FLUA-FLUB-RSV-ADV Combo Test (Nasal Secretions)

MI-S44

INTENDED USE

The FLUA-FLUB-RSV-ADV Combo Test (Nasal Secretions) is a rapid visual immunoassay for the qualitative presumptive detection of influenza virus A, influenza virus B, respiratory syncytial virus and adenovirus from human nasal/nasopharyngeal swabs or nasal washes/aspirates. This kit is intended to be used as an aid in the diagnosis of influenza A viral, influenza B viral, respiratory syncytial viral, adenoviral infection.

INTRODUCTION

Influenza is a highly contagious, acute, epidemic to pandemic viral respiratory infection caused by three genera of the Orthomyxoviridaefamily[1]. Influenza virus can be distinguished into influenza virus A, B and C on the basis of antigenic differences between their nucleoprotein and matrix proteins. For types A and B, the antigenic variation of hemagglutinin and neuraminidase is responsible for the emergence of new strains, while type C is antigenically stable[1].Type A influenza viruses are typically more prevalent than type B viruses and are associated with most serious influenza epidemics, while type B influenza infections are usually milder[2]. Influenza due to type C species is rare compared to types A or B[3].

RSV is a negative single-stranded RNA virus of the Paramyxoviridae family[4]. It is the causative agent of a highly contagious, acute, viral infection of the respiratory tract. Worldwide, RSV contributes to the deaths of up to 1,000,000 infants and children under the age of five each year[5]. It is also the major viral cause of nosocomial illness in children already hospitalized for other reasons[6]. Among children hospitalized with RSV infection, the mortality rate is estimated to be as low as 0.3% to 1.0%[7] and in the range of 2.5% to 4.0% for children with underlying cardiac or pulmonary disease[8]. RSV infection confers only limited protective immunity. Thus, persons can be repeatedly infected and develop serious disease throughout their lifetime[9].

RSV has only one serotype, and is divided into two antigenic subgroups, A and B[10]. Both groups circulate simultaneously in the community. Subtype B is characterized as the asymptomatic strains of the virus that the majority of the population experiences. The more severe clinical illnesses involve subtype A strains, which tend to predominate in most outbreaks. RSV may be suspected based on the time of year of the infection; prevalence usually coincides with the winter flu season.

Adenoviruses are common viruses that cause a range of illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup and bronchitis[11]. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection. Adenovirus strains are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission[12]. And outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring, and early summer; however, adenovirus infections can occur throughout the year[13].

The clinical presentation of infections caused by the heterogeneous group of the respiratory viruses can be very similar. Thus, the FLUA-FLUB-RSV-ADV Test(Nasal Secretions) offers a simple, qualitative diagnosisof influenza A/influenza B/respiratory syncytial virus or adenovirus infection.

PRINCIPLE

The FLUA-FLUB-RSV-ADV Combo Test has been designed to detect influenza A virus, influenza B virus, respiratory syncytial virus and adenovirus through visual interpretation of color development on the four internal test strips.

Influenza A test (FLU A): Anti-Influenza A antibodies are immobilized on the test region of the membrane. During testing, the extracted antigens, if present, will bind to anti-Influenza A antibodies conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-Influenza A antibodies at the detection zone. Excess colored particles are captured at the internal control zone. The presence of one colored band on the test region (T) indicates a positive result for the particular viral antigens, while its absence indicates a negative result.

Influenza B test (FLU B): Anti-Influenza B antibodies are immobilized on the test region of the membrane. During testing, the extracted antigens, if present, will bind to anti-Influenza B antibodies conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-Influenza B antibodies at the detection zone. Excess colored particles are captured at the internal control zone. The presence of one colored band on the test region (T) indicates a positive result for the particular viral antigens, while its absence indicates a negative result.

Respiratory syncytial virus test (RSV): Anti-respiratory syncytial virus antibodies are immobilized on the test region of the membrane. During testing, the extracted antigens, if present, will bind to anti-respiratory syncytial virus antibodies conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti respiratory syncytial virus antibodies at the detection zone. Excess colored particles are captured at the internal control zone.The presence of one colored band in the test region indicates a positive result for the particular viral antigens, while its absence indicates a negative result.

Adenovirus test (ADV): Anti-adenovirus antibodies are immobilized on the test region of the nitrocellulose membrane. During test procedure, the extracted antigens, if present, will bind to anti-adenovirus antibodies conjugated to colored particles on the label pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by anti-adenovirus antibodies at the detection zone. Excess colored particles are captured at the internal control zone. The presence of one colored band in the test region indicates a positive result for the particular viral antigens, while its absence indicates a negative result.

The colored band at the control region of each test strip 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
- Extraction tube
- Swab
- Package insert
- Extraction buffer
- Nozzle with filter
- Tube stand

Materials Required but Not provided

- Centrifuge
- Specimens collection container
- Timer

PRECAUTIONS

- For professional in vitro diagnostic use only.
- Do not use after expiration date indicated on the package. Do not use the test if its foil pouch is damaged. Do not reuse tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore, recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
- Read the entire procedure carefully prior to performing any tests.
- 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 the standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Extraction buffer contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of buffered saline or extracted samples, always flush with copious quantities of water to prevent azide building up.
- Do not interchange or mix reagents from different lots.
- Humidity and temperature can adversely affect results.
- The used testing materials should be discarded in accordance with local, state and/or federal 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 equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

Specimen Collection

Acceptable specimens for testing with The FLUA-FLUB-RSV-ADV Combo Test include samples from nasal/nasopharyngeal swabs, nasal washes/aspirates. Do not use specimens that are obviously contaminate with blood, as it may interfere with the flow of sample with the interpretation of test results. Use freshly collected specimens for best test performance. Rapid tests will have more reliable clinical performance when performed early in the course of infection[7]. To ensure optimal performance, use the swabs supplied in the kit.

Nasal Swab

- Insert the swab into the nostril that exhibits the most visible drainage, if secretion is not visible, into the nostril that is most congested. Gently push the swab until resistance is met at the level of turbinates (less than one inch into the nostril), rotate the swab a few times against nasal wall. Slowly withdraw the swab while continuing with a rotating motion.
- *Note: In patients whose nasal cavity is dry, wet the swab with a sterilized physiological saline solution (not supplied in the kit) in advance and then collect a sample with it.*

Nasopharyngeal (NP) Swab

- Insert the swab carefully 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.

Nasal Wash

- With the patient's head hyper-extended, instill sterile, normal saline into one nostril with a syringe. Use the minimal amount of saline that your procedure allows, as excessive volume will dilute the antigen in the specimen. To collect the nasal wash, place a clean, dry specimen container directly under the nose with slight pressure on the upper lip. Tilt the head forward allowing the fluid to run out of the nostril into the specimen container. Repeat for the other nostril and collect the wash into the same specimen container.
- *Note: The normal saline, syringe and specimen container is not supplied in the kit.*

Nasal Aspirate

- Insert one aspirating tube with a trap up to the depth of the nasal cavity. Connect another tube to the aspiration device making it a negative pressure. Aspirate a nasal fluid to the trap. Soak the nasal aspirate obtained to a sterile swab.
- *Note: The aspirating device is not supplied in the kit.*
- Samples may be stored at refrigerated (2~8℃) for up to 8 hours prior to testing. Specimens may also be stored frozen (-70℃ or colder) for longer.

TEST PROCEDURE

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

1. For each specimen swab, 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. Gently mix the bottle. Add extraction buffer up to the second fill line of the graduated extraction tube (approximately 20 drops extraction buffer).
3. **For Nasal/ Nasopharyngeal Swabs**
   a. Insert the swab into the extraction tube. Mix well and squeeze the swab several times by compressing the walls of the tube against the swab.
   b. Roll the swab head against the inside of the 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.

   For Nasal Wash/Aspirate Specimens
   a. Vortex or thoroughly mix specimen. Do not centrifuge, as the removal of cellular material may adversely affect test sensitivity.
   b. Transfer 1mL of specimen into the extraction tube using transfer pipette.
4. Insert filtered nozzle into sample extraction tube. Invert the tube and add 3 drops of test sample into the sample well by gently squeezing the tube.
5. Read results at 15 minutes.

INTERPRETATION OF RESULTS

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.

NOTE:

1. The intensity of color 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 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.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The FLUA-FLUB-RSV-ADV Combo Test is for professional *in vitro* diagnostic use, and should be

used for the qualitative detection of influenza A virus, influenza B virus, respiratory syncytial virus and adenovirus only.

2. Following certain antibiotic treatments, the concentration of influenza A/influenza B/respiratory syncytial viral or adenoviral antigens may decrease to the concentration below the minimum detection level of the test. Therefore, diagnosis should be made with caution during antibiotic treatment.
3. Failure to follow the TEST PROCEDURE and INTERPRETATION OF RESULTS may adversely affect test performance and/or invalidate the test result.
4. A high dose "hook effect" may occur where the color intensity of test band decreases as the concentration of antigen increases. If a "hook effect" is suspected, dilution of specimens may increase color intensity of the test band.
5. 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.

PERFORMANCE CHARACTERISTICS

Specimen Correlation

Clinical evaluation was performed to compare the results obtained by FLUA-FLUB-RSV-ADV Combo Test and PCR. The results were summarized below:

For Influenza A test:

Table 1:FLUA-FLUB-RSV-ADV Combo vs. Influenza A PCR

Relative Sensitivity:96.3 % (89.7%-98.7%)*
Relative Specificity: 98.0% (95.3%-99.1%)*
Overall Agreement: 97.5% (95.2%-98.8%)*
*95% Confidence Interval

		Influenza A PCR		
		+	-	Total
FLUA-FLUB-RSV-ADV Combo Test	+	78	5	83
	-	3	240	243
	Total	81	245	326

For Influenza B test:

Table 2 FLUA-FLUB-RSV-ADV Combo vs. Influenza B PCR

Relative Sensitivity:96.8 % (89.0%-99.1%)*
Relative Specificity: 97.7% (95.1%-99.0%)*
Overall Agreement: 97.5% (95.2%-98.8%)*
*95% Confidence Interval

		Influenza B PCR		
		+	-	Total
FLUA-FLUB-RSV-ADV Combo Test	+	60	6	66
	-	2	258	260
	Total	62	264	326

For Respiratory Syncytial Virus test:

Table 3 FLUA-FLUB-RSV-ADV Combo vs. RSV PCR

Relative Sensitivity:96.2 % (89.4%-98.7%)*
Relative Specificity: 98.0% (95.3%-99.1%)*
Overall Agreement: 97.5% (95.2%-98.8%)*
*95% Confidence Interval

		RSV PCR		
		+	-	Total
FLUA-FLUB-RSV-ADV Combo Test	+	76	5	81
	-	3	242	245
	Total	79	247	326

For Adenovirus test:

Table 4 FLUA-FLUB-RSV-ADV Combo vs. Adenovirus PCR

Relative Sensitivity:96.2 % (90.5%-98.5%)*
Relative Specificity: 97.3% (94.2%-98.8%)*
Overall Agreement: 96.9% (94.4%-98.3%)*
*95% Confidence Interval

		Adenovirus PCR		
		+	-	Total
FLUA-FLUB-RSV-ADV Combo Test	+	100	6	106
	-	4	216	220
	Total	104	222	326

Analytical Specificity (Cross Reactivity)

The following organisms were found negative when tested with The FLUA-FLUB-RSV-ADV Combo Test (Nasal Secretions)

Human metapneumovirus, Group C *Streptococcus*, Epstein-Barr virus , Norovirus, Enterovirus EV70, Enterovirus EV71, Parainfluenza virus 1/2/3/4, Enterovirus A16, Enterovirus A24, Enterovirus B1, Echovirus 6, SARS-coronavirus-2, HCoV-229E, HCoV-OC43, HCoV-NL63, Rhinovirus A30, Rhinovirus B52, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus pneumonia, Streptococcus pyogenes, Bordetellaparapertussis, Bordetella pertussis, Bordetella pertussis, Chlamydia, pneumoniae, Haemophilus influenza, Legionella pneumophila, Mycoplasma pneumonia, Mycobacterium tuberculosis*

Note:

For FLU A test: Influenza A test has no cross reactivity with Influenza B virus, respiratory syncytial virus and adenovirus.
For FLU B test: Influenza B test has no cross reactivity with Influenza A virus, respiratory syncytial virus and adenovirus.
For RSV test: RSV test has no cross reactivity with Influenza A virus, Influenza B virus and adenovirus.
For ADV test: ADV test has no cross reactivity with Influenza A virus, Influenza B virus and respiratory syncytial virus.

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal or nasopharynx cavity, were evaluated at the concentrations listed below. None of them were found to affect test performance of the FLUA-FLUB-RSV-ADV Combo Test (Nasal Secretions)

Substance	Concentration	Substance	Concentration
3 OTC nasal sprays	10%	Guaiacol glyceryl ether	20mg/mL
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Whole blood	4%
4-acetamidophenol	10mg/mL	Mupirocin	250μg/mL
Acetylsalicylic acid	10mg/mL	Oxymetazoline	25μg/mL
Albuterol	10mg/mL	Phenylephrine	10 mg/mL
Chlorpheniramine	5 mg/mL	Phenylpropanolamine	1mg/mL
Dexamethasone	50μg/mL	Zanamivir	10mg/mL
Dextromethorphan	10μg/mL	Adamantanamine	500 ng/mL
Diphenhydramine	5 mg/mL	Oseltamivir phosphate	10mg/mL
Doxylamine succinate	1 mg/mL	Tobramycin	10mg/mL
Flunisolide	25μg/mL	Triamcinolone	14mg/mL

LITERATURE REFERENCES

1. Couch RB. Orthomyxoviruses. In: Baron S, editor. Medical Microbiology.4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 58. Available from: http://www.ncbi.nlm.nih.gov/books/NBK8611/
2. Q Street Medical Associates. March 08, 2015. Flu Season. https://www.qstreetmds.com/flu-season
3. Wikipedi acontributors, "Influenza virus C," Wikipedia, The Free Encyclopedia,http://en.wikipedia.org/w/index.php?title=Influenzavirus_C&oldid=649896527 (accessed May 25, 2015).
4. Course BS3035: Virology, University of Leicester, http://www-micro.msb.le.ac.uk/3035/Paramyxoviruses.html.
5. Respiratory Syncytial Virus (RSV): Overview, Treatment, and Prevention Strategies, Mark J. Polak, MD.
6. Macartney K. et al. Nosocomial Respiratory Syncytial Virus Infections: The Cost-Effectiveness and Cost-Benefit of Infection Control. Pediatrics Vol. 106 No. 3 Sept 2000, pp. 520–526. http://pediatrics.aappublications.org/cgi/content/full/106/3/520.
7. Thompson W. et al. Mortality Associated With Influenza and Respiratory Syncytial Virus in the United States. JAMA, January 8, 2003 – Vol 289, No. 2.
8. Moler F.W. et al. Respiratory syncytial virus morbidity and mortality estimates in congenital heart disease patients: a recent experience. Crit Care Med. 1992 Oct; 20(10):1406–13.
9. Guidelines for Preventing Health-Care–Associated Pneumonia, 2003, page 43
10. Mark J. Polak, MD, Department of Pediatrics, West Virginia University School of Medicine, Morgantown, WV, USA
11. Wadell G.; et al. (1987). Whelan, Julie; Bock, Gregory (eds.). Novel diarrhoea viruses. New York: Wiley. p. 63.ISBN978-0-471-91094-7.
12. Voss, Jameson D.; Atkinson, Richard L.; Dhurandhar, Nikhil V. (1 November 2015)."Role of adenoviruses in obesity".Rev. Med. Virol.**25**(6): 379–387.doi:10.1002/rmv.1852.PMID26352001.
13. This article incorporates public domain materialfrom theUnited States Governmentdocument "https://www.cdc.gov/ncidod/dvrd/revb/respiratory/eadfeat.htm"."Archived copy". Archived from the originalon July 3, 2007. Retrieved2007-07-03.

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		