealth/reporting.

7.11 Reader Statuses and Troubleshooting

- All Metrix Reader (Gen 2) statuses for normal and abnormal operation are shown below. If troubleshooting fails to resolve any problem, please contact support. If your Metrix Reader (Gen 2) needs to be disposed of, please place in electronic waste.

Reader Statuses



Reader Starting Up

The Reader is starting up. Please wait until center light is solid white.



Reader Ready

The Reader is ready to begin a test.



Test in Progress

A test is currently in progress. Do not disturb the Reader or remove the sensor



Negative Result

SARS-CoV-2, Flu A, and Flu B viral RNA not detected.



COVID-19 Positive Result

SARS-CoV-2 viral RNA was detected.



Flu A Positive Result

Influenza A viral RNA was detected.



Flu B Positive Result

Influenza B viral RNA was detected.



COVID-19 and Flu A Positive Result

SARS-CoV-2 and Influenza A viral RNA were detected.



Flu A and Flu B Positive Result

Influenza A and Influenza B viral RNA were detected.



COVID-19 and Flu B Positive Result

SARS-CoV-2 and Influenza B viral RNA was detected.



COVID-19, Flu A, and Flu B Positive Result

SARS-CoV-2, Influenza A, and Influenza B viral RNA was detected.



Invalid Result

Test should be repeated.

Troubleshooting



Test Error

Remove sensor and **firmly** press down on collector. **Firmly** reinsert sensor into reader. If error persists, discard sensor and use a new test kit.



Canceled Test

The test did not complete. Ensure you are using the Metrix Reader (Gen 2). Discard the Sensor and run the test with a new Metrix COVID/Flu Test kit.



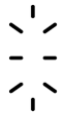
Hardware Failure

There is an error with the reader. Disconnect and reconnect the power.



No Power

Check all electrical connections. The reader is not receiving power.



Indicates flashing light

8. Quality Control

The Metrix COVID/Flu Test contains an internal control to monitor for sample lysis, reverse transcription, amplification, detection, and potential interference. External controls are not required for the test procedure.

9. Limitations

- Performance was evaluated with anterior nasal (nares) swab specimens only, using the instructions provided in this document. Failure to follow these procedures may cause false results.
- False negative results may occur if a specimen is improperly collected or handled. False negative results may occur if inadequate levels of virus are present in the specimen. False negative results may occur if the virus mutates in the regions targeted by the test.

- The test is a qualitative test and does not provide the quantitative value of detected organism present.
- Results from this test are read visually. Color-blind users may be unable to differentiate between all color status lights. Users with conditions affecting their vision, including color blind users should seek assistance to interpret results accurately.
- Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor are they the causative agents for clinical symptoms.
- Positive and negative predictive values are dependent upon disease prevalence. False negative results are more likely during peak activity when disease prevalence is high and false positive results are more likely during periods of low activity.
- Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause false positive results.
- 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/strains 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/Flu A/Flu B and their prevalence, which may change over time.

10. Performance Characteristics

10.1 Analytical Sensitivity (Limit of Detection)

The limit of detection (LoD) for SARS-CoV-2, Flu A, and Flu B were conducted to determine the lowest detectable viral concentrations at which at least 95% of all true positive replicates yield positive results using the Metrix COVID/Flu Test. All LoD studies were performed by diluting viruses into pooled negative nasal matrix and spiking 75 µL of the dilutions onto swabs.

LoD studies using single-analyte samples were first performed to establish the LoD for each target alone. This was done by in a stepwise fashion by first performing an LoD range-finding study for each target over a wide range of concentrations separated at two-fold dilution intervals (concentrations listed in genome equivalents, or GE, per swab). The tentative LoD was defined as the lowest concentration at which 3/3 replicates yielded positive results. The LoD was then confirmed using a narrow dilution series (two-fold above and below the tentative LoD) with 20 replicates. The confirmed LoD was defined as the lowest concentration at which at least 19/20 (95%) of replicates yielded positive results. The LoD was confirmed with another 20 replicates using a second lot of devices. Replicate negative samples were also included in all studies.

The LoD for SARS-CoV-2 (USA-WA/2020) was determined to be 500 GE/swab. The LoD for Flu A (A/Indiana/02/2020 and A/Hong Kong/2671/2019) was determined to be 1,000 GE/swab. The LoD for Flu B (B/Florida/78/2015 and B/Massachusetts/2/2012) was determined to be 2,000 GE/swab. Replicate negative samples were also run and all showed negative results in the tests as expected.

Limit of Detection: SARS-CoV-2 (USA-WA1/2020) Confirmatory Study

Lot	Swab Viral Load (GE/swab)	#Positive/#Tested	% Positive
1	1,000	20/20	100%
1	500	19/20	95%
1	250	10/20	50%
2	500	20/20	100%

Limit of Detection: Flu A, H1N1 (A/Indiana/02/2020) Confirmatory Study

Lot	Swab Viral Load (GE/swab)	#Positive/#Tested	% Positive
1	2,000	20/20	100%
1	1,000	19/20	95%
1	500	12/20	60%
2	1,000	19/20	95%

Limit of Detection: Flu A, H3N2 (A/Hong Kong/2671/2019) Confirmatory Study

Lot	Swab Viral Load (GE/swab)	#Positive/#Tested	% Positive
1	2000	20/20	100%
1	1,000	19/20	95%
1	500	9/20	45%
2	1,000	19/20	95%

Limit of Detection: Flu B, Victoria (B/Florida/78/2015) Confirmatory Study

Lot	Swab Viral Load (GE/swab)	#Positive/#Tested	% Positive
1	4,000	20/20	100%
1	2,000	19/20	95%
1	1,000	11/20	55%
2	2,000	19/20	95%

Limit of Detection: Flu B, Yamagata (B/Massachusetts/2/2012) Confirmatory Study

Lot	Swab Viral Load (GE/swab)	#Positive/#Tested	% Positive
1	4,000	20/20	100%
1	2,000	19/20	95%
1	1,000	13/20	65%
2	2,000	19/20	95%

A co-spike LoD study was also performed to confirm that the LoD for each target remains unchanged when the sample contains all three targets present at their respective LoDs as determined from the single-analyte studies outlined above.

10.2 Inclusivity (Analytical Sensitivity)

Both *in silico* and *in vitro* studies were conducted to demonstrate the reactivity of the Metrix COVID/Flu Test primers with SARS-CoV-2, Flu A, and Flu B.

(A) *In Silico* Study (Predicted Reactivity)

In order to evaluate reactivity, Metrix COVID/Flu Test primer sequences were aligned against complete SARS-CoV-2, Flu A, and Flu B sequences compiled from the NCBI database via a BLAST protocol. Reactivity towards each viral variant/strain was positively predicted if: (1) a primer set had at most one base pair mismatch on each primer in the set and (2) there were no mismatches within 5 nucleotides of the leading edge (3' end) for each primer.

a. SARS-CoV-2 Predicted Reactivity

A total of 1,009,808 complete SARS-CoV-2 sequences from between 1 JAN 2020 and 20 FEB 2024 were analyzed as described above. A total of 1,006,072 sequences (99.63%) were predicted to be reactive with the Metrix COVID/Flu Test. Additionally, >95% of all sequences analyzed for each Greek-letter are reactive with the Metrix COVID/Flu Test.

SARS-CoV-2 Variant	Number of Sequences Tested	Number of Reactive Sequences	Percent Reactive
Alpha	67,376	66,579	98.82%
Beta	397	396	99.75%
Delta	145,127	144,647	99.67%
Gamma	9,072	9,040	99.65%
Omicron	602,121	597,200	99.18%
Epsilon	11,046	10,906	98.73%
Eta	435	433	99.54%
Kappa	73	73	100.00%
Mu	26	25	96.15%
Zeta	365	365	100.00%
Total (All Variants)	1,009,808	1,006,072	99.63%

b. Influenza A and B Predicted Reactivity

A total of 23,506 complete Flu A (H1N1) sequences, 45,801 complete Flu A (H3N2) sequences, and 14,846 complete Flu B (Yamagata and Victoria) sequences from between 1 JAN 2009 and 20 FEB 2024 were analyzed as described above. More than 95% of all strains analyzed,

including those isolated to within the last 5 years, are reactive with the Metrix COVID/Flu Test.

Influenza Strain and Variant	Number of Sequences Tested	Number of Reactive Sequences	Percent Reactive
Influenza A (H1N1)	18,267	17,764	97.25%
Influenza A (H1N1)	5,239	5,017	95.76%
Influenza A (H3N2)	31,117	30,116	96.78%
Influenza A (H3N2)	14,684	14,198	96.69%
Influenza B (Yamagata + Victoria)	11,879	11,336	95.43%
Influenza B (Yamagata + Victoria)	2,967	2,873	96.83%

(B) *In Vitro* Study (Wet Testing)

Reactivity between the Metrix COVID/Flu Test and SARS-CoV-2, Flu A, and Flu B was also evaluated via wet testing. Targets were prepared in pooled negative nasal matrix at 3x LoD and were spiked onto dry swabs, which were then processed according to the instructions. Negative control samples (NTC) were also run in triplicate for each set of SARS-CoV-2, Flu A (H1N1 and H3N2), Flu B (Victoria and Yamagata) viruses tested. SARS-CoV-2 was evaluated using inactivated virus and influenza A (H1N1 and H3N2) and influenza B (Yamagata and Victoria) were evaluated using live virus. All NTC replicates produced negative results as expected, and all positive replicates for each virus produced positive results, confirming reactivity with the Metrix COVID/Flu Test.

Virus	Strain	Concentration (GE/swab)	#Positive / #Tested
SARS-CoV-2	USA-WA1/2020 (Lineage A)	1,500	3/3
	USA/CA_CDC-5574/2020 (Alpha, B.1.1.7.)	1,500	3/3
	MD-HP01542/2021 (Beta, B.1.351)	1,500	3/3
	MD-HP05285/2021 (Delta, B.1.617.2)	1,500	3/3
	GA-EHC-2811C/2021 (Omicron, B.1.1.529)	1,500	3/3

	NTC	0	0/3
Flu A (H1N1)	H1N1 A/Indiana/02/2020	3,000	3/3
	H1N1 A/Hawaii/66/2019	3,000	3/3
	H1N1 A/Idaho/07/2018	3,000	3/3
	H1N1 A/California/07/2009	3,000	3/3
	H1N1 A/Iowa/53/2015	3,000	3/3
	H1N1 A/Michigan/272/2017	3,000	3/3
	H1N1 A/Michigan/45/2015	3,000	3/3
	H1N1 A/St. Petersburg/61/2015	3,000	3/3
	H1N1 A/Wisconsin/505/2018	3,000	3/3
	H1N1 A/Wisconsin/588/2019	3,000	3/3
	NTC	0	0/3
Flu A (H3N2)	H3N2 A/Hong Kong/2671/2019	3,000	3/3
	H3N2 A/California/2/2014	3,000	3/3
	H3N2 A/Delaware/01/2021	3,000	3/3
	H3N2 A/Arizona/45/2018	3,000	3/3
	H3N2 A/Kansas/14/2017	3,000	3/3
	H3N2 A/Michigan/173/2020	3,000	3/3
	H3N2 A/New York/21/2020	3,000	3/3
	H3N2 A/Switzerland/9715293/2013	3,000	3/3
	H3N2 A/Tasmania/503/2020	3,000	3/3
	H3N2 A/Hong Kong/4801/2014	3,000	3/3
	NTC	0	0/3
Flu B (Victoria)	B/Florida/78/2015	6,000	3/3
	B/Texas/43/2019	6,000	3/3
	B/Hawaii/01/2018	6,000	3/3
	B/Michigan/01/2021	6,000	3/3
	B/Hong Kong/286/2017	6,000	3/3
	NTC	0	0/3
Flu B (Yamagata)	B/Massachusetts/2/2012	6,000	3/3
	B/Wisconsin/10/2016	6,000	3/3
	B/Indiana/17/2017	6,000	3/3
	B/Oklahoma/10/2018	6,000	3/3
	B/Texas/06/2011	6,000	3/3
	NTC	0	0/3

Influenza A H5 and H7 strains were also evaluated for *in vitro* inclusivity using either inactivated virus, genomic RNA, or double-stranded DNA (dsDNA) based on availability. Strains were initially tested at 3,000 GE/swab, and if 3/3 positive replicates did not produce positive results, the target concentration was increased until this criterion was met. The table below shows the concentrations at which each strain produce 3/3 positive results with the Metrix COVID/Flu Test.

Virus	Strain	Concentration (GE/swab)	X LoD	#Positive / #Tested
Influenza A H5 and H7	H5N1 (Inactivated Virus) A/Ohio/B24OSU-439/2024	10,000	10x	3/3
	H5N1 (Inactivated Virus) A/Ck/Scot/59	10,000	10x	3/3
	H5N3 (Genomic RNA) A/Duck/Singapore/645/97	100,000	100x	3/3
	H5N6 (dsDNA) A/Yangzhou/125/2022/segment 7	10,000	10x	3/3
	H7N2 (Genomic RNA) A/Equine/Prague/1/56/(HA) x A/Aichi/2/68 (NA) x A/Puerto Rico/8/34	3,000	3x	3/3
	H7N7 (dsDNA) A/Italy/3/2013/segment 7	100,000	100x	3/3
	NTC	0	N/A	0/3

10.3 Cross-Reactivity and Microbial Interference

The analytical specificity of the Metrix COVID/Flu Test was demonstrated by testing cross-reactivity and microbial interference with other microorganisms as well as substances that could potentially affect assay performance.

For the following tests, a negative sample refers to negative pooled nasal matrix (confirmed to be negative for SARS-CoV-2, Flu A, and Flu B) and a positive sample refers to negative pooled nasal matrix co-spiked with inactivated SARS-CoV-2 as well as live Flu A and Flu B viruses at two times their respective LoD. Positive and negative samples were spiked onto dry swabs which were then processed according to the test instructions.

All testing was performed with live microbes unless otherwise noted.

(A) Cross-Reactivity

The cross-reactivity of microorganisms was evaluated by running Metrix COVID/Flu Tests with triplicates of negative swab samples spiked with each of the 56 commensal microorganisms, tabulated below, at a high concentration. Viruses were tested at 5×10^5 TCID₅₀/mL or PFU/mL (37,500 TCID₅₀/swab or PFU/swab) and bacteria and fungi were tested at 5×10^6 CFU/mL (375,000 CFU/swab). Microbial cross-reactivity testing confirmed that none of the 56 organisms were reactive with the Metrix COVID/Flu Test at the concentrations tested.

Microorganism		# Tested / # Positive for Each Target			
		SARS-CoV-2	Flu A	Flu B	Internal Control
Virus	Adenovirus 7A	0/3	0/3	0/3	3/3
Virus	Adenovirus C1	0/3	0/3	0/3	3/3
Virus	Cytomegalovirus	0/3	0/3	0/3	3/3
Virus	Enterovirus Type 68	0/3	0/3	0/3	3/3
Virus	Epstein-Barr Virus	0/3	0/3	0/3	3/3
Virus	Herpes Simplex Virus Type 1	0/3	0/3	0/3	3/3
Virus	Herpes Simplex Virus Type 2	0/3	0/3	0/3	3/3
Virus	Human Coronavirus 229E (Inactivated Virus)	0/3	0/3	0/3	3/3
Virus	Human Coronavirus HKU1 (Synthetic RNA)	0/3	0/3	0/3	3/3
Virus	Human Coronavirus NL63 (Inactivated Virus)	0/3	0/3	0/3	3/3

Virus	Human Coronavirus NL63 (Synthetic RNA)	0/3	0/3	0/3	3/3
Virus	Human coronavirus OC43 (Inactivated Virus)	0/3	0/3	0/3	3/3
Virus	Human Metapneumovirus 16 (hMPV-16) Type A1	0/3	0/3	0/3	3/3
Virus	Measles (B3)	0/3	0/3	0/3	3/3
Virus	MERS-Coronavirus (Synthetic RNA)	0/3	0/3	0/3	3/3
Virus	Mumps	0/3	0/3	0/3	3/3
Virus	Parainfluenza 1	0/3	0/3	0/3	3/3
Virus	Parainfluenza 2	0/3	0/3	0/3	3/3
Virus	Parainfluenza 3	0/3	0/3	0/3	3/3
Virus	Parainfluenza 4	0/3	0/3	0/3	3/3
Virus	Parechovirus	0/3	0/3	0/3	3/3
Virus	Respiratory Syncytial Virus	0/3	0/3	0/3	3/3
Virus	Rhinovirus	0/3	0/3	0/3	3/3
Virus	SARS-Coronavirus (Extracted RNA)	0/3	0/3	0/3	3/3
Bacteria	<i>Bordetella parapertussis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Bordetella pertussis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Chlamydia pneumoniae</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Corynebacterium diphtheriae</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Coxiella burnetii</i> (Synthetic DNA)	0/3	0/3	0/3	3/3
Bacteria	<i>Eikenella corrodens</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Escherichia coli</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Fusobacterium necrophorum</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Haemophilus influenzae</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Lactobacillus salivarius</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Legionella pneumophila</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Moraxella catarrhalis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Mycobacterium tuberculosis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Mycoplasma genitalium</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Mycoplasma pneumoniae</i>	0/3	0/3	0/3	3/3

Bacteria	<i>Neisseria elongata</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Neisseria meningitidis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Neisseria mucosa</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Nocardia asteroides</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Porphyromonas gingivalis</i> (Genomic DNA)	0/3	0/3	0/3	3/3
Bacteria	<i>Prevotella oralis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Pseudomonas aeruginosa</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Staphylococcus aureus</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Staphylococcus epidermis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Streptococcus mitis</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Streptococcus mutans</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Streptococcus pneumoniae</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Streptococcus pyogenes</i>	0/3	0/3	0/3	3/3
Bacteria	<i>Streptococcus salivarius</i>	0/3	0/3	0/3	3/3
Fungus	<i>Aspergillus flavus</i>	0/3	0/3	0/3	3/3
Fungus	<i>Candida albicans</i>	0/3	0/3	0/3	3/3
Fungus	<i>Pneumocystis jirovecii</i> – <i>S. cerevisiae</i> *	0/3	0/3	0/3	3/3

* A gene specific to *Pneumocystis jirovecii* was inserted into the *S. cerevisiae* genome.

(B) Microbial Interference

The interference of microorganisms was evaluated by running Metrix COVID/Flu Tests with triplicates of positive swab samples spiked with each of the 56 commensal microorganisms, tabulated below, at a high concentration. Viruses were tested at 5×10^5 TCID₅₀/mL or PFU/mL (37,500 TCID₅₀/swab or PFU/swab) and bacteria and fungi were tested at 5×10^6 CFU/mL (375,000 CFU/swab). Microbial interference testing confirmed that none of the 56 organisms interfere with the detection of SARS-CoV-2, Flu A, or Flu B in the Metrix COVID/Flu Test at the concentrations tested.

Microorganism		# Tested / # Positive for Each Target			
		SARS-CoV-2	Flu A	Flu B	Internal Control
Virus	Adenovirus 7A	3/3	3/3	3/3	3/3
Virus	Adenovirus C1	3/3	3/3	3/3	3/3
Virus	Cytomegalovirus	3/3	3/3	3/3	3/3
Virus	Enterovirus Type 68	3/3	3/3	3/3	3/3
Virus	Epstein-Barr Virus	3/3	3/3	3/3	3/3
Virus	Herpes Simplex Virus Type 1	3/3	3/3	3/3	3/3
Virus	Herpes Simplex Virus Type 2	3/3	3/3	3/3	3/3
Virus	Human Coronavirus 229E (Inactivated Virus)	3/3	3/3	3/3	3/3
Virus	Human Coronavirus HKU1 (Synthetic RNA)	3/3	3/3	3/3	3/3
Virus	Human Coronavirus NL63 (Inactivated Virus)	3/3	3/3	3/3	3/3
Virus	Human Coronavirus NL63 (Synthetic RNA)	3/3	3/3	3/3	3/3
Virus	Human coronavirus OC43 (Inactivated Virus)	3/3	3/3	3/3	3/3
Virus	Human Metapneumovirus 16 (hMPV-16) Type A1	3/3	3/3	3/3	3/3
Virus	Measles (B3)	3/3	3/3	3/3	3/3
Virus	MERS-Coronavirus (Synthetic RNA)	3/3	3/3	3/3	3/3
Virus	Mumps	3/3	3/3	3/3	3/3
Virus	Parainfluenza 1	3/3	3/3	3/3	3/3
Virus	Parainfluenza 2	3/3	3/3	3/3	3/3
Virus	Parainfluenza 3	3/3	3/3	3/3	3/3
Virus	Parainfluenza 4	3/3	3/3	3/3	3/3
Virus	Parechovirus	3/3	3/3	3/3	3/3
Virus	Respiratory Syncytial Virus	3/3	3/3	3/3	3/3
Virus	Rhinovirus	3/3	3/3	3/3	3/3
Virus	SARS-Coronavirus (Extracted RNA)	3/3	3/3	3/3	3/3
Bacteria	<i>Bordetella parapertussis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Bordetella pertussis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Chlamydia pneumoniae</i>	3/3	3/3	3/3	3/3

Bacteria	<i>Corynebacterium diphtheriae</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Coxiella burnetii</i> (Synthetic DNA)	3/3	3/3	3/3	3/3
Bacteria	<i>Eikenella corrodens</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Escherichia coli</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Fusobacterium necrophorum</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Haemophilus influenzae</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Lactobacillus salivarius</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Legionella pneumophila</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Moraxella catarrhalis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Mycobacterium tuberculosis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Mycoplasma genitalium</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Mycoplasma pneumoniae</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Neisseria elongata</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Neisseria meningitidis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Neisseria mucosa</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Nocardia asteroides</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Porphyromonas gingivalis</i> (Genomic DNA)	3/3	3/3	3/3	3/3
Bacteria	<i>Prevotella oralis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Pseudomonas aeruginosa</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Staphylococcus aureus</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Staphylococcus epidermis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Streptococcus mitis</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Streptococcus mutans</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Streptococcus pneumoniae</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Streptococcus pyogenes</i>	3/3	3/3	3/3	3/3
Bacteria	<i>Streptococcus salivarius</i>	3/3	3/3	3/3	3/3
Fungus	<i>Aspergillus flavus</i>	3/3	3/3	3/3	3/3
Fungus	<i>Candida albicans</i>	3/3	3/3	3/3	3/3
Fungus	<i>Pneumocystis jirovecii</i> – <i>S. cerevisiae</i> *	3/3	3/3	3/3	3/3

* A gene specific to *Pneumocystis jirovecii* was inserted into the *S. cerevisiae* genome.

10.4 Analytical Specificity (Interfering Substances)

(A) Substance Cross-Reactivity

The potential interference of select substances was evaluated by running Metrix COVID/Flu Tests with triplicates of negative samples spiked with each of the 15 test substances at biologically relevant concentrations. Testing showed that 14 of the tested substances are not reactive with the Metrix COVID/Flu Test at the tested concentrations in the absence of the target analytes. Only FluMist generated false positive results with the Flu A and Flu B targets; however, this is expected because FluMist is a Flu A/B vaccine and contains attenuated Flu A/B virus.

Substance	Concentration	# Tested / # Positive for Each Target			
		SARS-CoV-2	Flu A	Flu B	Internal Control
Afrin	5% v/v	0/3	0/3	0/3	3/3
Chloraseptic Sore Throat Spray	5% v/v	0/3	0/3	0/3	3/3
Flonase Allergy Relief	5% v/v	0/3	0/3	0/3	3/3
FluMist Quadrivalent Vaccine (Nasal Spray)	5% v/v	0/3	3/3	3/3	3/3
Flunisolide	7.5% v/v	0/3	0/3	0/3	3/3
Human whole blood	1% v/v	0/3	0/3	0/3	3/3
Method All-Purpose Surface Cleaner	5% v/v	0/3	0/3	0/3	3/3
Mucin	1 mg/mL	0/3	0/3	0/3	3/3
Mupirocin	1 mg/mL	0/3	0/3	0/3	3/3
NeilMed NasoGel	1.25% v/v	0/3	0/3	0/3	3/3
Neo-Syneprine Cold and Sinus Extra Strength Spray	5% v/v	0/3	0/3	0/3	3/3
Relenza (Zanamivir)	1 mg/mL	0/3	0/3	0/3	3/3
Tamiflu	6 mg/mL	0/3	0/3	0/3	3/3
Tobramycin	2.5 mg/mL	0/3	0/3	0/3	3/3
Zicam Allergy Relief	5% v/v	0/3	0/3	0/3	3/3

The interference of select substances was evaluated by running Metrix COVID/Flu Tests with triplicates of positive samples spiked with each of the 15 test substances at biologically relevant concentrations. Testing showed that none of the 15 tested substances interfere with the detection of SARS-CoV-2, Flu A, or Flu B in the Metrix COVID/Flu Test at the concentrations listed below in the presence of the target analyte.

Substance	Concentration	# Tested / # Positive for Each Target			
		SARS-CoV-2	Flu A	Flu B	Internal Control
Afrin	5% v/v	3/3	3/3	3/3	3/3
Chloraseptic Sore Throat Spray	5% v/v	3/3	3/3	3/3	3/3
Flonase Allergy Relief	5% v/v	3/3	3/3	3/3	3/3
FluMist Quadrivalent Vaccine (Nasal Spray)	5% v/v	3/3	3/3	3/3	3/3
Flunisolide	7.5% v/v	3/3	3/3	3/3	3/3
Human whole blood	1% v/v	3/3	3/3	3/3	3/3
Method All-Purpose Surface Cleaner	5% v/v	3/3	3/3	3/3	3/3
Mucin	1 mg/mL	3/3	3/3	3/3	3/3
Mupirocin	1 mg/mL	3/3	3/3	3/3	3/3
NeilMed NasoGel	1.25% v/v	3/3	3/3	3/3	3/3
Neo-Synephrine Cold and Sinus Extra Strength Spray	5% v/v	3/3	3/3	3/3	3/3
Relenza (Zanamivir)	1 mg/mL	3/3	3/3	3/3	3/3
Tamiflu	6 mg/mL	3/3	3/3	3/3	3/3
Tobramycin	2.5 mg/mL	3/3	3/3	3/3	3/3
Zicam Allergy Relief	5% v/v	3/3	3/3	3/3	3/3

10.5 Shipping Study

To test the robustness of the test to shipping conditions, ten consumable shippers, each with 12 kits (for a total of 120 kits, 60 kits per temperature profile) and two reader kit shippers, each with 10 Readers

(for a total of 20 Readers, 10 Readers per temperature profile) were exposed to a Summer and Winter temperature profile. Each temperature profile is composed of five cycles, and kits were be exposed to in sequential order for the time listed in the tables below.

Summer Shipping Profile			
Temperature	Cycle Number	Cycle Time (hours)	Total Time (hours)
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

Winter Shipping Profile			
Temperature	Cycle Number	Cycle Time (hours)	Total Time (hours)
-20°C	1	8	8
18°C	2	4	12
-20° C	3	2	14
20° C	4	36	50
-20° C	5	6	56

Exposure to each temperature profile was evaluated with 20 low positive (2x LoD) replicates, 10 high positive (5x LoD) replicates, and 10 negative (NTC) replicates. All samples were prepared in negative clinical nasal matrix. Positive samples were co-spiked with SARS-CoV-2, influenza A, and influenza B. For each temperature profile, all 10/10 NTC replicates produced negative results. The NTC testing criteria passed for both the Summer and Winter profile in this test.

Temperature Profile	Target	# Positives per Total Number of Replicates		
		NTC	2x LoD	5x LoD
Summer Profile	SARS-CoV-2	0/10	20/20	10/10
	Flu A	0/10	20/20	10/10
	Flu B	0/10	20/20	10/10
Winter Profile	SARS-CoV-2	0/10	20/20	10/10
	Flu A	0/10	20/20	10/10
	Flu B	0/10	20/20	10/10

(A) Assay Workflow

Aptitude conducted a human factors usability study to evaluate the ability of users to successfully complete the workflow of the Metrix COVID-19 Test (which has the same workflow as the Metrix COVID/Flu Test). Forty-two subjects were recruited with a wide range of age and education demographics. Twenty-five subjects between the ages of 2 and 14 were assisted by a parent or a guardian during the test (ten of these subjects were aged 2 or 3 years). The study was conducted in a simulated home environment; subjects were provided with standard items found in a common home setting such as a sturdy work surface, electrical power outlets, tissues, handwashing area, and a trash can. Subjects were asked to perform a test following printed instructions found in the test kits, or a step-by-step video guide accessible on smart devices via a QR code in the printed instructions. All subjects (100%, 42/42) successfully completed all critical steps.

(B) Result Interpretation

Aptitude also conducted a human factors usability study focusing on result interpretation via the LEDs located on top of the Metrix Reader (Gen 2). Untrained users were provided with only a Metrix Reader (Gen 2) and Metrix COVID/Flu Test instructions for this study. Participants were asked to interpret simulated status results, presented to them in a randomized order using only the materials provided. All 25 participants (100%) correctly interpreted all statuses presented to them.

Because the Metrix COVID/Flu Test uses multi-color LEDs to report results, Aptitude also performed the above result interpretation study with color vision-impaired users. A total of 21 self-reported color vision-impaired users were enrolled in this study: 18 users reported red/green colorblindness, 1 user reported total colorblindness, and 2 users reported an inability to differentiate blue and purple. All color vision-impaired users (100%, 21/21) successfully interpreted all result statuses

for the Metrix COVID/Flu Test. Users indicated that the use of both spatial separation and temporal separation (i.e., flashing) of LEDs aided them in correctly identifying each result status.

10.7 Flex Studies

The Metrix COVID/Flu Test was evaluated for robustness against several sample preparation, test handling and environmental variables. Appropriate labelling precautions were introduced to mitigate potential errors that could occur when the test is performed without healthcare provider supervision.

10.8 Clinical Evaluation

To evaluate the clinical performance of the Metrix COVID/Flu Test, Aptitude conducted a prospective, multi-site study which enrolled symptomatic participants on an all-comers basis from November 20, 2023 to March 1, 2024. Each participant, by themselves or with the assistance of their parent/guardian, performed the entire test, from sampling to interpretation of results, following the test instructions without guidance or training.

A total of 556 prospectively enrolled participants successfully completed the Metrix COVID/Flu Test. For each subject, a comparator nasopharyngeal (NP) swab was collected and analyzed with a high-sensitivity FDA-Cleared SARS-CoV-2 and Flu A/B RT-PCR assay.

The Positive Percent Agreement (PPA) for SARS-CoV-2, Flu A, and Flu B was determined to be 95.5% (84/88), 95.8 (69/72), and 95.2 (40/42), respectively. The Negative Percent Agreement (NPA) for SARS-CoV-2, Flu A, and Flu B was determined to be 99.6% (466/468), 99.4% (481/484), and 99.4% (511/514), respectively. A total of 5 subjects (0.9%) were unevaluable due to invalid results.

A total of 9 samples collected from participants showed co-infection with SARS-CoV-2 and Flu A/B (5 Flu A and 4 Flu B).

Comparator Co-infection Result	Candidate Test Concurrence	Discordant Candidate Test Result
SARS-CoV-2 Positive and Influenza A Positive	4/5	Influenza A Positive (1/5)
SARS-CoV-2 Positive and Influenza B Positive	4/4	N/A

The individual comparator tables for SARS-CoV-2, Flu A, and Flu B are summarized below.

(A) Metrix COVID/Flu Test – SARS-CoV-2

SARS-CoV-2		Comparator: NP Swab on High Sensitivity FDA-Cleared SARS-CoV-2 and Flu A/B RT-PCR Assay		
		Positive	Negative	Total
Candidate: Metrix COVID/Flu Test	Positive	84	2	86
	Negative	4	466	470
	Total	88	468	556
PPA: 95.5% (95% CI: 88.9% – 98.2%)				
NPA: 99.6% (95% CI: 98.5% – 99.9%)				

(B) Metrix COVID/Flu Test – Flu A

Flu A		Comparator: NP Swab on High Sensitivity FDA-Cleared SARS-CoV-2 and Flu A/B RT-PCR Assay		
		Positive	Negative	Total
Candidate: Metrix COVID/Flu Test	Positive	69	3	72
	Negative	3	481	484
	Total	72	484	556
PPA: 95.8% (95% CI: 88.5% – 98.6%)				
NPA: 99.4% (95% CI: 98.2% – 99.8%)				

(C) Metrix COVID/Flu Test – Flu B

Flu B		Comparator: NP Swab on High Sensitivity FDA-Cleared SARS-CoV-2 and Flu A/B RT-PCR Assay		
		Positive	Negative	Total
Candidate: Metrix COVID/Flu Test	Positive	40	3	43
	Negative	2	511	513
	Total	42	514	556
PPA: 95.2% (95% CI: 84.2% - 98.7%)				
NPA: 99.4% (95% CI: 98.3% - 99.8%)				

11. References

1. Centers for Disease Control and Prevention. "Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (Covid-19)."
<https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>
2. Centers for Disease Control and Prevention. "Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition."
<https://www.cdc.gov/labs/BMBL.html>
3. Clinical and Laboratory Standards Institute. "Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline." Document M29
<https://clsi.org/standards/products/microbiology/documents/m29/>

12. Table of Symbols

The following symbols are used throughout this manual or on the product:

Symbol	Definition
	This symbol references the product's catalog number.
	This symbol indicates the product's batch/lot code.
	This symbol indicates the product's serial number.
	For In Vitro Diagnostic Use.
	This symbol indicates the name and location of the product manufacturer.
	This symbol indicates the date that the product was manufactured.
	This symbol indicates that you should consult the instructions for use.
	This symbol indicates the product's upper and lower temperature limitations.
	This symbol indicates the product's expiration date.
	This symbol indicates that you should not use the package if it is damaged.
	This symbol indicates that the product is intended for single use only. Do no reuse.
	This symbol indicates that the product should not be disposed of in a municipal trash bin when it has reached the end of its lifetime.
	This symbol indicates that the product should be kept dry.
	This symbol certifies that the electromagnetic interference from the device is under limits approved by the Federal Communications Commission.
	This symbol indicates direct current (DC) voltage.

13. Manufacturer, Support, and Ordering Information

Manufacturer



Aptitude Medical Systems, Inc.
125 Cremona Drive, Suite 100
Goleta, CA 93117

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Customer Support and Ordering Information

aptitudemetrix.com
888.934.2253
support@aptitudemetrix.com

