COVID-19 & Influenza A/B Antigen Combo Test Device

(Nasal Swab /Nasopharyngeal Swab)

MI-S43001

INTENDED USE

The COVID-19 & Influenza A/B Antigen Combo Test Device is an in vitro immunoassay. The assay is for the direct and qualitative detection of Influenza A and B and SARS-CoV-2 viral nucleoprotein antigens from nasopharyngeal and nasal swab specimens. This test is intended for individuals suspected of respiratory viral infection consistent with Influenza A and Influenza B infection or COVID-19, within the first 7 days of symptom onset.

Results are for identification of Influenza A, Influenza B and SARS-CoV-2 viral nucleoprotein antigen. Antigen is 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 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 positive results to the appropriate public health authority.

Negative results do not rule out Influenza A, Influenza B and/or 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 Influenza A, Influenza B and COVID-19 infections, and confirmed with a molecular assay, if necessary for patient management.

People who are suspected of being infected with SARS-CoV-2 virus:

Negative SARS-CoV-2 results should be treated as presumptive. To increase the chance that the negative result is accurate, test again 48 hours after the first negative result

The assay is for point of care use only, and the intended user is healthcare professional.

PRINCIPLE

The COVID-19 & Influenza A/B Antigen Combo Test Device detects Influenza A and B viral antigens and SARS-CoV-2 viral antigens through visual interpretation of color 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 colored particles are immobilized on the conjugated pad. During testing, the extracted antigens bind to anti-SARS-CoV-2 antibodies conjugated to colored 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 colored particles are captured in the internal control zone.

The presence of a colored band in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A colored 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 colored particles are immobilized on the conjugated pad. During testing, the extracted antigens bind to anti-influenza A and B antibodies conjugated to colored 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 colored 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.

MATERIALS

Materials Provided

- Individually packed test devices
- Individually packed swabs
- Positive control
- Package insert
- Extraction buffer
- Tube holder
- Negative control

Materials Required but not provided

- Clock, timer or stopwatch

PRECAUTIONS

- For *in vitro* Diagnostic Use Only.
- Read the Package Insert prior to use. Instructions should be read and followed carefully.
- Do not use the kit or components beyond the expiration date.
- Do not use the kit to the patients 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 result may occur if test reagents or components are improperly stored.
- Do not use the Extraction Buffer if it is discolored or turbid. Discoloration 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 transportation may yield false results. Avoid skin contact with buffer.
- If an infection with SARS-CoV-2 is suspected based on the 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 an infection with a novel influenza A virus is suspected based on the 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.

STORAGE AND STABILITY

- Store the COVID-19 & Influenza A/B Antigen Combo Test Device at 2~30℃ when not in use.
- **DO NOT FREEZE**.
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

SPECIMEN COLLECTION AND STORAGE

-Nasopharyngeal swab (NP swab):

1) Remove the swab from its packaging
2) Insert the swab into the nostril parallel to the palate, and gently push the swab into the posterior nasopharynx. Rotate 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 sufficient nasopharyngeal specimen is collected via both nasal cavities
4) Process the swab as soon as possible after collecting the specimen

-Nasal swab (NS swab):

1) Remove the swab from its packaging
2) Insert the swab into the nostril (1/2-3/4 inch). Gently twist the swab 5 times against the nasal wall. The swab should remain in the nostril for 15 seconds.
3) Repeat the sample collection procedure for the other nostril to ensure sufficient nasal specimen is collected via both nasal cavities
4) Process the swab as soon as possible after collecting the specimen

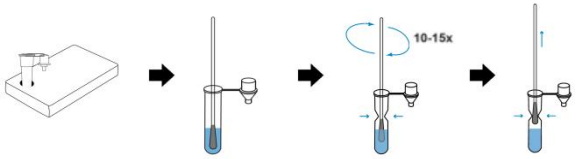
Note:

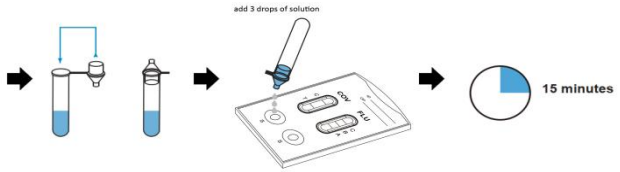
1. Only use the device with the provided, validated swabs.
2. Swabs specimens should be tested immediately after collection. Use freshly collected specimens for the best test performance.
3. If not tested immediately, swab specimens should be stored in a clean, dry, closed container at 2-8℃ for up to 4 hours, or at 15-30℃ for up to 2 hours after collection.
4. Do not use the specimens that are evidently contaminated with blood, as it may interfere with the flow of the sample and with the interpretation of the test results.

TEST PROCEDURE

Bring devices, reagents and specimens and/or controls to room temperature (15~30℃).

1. For each specimen, open the foil pouch just before testing, and remove the test device, and put it on a clean, level surface. Label the tube with the patient identification. For best results, the assay should be performed within one hour.

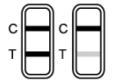




2. Tear the aluminum foil off the top of the pre-filled extraction buffer tube and insert the extraction buffer tube into the tube holder.
3. Place the swab into the tube. Rotate the swab while squeezing the lower part of the tube 10-15 times so that a slight pressure is exerted on the tip of the swab.
4. Remove the swab while squeezing the swab between the walls of the extraction buffer tube to expel as much liquid as possible from the swab.
5. Insert the nozzle back onto the top of the extraction buffer tube.
6. Invert the tube and add 3 drops of the solution into each of the two sample wells by gently squeezing the tube.
7. Read results at 15 minutes. Do not read the results after 30 minutes.

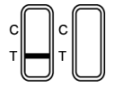
RESULT INTERPRETATION

For COVID-19 Antigen test



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

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).



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, discontinue using the kit immediately and contact your local distributor.

For Influenza A/B test

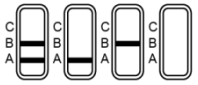
Influenza A Positive: One colored band appears in the control region (C), and another colored band in the A region (A).

Influenza B Positive: One colored band appears in the control region (C), and another colored band in the B region (B).

Influenza A+B Positive: One colored band appears in the control region (C), and two other colored 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 colored band appears in the control region (C), and band appears neither in the A region (A) nor B region (B).



Invalid: No colored 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 color intensity in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color 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 COVID-19 & Influenza A/B Antigen Combo Test Device has built-in (procedural) controls. Each strip in the test device has an internal standard zone to ensure proper sample flow. The user should confirm that the colored band located at the "C" region is present before reading the result.

External Positive and Negative Controls
Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working properly and the test is correctly performed. External positive and negative controls should be used in accordance with applicable accrediting organizations. However, Assure recommends that the labs receiving this test execute a control test for the material they receive. Before testing, a new operator is recommended to execute the control test.

LIMITATIONS OF THE TEST

1. The COVID-19 & Influenza A/B Antigen Combo Test Device is intended for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of influenza A and B and SARS-CoV-2 antigens. The intensity of color in a positive band should not be considered as a "quantitative or semi-quantitative" result.
2. The test does not differentiate between SARS-CoV and SARS-CoV-2.
3. Both viable and nonviable influenza A and B viruses and SARS-CoV-2 viruses are detectable with COVID-19 & Influenza A/B Antigen Combo Test Device.
4. 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 a physician after all clinical and laboratory findings have been evaluated.
5. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
6. 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.
7. Negative results do not preclude influenza A and B or SARS-CoV-2 infection and should be confirmed via a molecular assay.
8. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The 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.

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.

Strain	Source	LOD
SARS-CoV-2	hCoV-19/China/ZJ-NB841/2020	$2.0\times10^{2.4}$ $TCID_{50}$/mL
Influenza A (H1N1) virus	A/China/ZJ-HZ166/2018	2.0×10^{4} $TCID_{50}$/mL
Influenza A (H3N2) virus	A/China/ZJ-TZ314/2016	8.6×10^{4} $TCID_{50}$/mL
Influenza B Yamagata lineage virus	BY/China/ZJ-HZ415/2018	5.0×10^{5} $TCID_{50}$/mL
Influenza B Victoria lineage virus	BV/China/ZJ-HZ809/2019	4.4×105 $TCID_{50}$/mL

Hook-effect
No high dose hook effect was observed when testing the COVID-19 & Influenza A/B Antigen Combo Test Device with the following viral concentrations:

Viral strain	Concentration
SARS-CoV-2	$1\times10^{6.4}$ $TCID_{50}$/mL
Influenza A (H1N1) virus	2.0×10^{6} $TCID_{50}$/mL
Influenza A (H3N2) virus	8.6×10^{6} $TCID_{50}$/mL
Influenza B Victoria lineage virus	4.4×10^{6} $TCID_{50}$/mL
Influenza B Yamagata lineage virus	5.0×10^{6} $TCID_{50}$/mL

Clinical Evaluation:
Both the NP and nasal swab specimens were collected from the same individual. The results were summarized below:

For COVID-19 antigen detection:
Of the 506 total nasopharyngeal swabs or nasal swabs tested in the clinical evaluation study, 100 swab specimens were found to be positive by RT-PCR and 406 swab specimens were found to be negative by RT-PCR. Nasal and nasopharyngeal swabs collected from the same individual were tested with the COVID-19 & Influenza A/B Antigen Combo Test Device. The results are shown in Table 1 and Table 2.

Table 1: COVID-19 Antigen Test vs. RT-PCR

NP swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	95	1	96
	Negative	5	405	410
	Total	100	406	506

Diagnostic Sensitivity: 95.0% (88.8% ~ 97.8%)*
Diagnostic Specificity: 99.8% (98.6% ~ 100.0%)*
Overall Agreement: 98.8% (97.4% ~ 99.5%)*
*95% Confidence Interval

Table 2: COVID-19 Antigen Test vs. RT-PCR

NS swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	94	2	96
	Negative	6	404	410
	Total	100	406	506

Diagnostic Sensitivity: 94.0% (87.5% ~ 97.2%)*
Diagnostic Specificity: 99.5% (98.2% ~ 99.9%)*
Overall Agreement: 98.4% (96.9% ~ 99.2%)*
*95% Confidence Interval

For Influenza A antigen detection
Of the 506 total nasopharyngeal swabs or nasal swabs tested in the clinical evaluation study, 53 swab specimens were found to be positive by RT-PCR and 453 swab specimens were found to be negative by RT-PCR. Nasal and nasopharyngeal swabs collected from the same individual were tested with the COVID-19 & Influenza A/B Antigen Combo Test Device. The results are showed in Table 3 and Table 4.

Table 3: Influenza A Antigen Test vs. RT-PCR

NP swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	51	0	51
	Negative	2	453	455
	Total	53	453	506

Diagnostic Sensitivity: 96.2% (87.2% ~ 99.0%)*
Diagnostic Specificity: 100.0% (99.2% ~ 100.0%)*
Overall Agreement: 99.6% (98.6% ~ 99.9%)*
*95% Confidence Interval

Table 4: Influenza A Antigen Test vs. RT-PCR

NS swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	50	1	51
	Negative	3	452	455
	Total	53	453	506

Diagnostic Sensitivity: 94.3% (84.6% ~ 98.1%)*
Diagnostic Specificity: 99.8% (98.8% ~ 100.0%)*
Overall Agreement: 99.2% (98.0% ~ 99.7%)*
*95% Confidence Interval

For Influenza B antigen detection
Of the 506 total nasopharyngeal swabs or nasal swabs tested in the clinical evaluation study, 35 swab specimens were found to be positive by RT-PCR and 471 swab specimens were found to be negative by RT-PCR. Nasal and nasopharyngeal swabs collected from the same individual were tested with the COVID-19 & Influenza A/B Antigen Combo Test Device. The results are showed in Table 5 and Table 6.

Table 5: Influenza B Antigen Test vs. RT-PCR

NP swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	33	1	34
	Negative	2	470	472
	Total	35	471	506

Diagnostic Sensitivity: 94.3% (81.4% ~ 98.4%)*
Diagnostic Specificity: 99.8% (98.8% ~ 100.0%)*
Overall Agreement: 99.4% (98.3% ~ 99.8%)*
*95% Confidence Interval

Table 6: Influenza B Antigen Test vs. RT-PCR

NS swab		RT-PCR		Total
		Positive	Negative	
COVID-19 & Influenza A/B Antigen Combo Test Device	Positive	33	2	35
	Negative	2	469	471
	Total	35	471	506

Diagnostic Sensitivity: 94.3% (81.4% ~ 98.4%)*
Diagnostic Specificity: 99.6% (98.5% ~ 99.9%)*
Overall Agreement: 99.2% (98.0% ~ 99.7%)*
*95% Confidence Interval

Cross Reactivity:
Cross-reactivity of the COVID-19 & Influenza A/B Antigen Combo Test Device was evaluated by testing a panel of respiratory pathogens that could potentially cross- react with the analyte detection reagents in the test device. Each microorganism, virus or negative matrix was tested in triplicate. Testing showed no evidence of cross- reactivity at the concentrations tested.

Human coronavirus 229E	Enterovirus	*Bordetella pertussis*
Human coronavirus OC43	Respiratory syncytial virus A	*Mycoplasma pneumoniae*
Human coronavirus NL63	Respiratory syncytial virus B	*Chlamydia pneumoniae*
MERS-coronavirus	Haemophilus influenzae	*Legionella pneumophila*
Human coronavirus HKU1	*Streptococcus pneumoniae*	*Staphylococcus aureus*
Human Metapneumovirus	*Streptococcus pyogenes*	*Staphylococcus epidermidis*
Parainfluenza virus 1	Influenza A (H1N1)	*Candida albicans*
Parainfluenza virus 2	Influenza A (H3N2)	Adenovirus
Parainfluenza virus 3	Influenza B Victoria lineage	Rhinovirus
Parainfluenza virus 4	Influenza B Yamagata lineage	Pooled human nasal wash

To estimate the likelihood of cross-reactivity with the 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) to analyze the degree of protein sequence homology.
For *Pneumocystis jirovecii (PJP)*, the blast results showed that no homology exists between the SARS-CoV-2 nucleocapsid protein and the *Pneumocystis jirovecii (PJP)*. For Mycobacterium tuberculosis, the blast results showed that no homology exists between the SARS-CoV-2 nucleocapsid protein and *Mycobacterium tuberculosis.*

Note:
1) **For FLU A detection:** FLUA detection has no cross-reactivity with the influenza B and SARS-CoV-2.
2) **For FLU B detection:** FLUB detection has no cross-reactivity with the influenza A and SARS-CoV-2.
3) **For SARS-CoV-2 detection (COVID-19):** SARS-CoV-2 detection has no cross-reactivity with influenza A and influenza B.
4) A cross-reactivity with SARS was observed for the detection of SARS-CoV-2, but not for the detection of Flu A and Flu B

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 COVID-19 & Influenza A/B Antigen Combo Test Device.

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

LITERATURE REFERENCES

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

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse		

IVD

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