VITASSAY

Influenza A+B+ RSV+Adenovirus Resp.

Rapid test for the qualitative detection of Influenza type A, Influenza type B, RSV and Adenovirus from nasopharyngeal swabs.

IUE-7715043 Ed00 January 2017

CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Influenza A+B+RSV+Adenovirus Resp. is a rapid, immunochromatographic assay for the simultaneous qualitative detection of Influenza type A, Influenza type B, RSV and Adenovirus from nasal swabs.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of Influenza type A, influenza type B, RSV and/or Adenovirus infection.

INTRODUCTION

The clinical presentation of respiratory infections caused by different viral pathogens can be very similar, making etiological diagnosis difficult.

Influenza virus, respiratory syncytial virus (RSV) and adenovirus are of primary importance since infections produced by them range from mild respiratory illness to fatal pneumonia, and cause considerable morbidity and excess deaths in children, elderly people, and in immunocompromised individuals throughout the world.

Influenza A and B are two types of influenza viruses that cause epidemic human disease. Uncomplicated influenza illness is characterized by the abrupt onset of constitutional and respiratory signs and symptoms (e.g. fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis). Among children, otitis, nausea, and vomiting are also commonly reported with influenza illness.

RSV is a frequent cause of flu-like symptoms. It can sometimes cause lower respiratory tract illness, which can be severe, and should be considered in the differential diagnosis in such cases.

Typically adenovirus infections result in self-limiting respiratory, gastrointestinal or ocular infections, however, adenovirus can cause severe disseminated disease in immunocompromised patients.

PRINCIPLE

Vitassay Influenza A+B+RSV+Adenovirus Resp. is a qualitative immunochromatographic assay to make a presumptive diagnosis of Influenza type A, Influenza type B, RSV and/or Adenovirus infection.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Influenza type A.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Influenza type B.

Strip C: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against RSV.

Strip D: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Adenovirus.

During the process, the sample reacts with the antibodies against Influenza A (strip A) and/or Influenza B (strip B) and/or RSV (strip C), and/or Adenovirus (strip D) forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is Influenza type A positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A, if the sample is Influenza type B positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip B, if the sample is RSV positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip C and if the sample is Adenovirus positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip D. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for all the strips).

The presence of these green lines (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Influenza A+B+RSV+Adenovirus Resp.** Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/35.6-86ºF).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **10** Tests/kit **Vitassay Influenza A+B+RSV+Adenovirus Resp.** ▪ 10 Viasl with sample diluent. ▪ 10 Swabs. ▪ Instructions for use.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Timer. ▪ Vortex

SPECIMEN COLLECTION

Samples should be collected in clean and dry containers.

Samples should be process as soon as possible after collection. If this is not possible, the samples can be store in the refrigerator (2-8ºC/35.6-46.4ºF) for 8 hours prior testing.

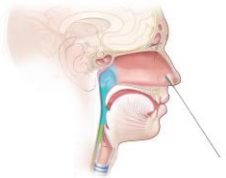
Samples must be brought to room temperature before testing.

Homogenize the samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

- Nasal swab method:

1. Remove the swab from its packing.
2. Use the sterile swab to collect the specimen from the nostril, rotating against the nasal wall (ensuring that swab contains cells as well as mucus).
3. Repeat the same procedure from the other nostril.
4. Process the swab as soon as possible after collecting the specimen.



PROCEDURE

Allow tests, samples, controls and diluent to reach room temperature (15-30ºC/59-86ºF) prior to testing.

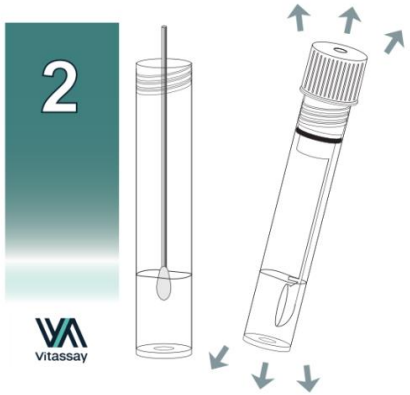
Do not open pouches until the performance of the assay.

- Nasal swab method:

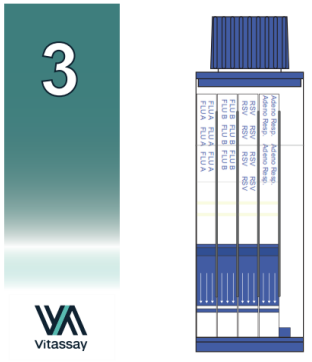
1. Open the cap of the vial for sample dilution with diluent B (figure 1).
2. Introduce the swab into the vial for sample dilution (figure 2) and mix the solution by rotating the swab forcefully against the side of the tube at least 1 minute. Best results are obtained when the specimen is vigorously extracted in the solution (figure 2). Extract as much liquid as possible from the swab, squeezing the sides of the tube or rotating the swab against the side of the tube as the swab is withdrawn. Discard the swab.
3. Close the vial with sample and diluent. Shake the vial to assure a good sample dispersion, sake during 60 seconds (figure 2).
4. Remove **Vitassay Influenza A+B+RSV+Adenovirus Resp.** from its sealed bag just before using it (figure 3).
5. Take the vial for sample dilution containing the diluted sample (figure 4), place it inside the multiplex tube (figure 5). Screw the cap of the multiplex tube tighly (figure 6). The bottom of the vial for sample dilution will break and the diluent+sample solution reaches the sample zone of the strips (figure 7).
6. Read the results at **10 minutes**. Do not read the test result later than 10 minutes.

If the test does not run due to solid particles (the sample is not homogenized), migration process can stop on one or more strips. In this case, tap the end of the multiplex tube on hard surface to allow migration to start again.

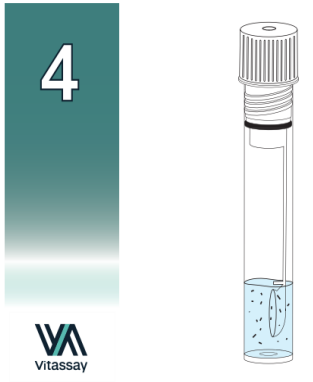
Open the cap of the vial for sample dilution.



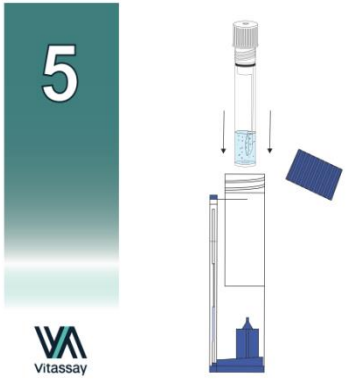
Introduce the swab into the vial for sample dilution and mix the solution by rotating the swab forcefully against the side of the tube. Extract as much liquid as possible form the swab. Mix 60 seconds.



Vitassay Influenza A+B+RSV+Adenovirus Resp.



Vial with the diluted sample inside.



Introduce the vial with the diluted sample into the multiplex.

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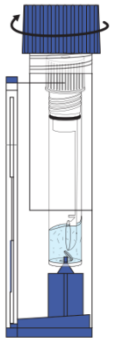

Close the cap and the bottom of the diluent vial will break.

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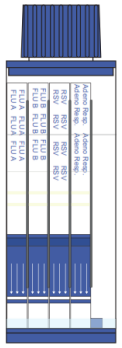
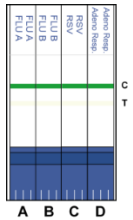
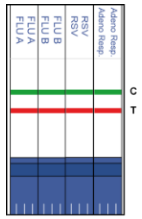
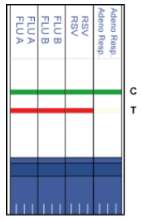
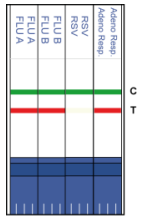
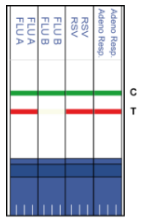
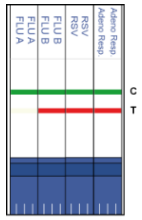
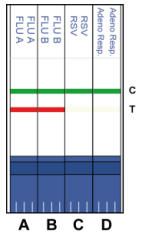


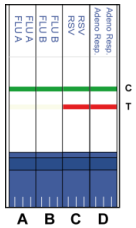
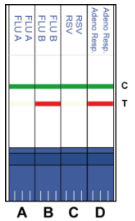
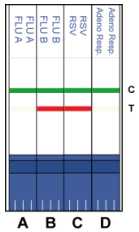
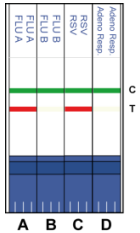
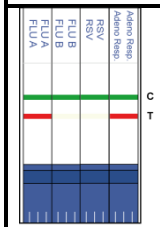
Reaction takes place. **Read results at 10 minutes.**

INTERPRETATION OF THE RESULTS

Strip A: Influenza A, Strip B: Influenza B, Strip C: RSV and Strip D: Adenovirus

	**NEGATIVE** Only one green line in the control zone (C) in the four strips (A,B,C and D)	There is no Influenza A, Influenza B, RSV and Adenovirus presence.
	**POSITIVE** In addition to the green line (control line C), a red line appears in each strip, test line (T)	There is Influenza A, Influenza B, RSV and Adenovirus presence.

	**NEGATIVE** Strip D (Adenovirus)→ green line **POSITIVE** Strip A (Influenza A)→ green/red lines Strip B (Influenza B)→ green/red lines Strip C (RSV)→ green/red lines	There is Influenza A, B and RSV presence. Infection caused by Infuenza A, B and RSV.
	**NEGATIVE** Strip C (RSV)→ green line **POSITIVE** Strip A (Influenza tipo A)→ green/red lines Strip B (Influenza tipo B)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is Influenza A, B and Adenovirus presence. Infection caused by Infuenza A, B and Adenovirus.
	**NEGATIVE** Strip B (Influenza B)→ green line **POSITIVO** Strip A (Influenza A)→ green/red lines Strip C (RSV)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is Influenza B, RSV and Adenovirus presence. Infection caused by Infuenza B, RSV and Adenovirus.
	**NEGATIVE** Strip A (Influenza A)→ green line **POSITIVE** Strip B (Influenza B)→ green/red lines Strip C (RSV)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is Influenza B, RSV and Adenovirus presence. Infection caused by Infuenza B, RSV and Adenovirus.
	**NEGATIVE** Strip C (RSV)→ green line Strip D (Adenovirus)→ green line **POSITIVE** Strip A (Influenza A)→ green/red lines Strip B (Influenza B)→ green/red lines	There is Influenza A and B presence. Infection caused by Infuenza A and B.

	**NEGATIVE** Strip A (Influenza A)→ green line Strip B (Influenza B)→ green line **POSITIVE** Strip C (RSV)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is RSV and Adenovirus presence. Infection caused by RSV and Adenovirus.
	**NEGATIVE** Strip A (Influenza A)→ green line Tira C (RSV)→ green line **POSITIVE** Strip B (Influenza tipo B)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is Influenza B and Adenovirus presence. Infection caused by Infuenza B and Adenovirus.
	**NEGATIVE** Strip A (Influenza A)→ green line Strip D (Adenoviurs)→ green line **POSITIVE** Strip B (Influenza B)→ green/red lines Strip C (RSV)→ green/red lines	There is Influenza B and RSV presence. Infection caused by Infuenza B and RSV.
	**NEGATIVE** Strip B (Influenza B)→ green line Strip D (Adenovirus)→ green line **POSITIVE** Strip A (Influenza A)→ green/red lines Strip C (RSV)→ green/red lines	There is Influenza A and RSV presence. Infection caused by Infuenza A and RSV.
	**NEGATIVE** Strip B (Influenza B)→ green line Strip C (RSV)→ green line **POSITIVE** Strip A (Influenza A)→ green/red lines Strip D (Adenovirus)→ green/red lines	There is Influenza A and Adenovirus presence. Infection caused by Infuenza A and Adenovirus.

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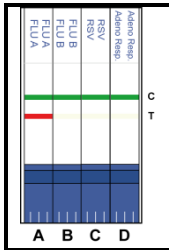
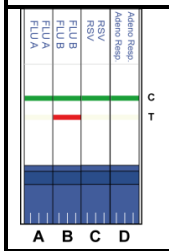
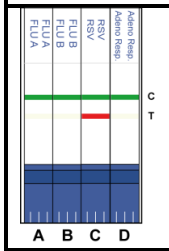
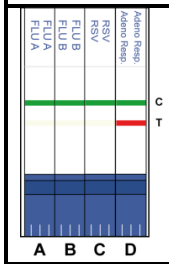
	**NEGATIVE** Strip B (Influenza B)→ green line Strip C (RSV)→ green line Strip D (Adenovirus)→ green line **POSITIVE** Strip A (Influenza A)→ green/red lines	There is Influenza A presence. Infection caused by Infuenza A.
	**NEGATIVE** Strip A (Influenza A)→)→ green line Strip C (RSV)→ green line Strip D (Adenovirus)→ green line **POSITIVE** Strip B (Influenza B)→ green/red lines	There is Influenza B presence. Infection caused by Infuenza B.
	**NEGATIVE** Strip A (Influenza A)→ green line Strip B (Influenza B→ green line Strip D (Adenovirus)→ green line **POSITIVE** Tira C (RSV)→ green/red lines	There is RSV presence. Infection caused by RSV.
	**NEGATIVE** Strip A (Influenza A)→ green line Strip B (Influenza B)→ green line Strip C (RSV)→ green line **POSITIVE** Strip D (Adenovirus)→ green/red lines	There is Adenovirus presence. Infection caused by Adenovirus.
Any other results	Invalid result either A, B, C or D, we recommend repeating the assay using the same sample with another test.	

Notes: The intensity of the red coloured test line in the result line region (T) will vary depending on the concentration of antigens in the specimen.

Positive results detailed in the above table should be followed up with additional confirmatory diagnostic procedures.

Single or dual simultaneous virus infections are more frequent than triple.

Invalid results: Total absence of any control coloured lines (green) indicates an invalid result, regardless of the appearance or not of the test lines (red**).** Wrong procedural techniques or deterioration of the reagents are mostly the main reasons for control line failure. Review the procedure and repeat the assay with a new test. If the problem persists, discontinue using the kit and contact your local distributor.

QUALITY CONTROL

Internal procedural control is included in **Vitassay Influenza A+B+RSV+Adenovirus Resp**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Influenza A+B+RSV+Adenovirus** must be carried out within 2 hours of opening the sealed bag.
- The intensity of test line may vary depending on the concentration of antigens.
- The quality of **Vitassay Influenza A+B+RSV+Adenovirus** depend on the quality of the sample; proper samples are from nasal swabs.
- Positive results determine the presence of Influenza type A, Influenza type B, RSV and/or Adenovirus respiratory infection. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the detection limit value. If symptoms or situation still persist, it is recommended that all negative results undergo confirmatory testing using other method and/or virus identification by cell culture and PCR.

EXPECTED VALUES

Respiratory infections caused by influenza virus type A, influenza virus type B, respiratory syncytial virus (RSV), parainfluenza virus are major causes of upper and lower respiratory tract diseases in infants and young children, causing croup, bronchiolitis, and pneumonia. Additionally, these viruses have all been identified as important causes of several lower respiratory tract diseases, with significant morbidity and mortality, in elderly and immunocompromised patients.

Sixty to ninety percent of the clinical syndrome of bronchiolitis is caused by respiratory syncytial virus (RSV) infection.

Adenoviruses are implicated in 4%-10% of cases of pneumonia, 2%-10% of cases of bronchiolitis, and 3%-9% of cases of croup.

Adenoviruses are less frequent cause of lower respiratory tract infection in children than are respiratory syncytial virus and parainfluenza virus.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

Nasopharyngeal samples were used in order to evaluate the results obtained by **Vitassay Influenza A + B + RSV + Adenovirus Resp.** with other immunochromatographic tests ((BinaxNOW® Influenza A&B (Alere), BinaxNOW® RSV (Alere) and Adenovirus Respi, (CorisBioConcept) and a immunofluorescence test (PathoDx®Adenovirus, Remel).

Results were as follows:

		BinaxNOW® Influenza A&B		
		Positive	Negative	Total
Vitassay Influenza A + B + RSV + Adenovirus Resp. (Influenza A+B)	Positive	5	0	5
	Negative	0	6	6
	Total	5	6	11

Vitassay Influenza A + B + RSV + Adenovirus Resp. vs BinaxNOW® Influenza A&B			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

		BinaxNOW® RSV		
		Positive	Negative	Total
Vitassay Influenza A + B + RSV + Adenovirus Resp. (RSV)	Positive	18	0	5
	Negative	1	10	11
	Total	19	10	29

Vitassay Influenza A + B + RSV + Adenovirus Resp. vs **BinaxNOW® RSV**			
Sensitivity	**Specificity**	**PPV**	**NPV**
95%	>99%	>99%	91%

		PathoDx®Adenovirus		
		Positive	Negative	Total
Vitassay Influenza A + B + RSV +Adenovirus Resp. (Adenovirus)	Positive	20	0	20
	Negative	0	5	5
	Total	20	5	25
		Adenovirus Respi		
		Positive	Negative	Total
Vitassay Influenza A +B +RSV + Adenovirus Resp. (Adenovirus)	Positive	20	0	20
	Negative	0	5	5
	Total	20	5	25

Vitassay Influenza A +B +RSV + Adenovirus Resp. vs PathoDx®Adenovirus Test and **Adenovirus Respi Test**			
Sensitivity	**Specificity**	**PPV**	**NPV**
>99%	>99%	>99%	>99%

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The results showed that **Vitassay Influenza A + B + RSV + Adenovirus** has a high sensitivity and specificity to detect Influenza type A, Influenza type B, RSV and Adenovirus.

Cross reactivity

No cross reactivity was detected against organisms that cause other respiratory infections:

Influenza type A (Strips B, C and D)	Influenza type B (Strips A, C and D)
Adenovirus (Strips A, B and C)	RSV (Strips A, B and D)

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

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