Rapid Response®

Influenza AB + COVID-19 Antigen – 3 in 1 Test

REF COF-19CPC5, COF-19CPC25 Product Insert

For *in vitro* diagnostic use only.

Intended Use

The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of nucleoprotein antigens from SARS-CoV-2, influenza A and/or influenza B in nasopharyngeal or anterior nasal swab specimens. This test is intended for individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider within the first seven days of the onset of symptoms. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. Testing is limited to clinical laboratories and point of care settings.

Results are for the identification of SARS-CoV-2, influenza A and influenza B nucleocapsid antigens. These 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 clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all 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. To increase the chance that the negative result is accurate, test again 48 hours after the first negative result. 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 COVID19. Negative influenza A and B test results should be treated as presumptive. It is recommended these results be confirmed by viral culture or a 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.

The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test is intended for use by healthcare professionals and laboratory personnel trained to perform the test.

Principle

The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test detects Influenza A and B viral antigens and SARS-CoV-2 viral antigens through visual interpretation of colour development.

A sample is added to the extraction buffer which is optimized to release the influenza A and B antigens and SARS-CoV-2 antigens from specimen.

For COVID-19 Antigen Test: Anti-SARS-CoV-2 antibodies are immobilized in the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to coloured particles are immobilized on the conjugated pad. During testing, the extracted antigens bind to anti-SARS-CoV-2 antibodies conjugated to coloured particles. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by the anti-SARS-CoV-2 antibodies in the test region. Excess coloured particles are captured in the internal control zone.

The presence of a coloured band in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A coloured band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

For Influenza A/B Antigen Test: Anti-influenza A and B antibodies are immobilized in the test region A and B of the nitrocellulose membrane, respectively. Anti-influenza A and B antibodies conjugated to coloured particles are immobilized on the conjugated pad. During testing, the extracted antigens bind to anti-influenza A and B antibodies conjugated to coloured particles on the sample pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by either anti-influenza A or anti-influenza B nucleoprotein monoclonal antibodies in the respective detection zone. Excess coloured particles are captured in the internal control zone.

The presence of a red band in the A and/or B region indicates a positive result for the particular viral antigens, while its absence indicates a negative result. A red band in the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking is working.

Precautions

- For *in vitro* diagnostic use only.
- Read the package insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- Do not use the kit to the age under 2 years old.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous results may occur if test reagents or components are improperly stored.
- Do not use the extraction buffer if it is discoloured or turbid. Discolouration or turbidity may be a sign of microbial contamination.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Failure to bring specimens and reagents to room temperature before testing may yield false results. Inaccurate or inappropriate specimen collection, storage, and transport may yield false results. Avoid skin contact with buffer.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.
- If infection with a novel Influenza A 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 departments for testing.
- Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of Influenza A and B and SARS-CoV-2 specimens are NOT recommended, except in a BSL3 laboratory using BSL3 work practices.

Materials

Materials provided

- Individually packed test cassettes
- Pre-filled extraction buffer tubes
- Tube stand
- Product insert
- Disposable swabs

Materials required but not provided

- Timer
- External control Swabs (COF-C03): Users can contact BTNX at sales@btnx.com to place an order for control swabs.

Storage and Stability

- Store the Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test at 2~30°C when not in use.
- **Do not freeze.**
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

Collection and Storage of Specimens

Nasopharyngeal specimen (NP specimen):

1. Remove the Disposable swab from its packing
2. Insert the swab into the nostril parallel to the palate, and gently push the swab into the posterior nasopharynx. Rotating against the nasal wall (to ensure swab contains cells as well as mucus)
3. Repeat the sample collection procedure for the other nostril to ensure that sufficient specimen has been collected from both nasal cavities.
4. Process the swab immediately after collecting the specimen.

Nasal specimen (NS specimen):

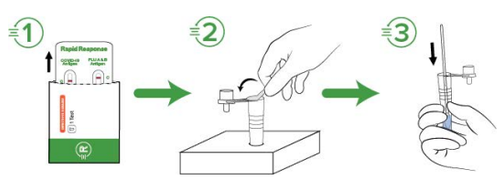
1. Have the patient blow their nose.
2. Remove the Disposable swab supplied in the kit from its packaging opening from the indicated end.
3. Insert the entire absorbent tip of the swab inside one of the patient's nostrils (typically between ⅗ to 1 inch (1.5 to 2.5cm). Using a circular motion, the nasal orifice should be swabbed for a minimum of five seconds.
4. Compress the nostril with the fingers to trap the swab tip and rotate in a circular path against the nasal wall at least 5 times for approximately 10 seconds. Be sure to collect any nasal drainage that may be present on the swab.
5. Remove the swab and repeat steps 3 and 4 in the other nostril. Do not return the Nasal swab to the original paper packaging.

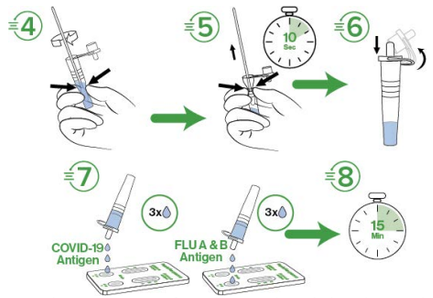
NOTE:

1. The swab provided with the kit can be used to collect either Nasopharyngeal or Nasal specimens.
2. Swabs specimens should be tested immediately after collection. Use freshly collected specimens for best test performance.
3. If not tested immediately, swab specimens may be stored at 2-8°C for up to 8 hours after collection.
4. Do not use specimens that are obviously contaminated with blood, as it may interfere with the flow of sample with the interpretation of test results.

Test Procedure

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

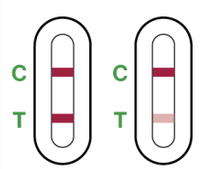




1. Remove the test cassette from the pouch and place it on a clean, flat surface. Once opened use the cassette within 1 hour.
2. Tear the aluminum foil off the top of the pre-filled extraction buffer tube and insert the extraction buffer tube into the tube holder.
3. Place the swab with the specimen into the liquid inside the pre-filled extraction buffer tube.
4. While holding the tube rotate the swab while squeezing the lower part of the tube 10-15 times so that a slight pressure is exerted on the tip of the swab to release the specimen.
5. Remove the swab while squeezing the swab against the walls of the tube to expel as much liquid as possible from the swab. Discard the swab in accordance with your biohazard waste disposal protocol.
6. Secure the nozzle dropper cap tightly onto the top of the extraction tube.
7. Invert the dropper vertically and add 3 drops of the sample solution (approx. 80µL) to each of the two sample wells (S).
8. Immediately start a timer for 15 minutes. Read the result at 15 minutes. Do not interpret the result after 30 minutes.

Results Interpretation

For COVID-19 Antigen test

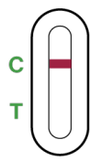
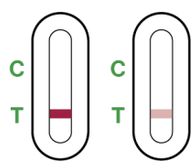


POSITIVE: Two coloured bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

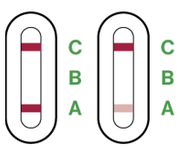
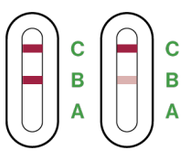
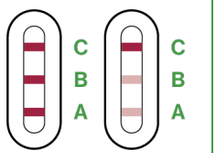
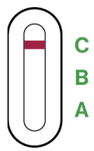
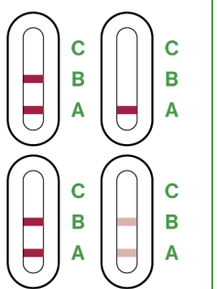
IMPORTANT: Look very closely! Any faint coloured line in the test region should be considered positive.

A positive test result for COVID-19 indicates that antigens from SARS-CoV-2 were detected, and the person is very likely to be infected with the virus and presumed contagious. Test results should always be considered in the context of clinical observations and epidemiological data (such as local prevalence rates and current outbreak/epicenter locations) in making a final diagnosis and patient management decisions. Patient management should follow current health guidelines. You do not need to perform repeat testing if you have a positive result at any time. Additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or

Document Number: H10110443800
Effective Date: 2023-06-15

	residing in communities with low prevalence of infection.
	**NEGATIVE:** Only one coloured band appears, in the control region (C). No apparent coloured band appears in the test region (T). **To increase the chance that the negative result for COVID-19 is accurate, you should:** • **Test again in 48 hours if the patient present symptoms on the first day of testing.** A negative result is presumptive, meaning it is not certain that the patient does not have COVID-19. They may still have COVID-19 and may still be contagious. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR. If all repeat tests are negative and the patient is concerned they have COVID-19, they may choose to test again using an antigen test or consult with your health care provider regarding molecular testing.
	**INVALID:** Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, consult a healthcare professional. If you still have symptoms, you should self-isolate at home and avoid contact with others prior to retesting.

For Influenza A/B test

	**Influenza A Positive:** One coloured band appears in the control region (C), and another coloured band in the A region (A).
	**Influenza B Positive:** One coloured band appears in the control region (C), and another coloured band in the B region (B).
	**Influenza A+B Positive:** One coloured band appears in the control region (C), and two other coloured bands appear in both A region (A) and B region (B). **NOTE:** Co-infection with influenza A and B is very rare. A clinical specimen that generates positive results for both A and B should be considered an invalid result, and another test should be performed. If the test is again positive for both influenza A and B, the specimen should be re-tested by another method prior to reporting of results.
	**Negative:** Only one coloured band appears in the control region (C), and band appears neither in the A region (A) nor B region (B).
	**Invalid:** No coloured band appears in the control region (C), whether a test band(s) is present or not. Repeat invalid tests with a new sample, new test device and reagent. Insufficient sample volume, inaccurate operating procedure or expired tests may yield an invalid result. Contact your local distributor if the problem continues.

NOTE:

1. The colour intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of colour in the test region should be considered positive. Note that this is a qualitative test only and cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

Quality Control

Internal Procedural Controls

The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test has built-in (procedural) controls. Each test device has an internal standard zone to ensure proper sample flow. The user should confirm that the coloured band located at the "C" region is present before reading the result.

External Positive and Negative Controls

Good laboratory practice suggests that positive and negative external controls are run routinely to ensure that the test is correctly performed. External positive and negative controls should be used in accordance with applicable accrediting organizations. However, BTNX recommends that labs receiving this test execute a control test for each lot of kits that they receive.

Limitations

1. The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test is for professional *in vitro* diagnostic use and should only be used for the qualitative detection of influenza A and B and SARS-CoV-2 antigen. The intensity of colour in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable influenza A and B viruses and SARS-CoV-2 viruses are detectable with Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. Negative results do not preclude influenza A and B or SARS-CoV-2 infection and should be confirmed via molecular assay.
7. The performance of Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 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.
8. The Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test cannot differentiate between SARS and SARS-COV-2.

Serial Testing (Repeat Testing) Information and Limitations

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

Performance Characteristics

Analytical Sensitivity (Limit of Detection):

The limit of detection was determined by evaluating different concentrations of quantified SARS-CoV-2, two subtypes of influenza A virus and two subtypes of influenza B virus. The concentrations identified as the LOD levels for each strain tested are listed below.

Viral strain	LoD concentration
SARS-CoV-2 (hCoV-19/China/ZJ-NB841/2020)	$2\times10^{2.4}$ $TCID_{50}$/mL
Influenza A virus (H1N1: A/China/ZJ-HZ166/2018)	2.0×10^{4} $TCID_{50}$/mL
Influenza A virus (H3N2: A/China/ZJ-TZ314/2016)	8.6×10^{4} $TCID_{50}$/mL
Influenza B virus (Yamagata lineage: BY/China/ZJ-HZ415/2018)	5.0×10^{5} $TCID_{50}$/mL
Influenza B virus (Victoria lineage: BV/China/ZJ-HZ809/2019)	4.4×10^{5} $TCID_{50}$/mL

Analytical Inclusivity

The analytical inclusivity of SARS-CoV-2 variants was evaluated by testing the quantified strains of SARS-CoV-2 isolated from clinical samples and establishing the LOD levels for each one tested as below:

Strains of SARS-CoV-2	LoD concentration
BA.4.1	1.19×10^{2} $TCID_{50}$/mL
BA.5.2.1	9.33×10^{1} $TCID_{50}$/mL
BA.2.75	3.6×10^{2} $TCID_{50}$/mL
BF.7	4.0×10^{2} $TCID_{50}$/mL
XBB	4.8×10^{2} $TCID_{50}$/mL
XBB.1.5	3.5×10^{2} $TCID_{50}$/mL
BQ.1	2.05×10^{2} $TCID_{50}$/mL
BQ.1.1	5.2×10^{2} $TCID_{50}$/mL

Inclusivity for multiple strains for Influenza A and B were also quantified and evaluated to establish the LOD levels for each one as below:

Viral Culture		Viral concentration ($TCID_{50}$/mL)
Influenza A (H1N1)	A/Michigan/45/2015	2.0×10^4
	A/California/07/2009	2.06×10^4
	A/Brisbane/02/2018	1.80×10^4
	A/Victoria/2570/2019	2.25×10^4
	A/Wisconsin/588/2019	2.21×10^4
	A/Guangdong-Maonan/SWL1536/2019	1.00×10^4
	A/Hawaii/70/2019	1.20×10^4
Influenza A virus (H3N2)	A/Singapore/INFIMH-16-0019/2016	2.66×10^4
	A/Hong Kong/4801/2014	8.60×10^4
	A/Hong Kong/2671/2019	1.46×10^4
	A/Hong Kong/45/2019	2.16×10^4
	A/Switzerland/9715293/2013	2.44×10^4
	A/Darwin/6/2021	1.58×10^4
	A/Darwin/9/2021	1.83×10^4
	A/Cambodia/e0826360/2020	1.24×10^4
	A/Kansas/14/2017	1.35×10^4
Influenza B virus (Victoria)	B/Colourado/06/2017	4.40×10^5
	B/Brisbane/60/2008	8.25×10^4
	B/Washington/02/2019	1.20×10^5
	B/Austria/1359417/2021	6.88×10^4
Influenza B virus (Yamagata)	B/Massachusetts/2/2012	1.53×10^5
	B/Phuket/3073/2013	5.00×10^5

Clinical Evaluation:

A total of 421 individuals among multiple sites were enrolled to verify the performance of Rapid Response® Influenza AB + COVID-19 Antigen – 3 in

1 Test. To be enrolled in the study, subjects were either asymptomatic or presenting symptoms related to COVID-19 or Influenza A/B within seven (7) days of the onset of symptoms, such as fever, cough, unexplained loss of taste and smell, sore throat, headache, shortness of breath, muscle/joint ache, or diarrhea. Three specimens were collected from each individual – 1 Nasal specimen for the antigen test, 2 NP specimens, one for the antigen test and another for Rt-PCR. Individual performance characteristics of the NP and Nasal specimen for each of SARS-COV-2, Influenza A and Influenza B are shown in the tables below.

Table 1: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for SARS-COV-2 – NP specimen

NP specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	89	1	90
	Negative	5	326	331
	Total	94	327	421

Diagnostic Sensitivity: 94.7% (88.1% ~ 97.7%) *
Diagnostic Specificity: 99.7% (98.3% ~ 99.9%) *
Overall Agreement: 98.6% (96.9% ~ 99.3%) *

Table 2: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for SARS-COV-2 – Nasal specimen

NS specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	88	1	89
	Negative	6	326	332
	Total	94	327	421

Diagnostic Sensitivity: 93.6% (86.8% ~ 97.0%) *
Diagnostic Specificity: 99.7% (98.3% ~ 99.9%) *
Overall Agreement: 98.3% (96.6% ~ 99.2%) *

Table 3: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for Influenza A – NP specimen

NP specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	51	0	51
	Negative	2	368	370
	Total	53	368	421

Diagnostic Sensitivity: 96.2% (87.2% ~ 99.0%)*
Diagnostic Specificity: 100.0% (99.0% ~ 100.0%)*
Overall Agreement: 99.5% (98.3% ~ 99.9%)*

Table 4: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for Influenza A – Nasal specimen

NS specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	50	1	51
	Negative	3	367	370
	Total	53	368	421

Diagnostic Sensitivity: 94.3% (84.6% ~ 98.1%) *
Diagnostic Specificity: 99.7% (98.5% ~ 100.0%) *
Overall Agreement: 99.0% (97.6% ~ 99.6%) *

Table 5: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for Influenza B – NP specimen

NP specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	28	1	29
	Negative	2	390	392
	Total	30	391	421

Diagnostic Sensitivity: 93.3% (78.7% ~ 98.2%)*
Diagnostic Specificity: 99.7% (98.6% ~ 100.0%)*
Overall Agreement: 99.3% (97.9% ~ 99.8%)*

Table 6: Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test Vs RT-PCR for Influenza B – Nasal specimen

NS specimen		RT PCR		
		Positive	Negative	Total
Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test	Positive	28	2	30
	Negative	2	389	391
	Total	30	391	421

Diagnostic Sensitivity: 93.3% (78.7% ~ 98.2%)*
Diagnostic Specificity: 99.5% (98.2% ~ 99.9%)*
Overall Agreement: 99.0% (97.6% ~ 99.6%)*
*95% Confidence Interval

Serial-testing clinical performance for SARS-COV-2:

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.
At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.
Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.
Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RT-PCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing. Performance of the antigen test with serial testing in individuals is described in following table.

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

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

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

Cross Reactivity and Microbial interference:

Cross-reactivity and microbial interference of the Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 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 the Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test. Each organism and virus were tested in the absence or presence of heat inactivated SARS-CoV-2, Influenza A (H1NI and H3N2 strains), and Influenza B (Victoria and Yamagata strains) at 2 x LoD.
The following microorganisms were tested:

Microorganisms	Target Concentration
Human coronavirus 229E	1×10^5 $TCID_{50}$/mL
Human coronavirus OC43	1×10^5 $TCID_{50}$/mL
Human coronavirus NL63	1×10^5 $TCID_{50}$/mL
MERS-coronavirus*	1×10^6 $TCID_{50}$/mL
SARS-coronavirus*	7.9×10^1 $TCID_{50}$/mL
Human coronavirus HKU1	1×10^5 $TCID_{50}$/mL
Adenovirus	1×10^5 $TCID_{50}$/mL
Human Metapneumovirus	1×10^5 $TCID_{50}$/mL
Parainfluenza virus 1	1×10^5 $TCID_{50}$/mL
Parainfluenza virus 2	1×10^5 $TCID_{50}$/mL
Parainfluenza virus 3	1×10^5 $TCID_{50}$/mL
Parainfluenza virus 4	1×10^5 $TCID_{50}$/mL
Influenza A (H1N1)	1×10^5 $TCID_{50}$/mL
Influenza A (H3N2)	1×10^5 $TCID_{50}$/mL
Influenza B Victoria lineage	1×10^5 $TCID_{50}$/mL
Influenza B Yamagata lineage	1×10^5 $TCID_{50}$/mL
Enterovirus	1×10^5 $TCID_{50}$/mL
Respiratory syncytial virus A	1×10^5 $TCID_{50}$/mL
Respiratory syncytial virus B	1×10^5 $TCID_{50}$/mL
Rhinovirus	1×10^5 $TCID_{50}$/mL
Haemophilus influenzae	1×10^6 CFU/ml
Streptococcus pneumoniae	1×10^6 CFU/ml
Streptococcus pyogenes	1×10^6 CFU/ml
Candida albicans	1×10^6 CFU/ml
Bordetella pertussis	1×10^6 CFU/ml
Mycoplasma pneumoniae	1×10^6 CFU/ml
Chlamydia pneumoniae	1×10^6 CFU/ml
Legionella pneumophila	1×10^6 CFU/ml
Staphylococcus aureus	1×10^6 CFU/ml
Staphylococcus epidermidis	1×10^6 CFU/ml
Pooled human nasal wash	/

*SARS and MERS were only tested with SARS-CoV-2 and not with Influenza A or Influenza B.
No cross reactivity or microbial interference was observed for any of the organisms tested at the concentrations mentioned above with Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test, except for SARS-coronavirus which exhibited cross-reactivity with COVID-19 when tested at 7.9 x 10^1 $TCID_{50}$/mL. No cross-reactivities or microbial interference was observed for Influenza A or Influenza B.
To estimate the likely-hood of cross-reactivity with SARS-COV-2 of organisms that were not available for wet testing, *in-silico* analysis using the basic local alignment search tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to identify the degree of protein sequence homology.
Blast results showed no homology between the SARS-COV-2 nucleocapsid protein and either of Pneumocystis jirovecii (PJP) or Mycobacterium tuberculosis (Mt).
Blast results showed no homology between the Influenza A nucleocapsid protein and either of Pneumocystis jirovecii (PJP) or Mycobacterium tuberculosis (Mt).
Blast results showed no homology between the Influenza B nucleocapsid protein and either of Pneumocystis jirovecii (PJP) or Mycobacterium tuberculosis (Mt).

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the respiratory tract, were evaluated at the concentrations listed below. None of them were found to affect test performance of the Rapid Response® Influenza AB + COVID-19 Antigen – 3 in 1 Test.

Document Number: H1011044380O
Effective Date: 2023-06-15

Potential Interfering Substances	Concentration
Whole Blood	4%
Mucin	0.5%
Cepacol® Sore Throat Lozenges (benzocaine/menthol)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
Nasal Drops (Phenylephrine)	15% v/v
Nasal Spray (Oxymetazoline)	15% v/v
Nasal Spray (Cromolyn)	15% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL
Body Hand lotion (Cerave)	0.5%(w/v)
Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v
Hand Lotion (Eucerin)	5% w/v
Hand soap liquid gel (soft soap)	10%(w/v)
Hand Sanitizer, 80% ethanol, fast drying	15% v/v

High Dose Hook Effect

No high dose hook effect was observed when testing the Rapid Response Influenza AB + COVID-19 Antigen – 3 in 1 Test with the following viral concentrations:

Viral strain	LoD concentration
SARS-CoV-2 (hCoV-19/China/ZJ-NB841/2020)	$2\times10^{5.4}$ $TCID_{50}$/mL
Influenza A virus (H1N1: A/China/ZJ-HZ166/2018)	2.0×10^{6} $TCID_{50}$/mL
Influenza A virus (H3N2: A/China/ZJ-TZ314/2016)	8.6×10^{6} $TCID_{50}$/mL
Influenza B virus (Yamagata lineage: BY/China/ZJ-HZ415/2018)	5.0×10^{6} $TCID_{50}$/mL
Influenza B virus (Victoria lineage: BV/China/ZJ-HZ809/2019)	4.4×10^{6} $TCID_{50}$/mL

Competitive Inhibition Study

High concentrations of the three viruses were tested to determine if they would interfere with the detection of the two other viruses at lower concentrations.

Viral culture used in the study		
Influenza virus	**Strain**	**Titer of viral culture**
Influenza A (H3N2)	A/Switzerland/9715293/2013	1.3×10^{7} $TCID_{50}$/mL
Influenza B (Victoria)	B/Brisbane/60/2008	1.1×10^{7} $TCID_{50}$/mL
SARS-CoV-2	hCoV-19/China/ZJ-NB841/2020	$1\times10^{6.4}$ $TCID_{50}$/mL

The three samples were diluted to 100x LoD for the high concentration and 2x LoD for the low concentration of the other two viruses. High concentrations of either virus did not interfere with the detection of the other two viruses at lower concentrations, thus no competitive inhibition was observed.

Bibliography

1. Forni, D., Cagliani, R., Clerici, M. & Sironi, M. Molecular evolution of human coronavirus genomes. Trends Microbiol. 25, 35–48 (2017).
2. Ithete, N. L. et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg. Infect. Dis. 19, 1697–1699 (2013).

Glossary of Symbols

Consult instructions for use	Test per Kit	Do Not Reuse	
Store between 2°C to 30°C	Use by	REF	Catalogue #
LOT	Lot Number	IVD	*In vitro* diagnostic medical device

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Document Number: H10110443800
Effective Date: 2023-06-15