Influenza A+B Antigen Rapid Test Device
Package Insert

Cat: IFL-522 **Specimens:** Nasopharyngeal swabs/ Nasal swab
Version: 03 **Effective Date:** 2019-09

For professional *in vitro* diagnostic use only.

INTENDED USE

The Influenza A+B Antigen Rapid Test Device is a rapid visual immunoassay for the qualitative, presumptive detection of influenza A and B viral antigens form throat swabs and nasopharyngeal swab specimens. The test is intended for use as an aid in the rapid differential diagnosis of acute influenza type A and type B virus infection.

INTRODUCTION

Influenza is a highly contagious, acute, viral infection of the respiratory tract. The causative agents of the disease are immunologically diverse, single-strand RNA viruses known as influenza viruses. There are three types of influenza viruses: A, B, and C. Type A viruses are the most prevalent and are associated with most serious epidemics. Type B viruses produce a disease that is generally milder than that caused by type A. Type C viruses have never been associated with a large epidemic of human disease. Both type A and B viruses can circulate simultaneously, but usually one type is dominant during a given season. Influenza antigens may be detected in clinical specimens by immunoassay. The Influenza A+B Test is a lateral-flow immunoassay using highly sensitive monoclonal antibodies that are specific for influenza antigens. The test is specific to influenza types A and B antigens with no known cross-reactivity to normal flora or other known respiratory pathogens.

PRINCIPLE

The Influenza A+B Rapid Test Device detects influenza A and B viral antigens through visual interpretation of color development on the strip. Anti-influenza A and B antibodies are immobilized on the test region A and B of the membrane respectively. During testing, the extracted specimen reacts with anti- influenza A and B antibodies conjugated to colored particles and precoated onto the sample pad of the test. The mixture then migrates through the membrane by capillary action and interacts with reagents on the membrane. If there is sufficient influenza A and B viral antigens in the specimen, colored band(s) will form at the according test region of the membrane. The presence of a colored band in the A and/or B region indicates a positive result for the particular viral antigens, while its absence indicates a negative result. The appearance of 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 has occurred.

KIT COMPONENTS

Individually packed Test Devices	Each test contains colored conjugates and reactive reagents precoated at the corresponding regions
Extraction solution	For specimens extraction.
Extraction tubes	For specimen preparation
Sterile nasal swabs	For specimen collection
Package insert	For operating instructions

MATERIALS REQUIRED BUT NOT PROVIDED

Timer	For timing use
Pipette	Capable of delivering 300 ul

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
- The extraction reagent solution contains a salt solution if the solution contacts the skin or eye, flush with copious amounts of water.
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in the area where the specimens and kits are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- 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 department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ is available to receive and culture specimens.
- Do not interchange or mix reagents from different lots.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded in accordance with local regulations.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- **Specimen Collection**

Nasal swab sample:

For proper test performance, use the swabs supplied in the kit.

It is important to obtain as much secretion as possible. Therefore, to collect a nasal swab sample, insert the sterile swab into the nostril that presents the most secretion under visual inspection. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). rotate the swab a few times against nasal wail.

Nasopharyngeal swab sample:

It is important to obtain as much secretion as possible. Therefore, to collect a nasopharyngeal swab sample, carefully insert the sterile swab into the nostril that presents the most secretion under visual inspection. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. Rotate the swab several times.

- **Specimen Transport and Storage:**

Specimens should be tested as soon as possible after collection. If transport of the samples is required, the following transport media are recommended and have been tested and shown not to interfere with the performance of the test: Hank' s BalanceMKd salt solution, M5 Media, or saline. Alternatively, samples may be stored refrigerated (2-8 ℃), or at room temperature(15-30℃), in a clean, dry, closed container for up to eight hours prior to testing. Nasal wash/aspirate specimens may also be stored frozen(-70℃ or colder) for up to one month.

PROCEDURE

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

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed within one hour.
2. Gently mix Extraction reagent solution. Add 6 drops of the Extraction Solution into the Extraction tube.
3. Place the patient swab specimen into the Extraction Tube. Roll the swab at least 10 times while pressing the swab against the bottom and side of the Extraction Tube. Roll the swab head against the inside of the Extraction Tube as you remove it. Try to release as much liquid as possible. Dispose of the used swab in accordance with your biohazard waste disposal protocol.
4. Put on the tube tip, then add 3 drops of extracted sample into the sample well. Do not handle or move the Test Device until the test is complete and ready for reading.
5. As the test begins to work, color will migrate across the membrane. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT:

Flu A Positive:* A colored band appears in the control band region (C) and another colored band appears in the A region.

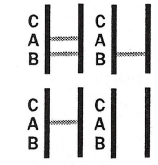
Flu B Positive:* A colored band appears in the control band region (C) and another colored band appears in the B region.

Flu A+B Positive:* A colored band appears in the control band region (C) and two other colored bands appear in the A region and B regions, respectively.

NEGATIVE RESULT:

Only one colored band appears, in the control band region (C). No band appears in either test band region (A/B).

INVALID RESULT:



Control band fails to appear. Results from any test which has not produced a control band at the specified reading 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.

NOTE:

1. The intensity of color in the test region (A/B) may vary depending on the concentration of analyses present in the specimen. Therefore, any shade of color in the test region (A/B) should be considered positive. Please 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 are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.

LIMITATIONS OF THE TEST

1. The Flu A+B Rapid Test Device is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of influenza A and/or B.
2. The etiology of respiratory infection caused by microorganisms other than influenza A or B virus will not be established with this test. The Flu A+B Rapid Test Device is capable of detecting both viable and non-viable influenza particles. The performance of the Flu A+B Rapid Test Device depends on antigen load and may not correlate with cell culture performed on the same specimen.
3. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at anytime rule out the presence of influenza A and/or B viral antigens in specimen, as they may be present below the minimum detection level of the test. As with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. The validity of Flu A+B Rapid Test Device has not been proven for identification or confirmation of cell culture isolates.
5. Inadequate or inappropriate specimen collection, storage, and transport may yield false negative test result.
6. Although this test has been shown to detect cultured avian influenza viruses, including avian influenza A subtype H5N1 virus, the performance characteristics of this test with specimens from humans infected with H5N1 or other avian influenza viruses are unknown.
7. Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
8. Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.
9. Positive and negative predictive values are highly dependent on prevalence. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.

PERFORMANCE CHARACTERISTICS

Table: Flu A+B Rapid Test vs. other commercial brand

Flu A

Relative Sensitivity: (99.7%~100%)	100%
Relative Specificity: (99.8%~100%)	100%
Overall Agreement: (99.8%~100%)	100%
***95% Confidence Interval**	

		Flu A+B Rapid Test +	-	**Total**
Other brand	+	52	0	52
	-	0	103	103
		52	103	155

Flu B

Relative Sensitivity: (99.6%~100%)	100%
Relative Specificity: (99.8%~100%)	100%
Overall Agreement: (99.8%~100%)	100%
***95% Confidence Interval**	

		Flu A+B Rapid Test +	-	**Total**
Other brand	+	27	0	27
	-	0	103	103
		27	103	130

Limit of detection (LOD)

LOD studies determine the lowest detectable concentration of Flu A+B at which approximately 95% of all (true positive) replicates test positive. Heat inactivated Influenza A and Influenza B virus, with a stock concentration of 5.1 X 10^5 $TCID_{50}$ / ml, was spiked into negative specimen and serially diluted. Each dilution was ran in triplicate on the Flu A+B Ag test. The Limit of Detection of the Flu A+B Antigen Rapid Test device (Swab) is 1.0X 10^2 $TCID_{50}$ / ml for Flu A and 1.25 X 10^2 $TCID_{50}$ / ml for Flu B.

ANALYTICAL SPECIFICITY AND CROSS-REACTIVITY

The Flu A+B Rapid Test Device was evaluated with a total of 30 bacterial and viral isolates. Bacterial isolates were evaluated at a concentration between 10^7 and 10^9 org/mL. Viral isolates were evaluated at a concentration of at least 10^4–10^8 $TCID_{50}$/mL. Adenovirus 18 and Parainfluenza virus 3 were tested at 10^2 $TCID_{50}$/mL. None of the organisms or viruses listed below gave a positive result in the Flu A+B Rapid Test Device.

Bacterial Panel:

Acinetobacter calcoaceticus	Bacteroides fragilis
Neisseria gonorrhoeae	Neisseria meningitidis
Pseudomonas aeruginosa	Staphylococcus aureus
Streptococcus pneumoniae	Streptococcus sanguis
Proteus vulgaris	Streptococcus sp. Gp. B
Streptococcus sp. Gp. C	Streptococcus sp. Gp. G
Mycobacterium tuberculosis	Mycoplasma orale

Viral Panel:

Human Adenovirus B	Human Rhinovirus 2
Human Adenovirus C	Human Rhinovirus 14
Adenovirus type 10	Human Rhinovirus 16
Adenovirus type 18	Measles
Human Coronavirus OC43	Mumps
Human Coxsackievirus A9	Sendai virus
Coxsackievirus B5	Parainfluenza virus 2
Human herpesvirus2	Parainfl uenza virus 3

ANALYTICAL SENSITIVITY

Analytical sensitivity was established using a total of 14 human epidemic strains of influenza viruses: 9 influenza A and 5 influenza B.

Viral Strain	**Viral Type**
Taiwan/1/86	A
Beijing/262/95	A
H1N1 Strain A/ New Caledonia/20/99 IVR 116	A
H1N1 Solomon Islands/03/06	A
H3N2 Strain A/ Shangdong/9/93	A
H3N2 Strain A/ Panama/2007/99	A
H3N2 Strain A/ Kiev/301/94	A
Wisconsin/67/05	A
Brisbane/10/06	A
Panama	B
Lee	B
Hong Kong	B
Maryland	B
Stockholm	B

INTERFERING SUBSTANCES

Whole blood, and several over-the-counter (OTC) products and common chemicals were evaluated and did not interfere with the Influenza A+B Test at the levels tested: whole blood (2%); three OTC mouthwashes (25%); three OTC throat drops (25%); three OTC nasal sprays (10%); 4-Acetamidophenol (10 mg/mL); Acetylsalicylic Acid (20 mg/mL); Chlorpheniramine (5 mg/mL); Dextromethorphan (10 mg/mL); Diphenhydramine (5 mg/mL); Ephedrine (20 mg/mL); Guaiacol glyceryl ether (20 mg/mL); Oxymetazoline (10 mg/mL); Phenylephrine (100 mg/mL); and Phenylpropanolamine (20 mg/mL).

LITERATURE REFERENCES

1. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003: 289: 179-86
2. McElhaney, J.E., Gravenstein, S., Krause, P., Hooton, J.W., Upshaw, C.M., and Drinka, P. 1998. Assessment of markers of the cell-mediated immune response after influenza virus infection in frail older adults. Clin. Diag. Lab. Immunol. 5:840-844.
3. Wright, K.E., Wilson, G.A.R., Novosad, D., Dimock, C., Tan, D., and Weber, J.M. 1995. Typing and subtyping of influenza viruses in clinical samples by PCR. J. Clin. Microbiol. 33:1180-1184.
4. Kendal, A.P. 1985. Influenza Viruses. p. 341-357. Laboratory Diagnosis of Viral Infections, In H. Lennette, (ed.) Marcel Dekker, Inc., New York.
5. McQuillen, J., Madeley, C.R., and Kendal, A.P. 1985. Monoclonal antibodies for the rapid diagnosis of influenza A and B virus infections by immunofluorescence. Lancet. ii: 911-914.
6. Guenthner, S.H., and Linnemann, C.C., Jr. 1988. Indirect immunofluorescence assay for rapid diagnosis of influenza virus. Laboratory Medicine. 19: 581-583
7. Minnick, L.L., and Ray, C.G. 1986. Early testing of cell cultures for detection of hemadsorbing viruses. J. Clin. Microbiol. 25: 421-422.
8. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S Government Printing Office, Washington, D.C.