Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

OneStep Influenza A & B (Swab/Nasal Aspirate) RapiCard™ InstaTest

REF 178901-20-44

IVD See external Label 2°C 30°C Σ 20Tests

Specificity	**99.2% Type A** **99.4 % Type B**
Sensitivity	**100% Type A** **97.9 % Type B**

INTENDED USE

The Cortez Diagnostics Inc. OneStep Influenza A+B RapiCard is a rapid chromatographic immunoassay for the qualitative detection of influenza A and B antigens in nasal swab or throat swab or nasal aspirate specimens. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections.

A rapid test for the qualitative detection of Influenza A and Influenza B virus in nasal swab, throat swab or nasal aspirate *specimens.*

For professional in vitro diagnostic use only.

SUMMARY AND EXPLANATION

Influenza (commonly known as 'flu') is a highly contagious, acute viral infection of the respiratory tract. It is a communicable disease easily transmitted through the coughing and sneezing of aerosolized droplets containing live virus.1 Influenza outbreaks occur each year during the fall and winter months. Type A viruses are typically more prevalent than type B viruses and are associated with most serious influenza epidemics, while type B infections are usually milder.

The gold standard of laboratory diagnosis is 14-day cell culture with one of a variety of cell lines that can support the growth of influenza virus.2 Cell culture has limited clinical utility, as results are obtained too late in the clinical course for effective patient intervention. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is a newer method that is generally more sensitive than culture with improved detection rates over culture of 2-23%.3 However, RT-PCR is expensive, complex and must be performed in specialized laboratories.

The Influenza A+B RapiCard Test (Swab/Nasal Aspirate) qualitatively detects the presence of Influenza A and/or Influenza B antigen in nasal swab or throat swab or nasal aspirate specimens, providing results within 15 minutes. The test uses antibodies specific for Influenza A and Influenza B to selectively detect Influenza A and Influenza B antigen in nasal swab, throat swab or nasal aspirate specimens.

TEST PRINCIPLE

The Influenza A+B RapiCard (Swab/Nasal Aspirate) is a qualitative, lateral flow immunoassay for the detection of Influenza A and Influenza B nucleoproteins in nasal swab, throat swab or nasal aspirate specimens. In this test, antibodies specific to the Influenza A and Influenza B nucleoproteins are separately coated on the test line regions of the test card. During testing, the extracted specimen reacts with the antibodies to Influenza A and/or Influenza B that are coated onto particles. The mixture migrates up the membrane to react with the antibodies to Influenza A and/or Influenza B on the membrane and generate one or two colored lines in the test regions. The presence of this colored line in either or both of the test regions indicates a positive result. To serve as a procedural control, a colored line will always appear in the control region if the test has been performed properly.

REAGENTS

The test card contains anti-Influenza A and B particles and anti-Influenza A and B coated on the membrane.

SPECIMEN COLLECTION AND PREPARATION

1. Nasopharyngeal swab sample
   Insert a sterilized swab into a nasal cavity securely from a nostril and collect mucoepidermis wiping turbinate several times.
2. Pharyngeal swab sample
   Insert a sterilized swab into pharynx and collect mucoepidermis mainly wiping flare region of post-pharyngeal wall and palatine tonsil several times, and be careful not to make saliva attach to the swab.
3. Nasopharyngeal aspirate
   Connect an aspiration catheter to an aspiration trap that is attached to an aspiration device, insert the catheter to nasal cavity from a nostril, start the aspiration device and then collect nasal aspirate sample. Dip a sterilized swab into the collected nasal aspirate sample and make the specimen cling to the swab.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. RapiCards
2. Extraction Reagent
3. Extraction Tubes
4. Sterile Swabs
5. Package insert
6. Workstation
7. Flu A positive control swab
8. Flu B positive control swab
9. Flu A+B negative control swab
10. Extraction Tube Tip

Materials required but not provided

1. Timer
2. Aspiration Device

ASSAY PROCEDURE

Allow the test, specimen, extraction buffer to equilibrate to room temperature (15-30°C) prior to testing.

1. Remove the RapiCard test from the sealed foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed immediately after opening the foil pouch.
2. Place the Extraction Tube in the workstation. Hold the extraction reagent bottle upside down vertically. Squeeze the bottle and let the solution drop into the extraction tube freely without touching the edge of the tube. Add 10 drops of solution (Approx. 400ul) to the Extraction Tube. See illustration 1.
3. Place the swab specimen in the Extraction Tube. Rotate the swab for approximately 10 seconds while pressing the head against the

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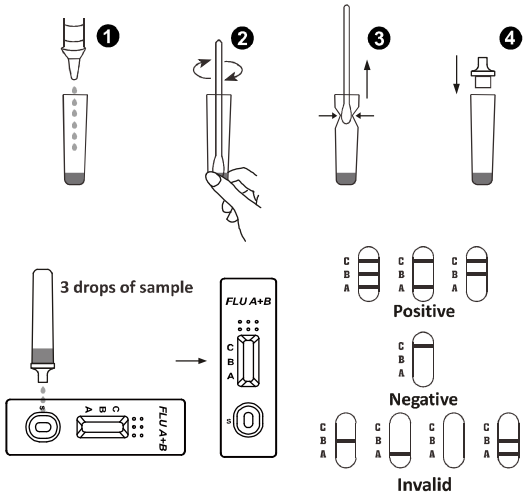
inside of the tube to release the antigen in the swab. See illustration 2.

4. Remove the swab while squeezing the swab head against the inside of the Extraction Tube as you remove it to expel as much liquid as possible from the swab. Discard the swab in accordance with your biohazard waste disposal protocol. See illustration 3.
5. Fit the dropper tip on top of the extraction tube. Place the Rapicard on a clean and level surface. See illustration 4
6. Add three drops of the solution (approx.120ul) to the sample well and then start the timer. Read the result at 15 minutes. Do not interpret the result after 20 minutes.



RESULTS

- **POSITIVE Influenza A:* Two distinct colored lines appear**. One colored line should be in the control region (C) and another colored line should be in the Influenza A region (A). A positive result in the Influenza A region indicates that Influenza A antigen was detected in the sample.
- **POSITIVE Influenza B:* Two distinct colored lines appear**. One colored line should be in the control region (C) and another colored line should be in the Influenza B region (B). A positive result in the Influenza B region indicates that Influenza B antigen was detected in the sample.
- **POSITIVE Influenza A and Influenza B:* Three distinct colored lines appear**. One colored line should be in the control region (C) and two colored line should be in the Influenza A region (A) and Influenza B region (B). A positive result in the Influenza A region and Influenza B region indicates that Influenza A antigen and Influenza B antigen were detected in the sample.

***NOTE**: The intensity of the color in the test line regions (A or B) will vary based on the amount of Flu A or B antigen present in the sample. So any shade of color in the test regions (A or B) should be considered positive.

- **Negative: One colored line appears in the control region (C).** No apparent colored line appears in the test line regions (A or B).
- **Invalid: Control line fails to appear.** Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test card. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal Quality Control

Internal procedural controls are included in the test. A red line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique. A clear background is an internal negative procedural control. If the test is working properly, the background in the result area should be white to light pink and not interfere with the ability to read the test result.

External Quality Control

It is recommended that a positive and negative external control be run every kit, and as deemed necessary by your internal laboratory procedures. External positive and negative controls are supplied in the kit.

Procedure for External Quality Control Testing

1. Add 10 full drops of (approx. 400ul) extraction reagent into the extraction tube, holding the bottle upright.
2. Add the Influenza A positive, Influenza B positive or negative control swab into the extraction tube.
3. Agitate the swab vigorously 15 times while pressing the head against the bottom of the tube to release in the swab.
4. Press the swab against the side of the tube and squeeze the bottom of the tube while removing the swab so that most of the liquid stays in the tube. Discard the swab.
5. Fit the dropper tip on top of the extraction tube. Place the RapiCard test on a clean and level surface.
6. Add three drops of the solution (approx. 120ul) to the sample well and then start the timer. Read the result at 15 minutes. Do not interpret the result after 20 minutes.

PERFORMANCE CHARACTERISTICS

Sensitivity, Specificity and Accuracy

The Cortez Diagnostics, Inc. Influenza A+B Rapid RapiCard (Swab/Nasal Aspirate) has been evaluated with specimens obtained from the patients. RT-PCR is used as the reference method for the Influenza A+B RapiCard Test (Swab/Nasal Aspirate). Specimens were considered positive if RT-PCR indicated a positive result. Specimens were considered negative if RT-PCR indicated a negative result

Nasal Swab Specimen

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
COR Test Flu A+B	Positive	100	2	102	85	2	87
	Negative	1	180	181	2	200	202
Total		101	182	283	87	202	289
Relative Sensitivity		99.0%			97.7%		
Relative Specificity		98.9%			99.0%		
Accuracy		98.9%			98.6%		

Throat Swab Specimen

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
COR Test Flu A+B	Positive	58	1	59	65	1	66
	Negative	3	150	153	4	162	166
Total		61	151	212	69	163	232
Relative Sensitivity		95.1%			94.2%		
Relative Specificity		99.3%			99.4%		
Accuracy		98.1%			97.8%		

Nasal Aspirate Specimen

		Type A			Type B		
		RT-PCR		Total	RT-PCR		Total
		Positive	Negative		Positive	Negative	
CORTest Flu A+B	Positive	46	2	48	94	1	95
	Negative	0	241	241	2	158	160
Total		46	243	289	96	159	255
Relative Sensitivity		100%			97.9%		
Relative Specificity		99.2%			99.4%		
Accuracy		99.3%			98.8%		

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Reactivity with Human Influenza Strain

The Influenza A+B RapiCard Test (Swab/Nasal Aspirate) was tested with the following human influenza strains and a discernible line at appropriate test-line regions was observed:

Influenza A Virus	*Influenza B Virus*
A/NWS/33 10(H1N1)	Bright
A/Hong Kong/8/68(H3N2)	B/R5
A/Port Chalmers/1/73(H3N2)	B/Russia/69
A/WS/33(H1N1)	B/Lee/40
A/New Jersey/8/76(HswN1)	B/Hong Kong/5/72
A/Mal/302/54(H1N1)	

Specificity Testing with Various Viral Strains

Description	Test Level
Human adenovirus C	5.62 x 10^5 TCID50/ml
Human adenovirus B	1.58 x 10^4 TCID50/ml
Adenovirus type 10	3.16 x 10^3 TCID50/ml
Adenovirus type 18	1.58 x 10^4 TCID50/ml
Human coronavirus OC43	2.45 x 10^6 LD50/ml
Coxsackievirus A9	2.65 x 10^4 LD50/ml 1.58 x 10^5 TCID50/ml
Coxsackievirus B5	1.58 x 10^7 TCID50/ml
Human herpesvirus 5	1.58 x 10^4 TCID50/ml
Echovirus 2	3.16 x 10^5 TCID50/ml
Echovirus 3	1 x 10^4 TCID50/ml
Echovirus 6	3.16 x 10^6 TCID50/ml
Herpes simplex virus 1	1.58 x 10^6 TCID50/ml
Human herpesvirus 2	2.81 x 10^5 TCID50/ml
Human Rhinovirus 2	2.81 x 10^4 TCID50/ml
Human Rhinovirus 14	1.58 x 10^6 TCID50/ml
Human Rhinovirus 16	8.89 x 10^6 TCID50/ml
Measles	1.58 x 10^4 TCID50/ml
Mumps	1.58 x 10^4 TCID50/ml
Sendai virus	8.89 x 10^7 TCID50/ml
Parainfluenza virus 2	1.58 x 10^7 TCID50/ml
Parainfluenza virus 3	1.58 x 10^8 TCID50/ml
Respiratory syncytial virus	8.89 x 10^4 TCID50/ml
Human respiratory syncytial virus	1.58 x 10^5 TCID50/ml
Rubella	2.81 x 10^5 TCID50/ml
Varicella-Zoster	1.58 x 10^3 TCID50/ml

TCID50 = Tissue Culture Infectious Dose is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated.

LD50 = Lethal Dose is the dilution of virus that under the conditions of the assay can be expected to kill 50% of the suckling mice inoculated.

Precision

Intra-Assay & Inter-Assay

Within-run and Between-run precision has been determined by using five specimens of Influenza standard control. Three different lots of the Influenza RapiCard Test (Swab/Nasal Aspirate) have been tested using negative, Influenza A weak, Influenza B Weak, Influenza A Strong and Influenza B Strong. Ten replicates of each level were tested each day for 3 consecutive days. The specimens were correctly identified>99% of the time.

Cross-reactivity

The following organisms were tested at 1.0x10^8org/ml and all found to be negative when tested with the Influenza A+B Rapid RapiCard Test (Swab/Nasal Aspirate):

Arcanobacterium	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus subspaureus*
Corynebacterium	*Staphylococcus epidermidis*
Enterococcus faecalis	*Staphylococcus saprophylicus*
Enterococcus faecium	*Streptococcus agalactiae*
Escherichia coli	*Streptococcus bovis*
Haemophilus	*Streptococcus dysgalatiae / subsp.dysgalatiae*
Moraxella catarrhalis	*Streptococcus oralis formerly Streptococcus*
Neisseria gonorrhoeae	*Streptococcus pneumoniae*
Neisseria lactamica	*Streptococcus pygenes*
Nesseria subllava	*Streptococcus salivarius*
Proleus vulgaris	*Streptococcus sp group F.type 2*

LIMITATIONS OF PROCEDURE

1. The Cortez Diagnostics, Inc. Influenza A+B Rapid RapiCard Test (Swab/Nasal Aspirate) is for professional in vitro diagnostic use only. The test should be used for the detection of Influenza A and/or B virus in nasal swab, throat swab or nasal aspirate specimens. Neither the quantitative value nor the rate of increase in Influenza A and/or B virus concentration can be determined by this qualitative test.
2. The Influenza A+B RapiCard (Swab/Nasal Aspirate) will only indicate the presence of Influenza A and/or B virus in the specimen from both viable and non-viable Influenza A and B strains.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. A negative result obtained from this kit should be confirmed by culture. A negative result may be obtained if the concentration of the Influenza A and/or B virus present in the nasal swab is not adequate or is below the detectable level of the test.
5. Excess blood or mucus on the swab specimen may interfere with test performance and may yield a false positive result.
6. The accuracy of the test depends on the quality of the swab sample. False negatives may result from improper sample collection or storage.
7. The use of over-the-counter and prescription nasal sprays at high concentrations can interfere with results, leading to either invalid or incorrect test results.
8. A positive result for influenza A and/or B does not preclude an underlying co-infection with another pathogen, therefore the possibility of an underlying bacterial infection should be considered.

EXPECTED VALUES

The Influenza A+B RapiCard (Swab/Nasal Aspirate) has been compared with a leading commercial RT-PCR test. The correlation between these two systems is over 97%.

PRECAUTIONS

Please read all the information in this package insert before performing the test.

1. For professional in vitro diagnostic use only. Do not use after the expiration date.
2. The test should remain in the sealed pouch until ready to use.
3. All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
4. The used test should be discarded according to local regulations.

STORAGE

Store as packaged at room temperature or refrigerated (2-30°C). The test is stable through to the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

REFERENCES

1. Williams, KM, Jackson MA, Hamilton M. (2002) Rapid Diagnostic Testing for URIs in Children; Impact on Physician Decision Making and Cost. Infec. Med. 19(3): 109-111.

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2. Betts, R.F. 1995. Influenza virus, p. 1546-1567. In G.L. Mandell, R.G. Douglas, Jr. and J.E. Bennett (ed.), Principle and practice of infectious diseases, 4th ed. Churchill Livingstone, Inc., New York, N.Y.
3. WHO recommendations on the use of rapid testing for influenza diagnosis, World Health Organisation, July 2005.

ISO 13485 ISO 9001 **Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA**	
Date Adopted	**2016-03-07**
REF **178901-20-44**	**CORTEZ- OneStep Influenza A & B (Swab,Nasal Aspirate) RapiCard™ InstaTest**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu**
Revision Date: 2016-01-14	