FLUA-FLUB-RSV-ADV-MP
Combo Test

For professional in vitro diagnostic use only.

INTENDED USE

The FLUA-FLUB-RSV-ADV-MP Combo Test is an in vitro immunoassay. The assay is for the direct and qualitative detection of influenza A virus, influenza B virus, Respiratory Syncytial Virus (RSV), Adenovirus (ADV), Mycoplasma pneumoniae (MP) antigens from human nasal/nasopharyngeal swabs or nasal washes/aspirates. This test is intended for professional use only.

PRINCIPLE

The FLUA-FLUB-RSV-ADV-MP Combo Test detect FLU A/B, RSV, ADV and MP antigens through visual interpretation of color development. A sample is added to the extraction buffer which is optimized to release the target antigens.
The FLUA-FLUB-RSV-ADV-MP Combo Test is composed of three test strips: 1) FLU A/B test strip, 2) RSV/ADV test strip and 3) MP test strip.
For FLU A/B Test: Anti-influenza A and B antibodies are immobilized on 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 antibodies at the respective detection zone. Excess colored particles are captured at the internal control zone.
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. 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 ADV/RSV Test: Anti-respiratory syncytial virus antibodies and anti-adenovirus antibodies are immobilized in the test region RSV and ADV of the nitrocellulose membrane, respectively. Anti-respiratory syncytial virus antibodies and anti-adenovirus antibodies conjugated to colored particles are immobilized on the conjugated pad.
During testing, the extracted antigens, if present, will bind to anti-respiratory syncytial virus antibodies or 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-respiratory syncytial virus antibodies or anti-adenovirus antibodies in the detection zone. Excess colored particles are captured at the internal control zone.
The presence of a red band in the RSV and/or ADV 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.
For MP test: Anti-*M. pneumoniae* antibodies are immobilized on the test region of the nitrocellulose membrane. *M. pneumoniae* antibodies conjugated to colored particles are immobilized on the conjugated pad.
During testing, the extracted antigens bind to *M. pneumoniae* antibodies conjugated to colored particles on the conjugated pad. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by *M. pneumoniae* monoclonal antibodies at the detection zone. Excess colored particles are captured at the internal control zone.
The presence of a colored band in the test region indicates a positive result, 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.

MATERIALS

Materials Provided

- Individually packed test devices
- Individually packed swabs
- Package insert
- Extraction buffer
- Tube stand

Materials Required but Not provided

- Clock, timer or stopwatch

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.
- The device contains material of animal origin and should be handled as a potential biohazard. Do not use if pouch is damaged or open.
- 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 decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false test results.
- Avoid skin contact with buffer.

STORAGE AND STABILITY

- Store The FLUA-FLUB-RSV-ADV-MP Combo 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.

SPECIMEN COLLECTION AND STORAGE

Specimen Collection:

Acceptable specimens for testing with the FLUA-FLUB-RSV-ADV-MP 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.

To ensure optimal performance, use the swabs supplied in the kit. Alternatively, sterile nylon, foam or rayon nasal swabs can be used for specimen collection. Do not use calcium alginate swabs.

- **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 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 are 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.

Specimen Storage

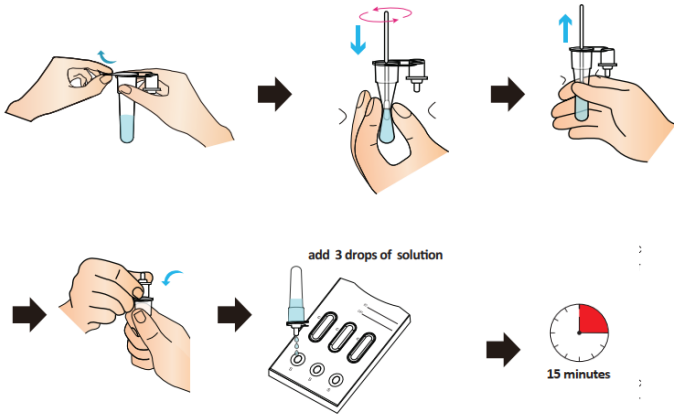
For nasal wash/aspirate, sample volumes of 1~3 mL are recommended. If transport medium is used, minimal dilution of specimens (1 mL) is recommended.

Specimens should be tested as soon as possible after collection. If storage is necessary, samples may be stored at refrigerated (2~8°C) for 3 days, or at room temperature (15~30°C), in a clean, dry, closed container for up to 4 hours prior to testing. Nasal wash or aspirate specimens may also be stored frozen (-70°C or colder) for up to one month.

TEST PROCEDURE

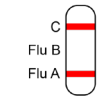
Bring devices, reagents and specimens and/or controls to room temperature (15~30°C) before use.

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. Peel off the aluminum foil cover of the extraction buffer.
3. insert the swab into the extraction tube. Mix well and **squeeze the swab 10-15 times** by compressing the walls of the tube against the swab.
4. Roll the swab head against the inner wall 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.
5. Insert the nozzle to the buffer tube. **Add 3 drops of solution** into each sample well by gently squeezing the tube.
6. Read results at 15 minutes. do not read results after 20 minutes

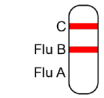


RESULT INTERPRETATION

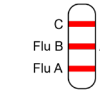
For FLU A/B Test



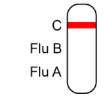
FLU A Positive: One colored band appears in the control region (C), and another colored band in the FLU A test region.



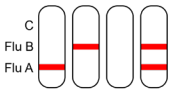
FLU B Positive: One colored band appears in the control region (C), and another colored band in the FLU B test region.



FLU A+B Positive: One colored band appears in the control region (C), and two other colored bands appear in both FLU A and FLU B test region.



Negative: Only one colored band appears in the control region (C), and no band appears either in the FLU A region or FLU B region.



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.

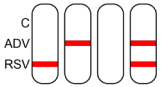
For ADV /RSV Test:

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

RSV Positive: One colored band appears in the control region (C), and another red band in the RSV test region.

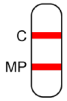
RSV+ADV Positive: One colored band appears in the control region (C), and two other colored bands appear in both ADV region and RSV region.

Negative: Only one colored band appears in the control region (C), and no band appears either in the ADV region or RSV region.

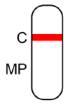


Invalid: No colored band appears in the control region (C), whether a test band(s) is present or not. Repeat invalid tests with a new sample, new test device and reagent. Insufficient sample volume, inaccurate operating procedure or expired tests may yield an invalid result. Contact your local distributor if the problem continues.

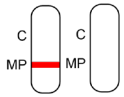
For MP Test



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



Negative: Only one colored band appears in the control region (C). No colored band appears in the MP test region.



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 color intensity in the test region 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 FLUA-FLUB-RSV-ADV-MP Combo Test has built-in (procedural) controls. Each strip on 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 and that the test is correctly performed.

LIMITATIONS OF THE TEST

1. The FLUA-FLUB-RSV-ADV-MP Combo Test is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of viral antigens of FLU A/B, RSV, ADV, and MP antigens. The intensity of color in a positive band should not be evaluated as "quantitative or semi-quantitative".
2. Both viable and nonviable viruses are detectable with The FLUA-FLUB-RSV-ADV-MP Combo 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 the viral infections and should be confirmed by other methods such as molecular assays.

PERFORMANCE CHARACTERISTICS

Clinical Evaluation:

For FLU A detection

Of the 326 total nasopharyngeal swab from influenza A viral infection patients, 81 were found to be positive by PCR and 245 were found to be negative by PCR. These swabs were tested with the device. The result is shown in table below.

Table1: FLU A Test vs. PCR

		PCR+	PCR-	Total
FLU A Test	**Influenza A+**	78	5	83
	Influenza A-	3	240	243
	Total	81	245	326

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

For FLU B detection

Of the 326 total nasopharyngeal swab from influenza B viral infection patients, 62 were found to be positive by PCR and 264 were found to be negative by PCR. These swabs were tested with the device. The result is shown in table below.

Table2: FLU B Test vs. PCR

		PCR +	PCR-	Total
FLU B Test	**Influenza B+**	60	6	66
	Influenza B-	2	258	260
	Total	62	264	326

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

For ADV detection:

104 were found to be positive by PCR and 279 were found to be negative by PCR. These swabs were tested with the device. The result is shown in table below.

Table 3: ADV Test vs. PCR

		PCR		Total
		Positive	Negative	
ADV Test	**Positive**	99	9	108
	Negative	5	270	275
	Total	104	279	383

Relative sensitivity: 95.2% (89.2%-97.9%)*
Relative specificity: 96.8% (94.0%-98.3%)*
Overall agreement: 96.3% (94.0%-97.8%)*
*95% Confidence Interval

For RSV detection:

79 were found to be positive by PCR and 304 were found to be negative by PCR. These swabs were tested with the device. The result is shown in table below.

Table4: RSV Test vs. PCR

		PCR		Total
		Positive	Negative	
RSV Test	**Positive**	76	7	83
	Negative	3	297	300
	Total	79	304	383

Relative sensitivity: 96.2% (89.4%-98.7%)*
Relative specificity: 97.7% (95.3%-98.9%)*
Overall agreement: 97.4% (95.3%-98.6%)*
*95% Confidence Interval

For MP detection:

295 nasopharyngeal swabs were tested on the device and confirmed by RT-PCR. 91 samples presented positive results and 204 samples presented negative results by the device. In addition, 99 samples presented positive results and 196 samples presented negative results by RT-PCR.

Table 5: MP Test vs. PCR

		PCR		Total
		Positive	Negative	
MP Test	**Positive**	90	1	91
	Negative	9	195	204
	Total	99	196	295

Relative Sensitivity: 90.9 % (83.6%~95.1%) *
Relative Specificity: 99.5 % (97.2%~99.9%)*
Overall Agreement: 96.6 % (93.9%~98.1 %)*
*95% Confidence Interval

Cross Reactivity:

The following organisms were found negative when tested with the assay. Samples positive for the following organisms were found negative when tested with The FLUA-FLUB-RSV-ADV-MP Combo Test.

Echovirus 6	HCoV-NL63	*Streptococcus pneumonia*
Coxsackie virusA16/ A24/ B1	HCoV-229E	*Bordetella parapertussis*
Enterovirus 70/ 71	Rhinovirus A30/B52	*Bordetella pertussis*
Human metapneumovirus	Group C *Streptococcus*	*Chlamydia, pneumoniae*
Norovirus	*Streptococcus pyogenes*	*Haemophilus influenza*
Epstein-Barr virus	*Staphylococcus aureus*	*Legionella pneumophila*
Measles virus	*Staphylococcus epidermidis*	Parainfluenza virus 1/2/3/4
Streptococcus agalactiae	*Mycobacterium tuberculosis*	HCoV-OC43

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 FLUA-FLUB-RSV-ADV-MP Combo Test.

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

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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		

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