REF V17XX

RAPID TEST FLU A_B

Lateral Flow Test | IVD

For the detection of Influenza virus antigens in human nasal or nasopharyngeal specimen.

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A. and complies with the specifications on the Standard EN ISO 13485:2016

Use only the current version of Product Data Sheet enclosed with the kit.

Rapid Test FLU A_B, V17XX, is a qualitative Lateral Flow test for the detection of Influenza A/B antigens in nasal or nasopharyngeal specimen.

The Lateral flow kit contains all reagents required for the immunoassay method.

Specimen: Nasal or nasopharyngeal swab.

- For professional use only
- For in vitro diagnostic use only
- Rapid Test FLU A_B is a sensitive screening assay for the detection of Influenza virus. Results should not be used as the only source to diagnose or to determine infection status.
- Negative result do not rule out Influenza infection
- Test should only be conducted by medical personnel
- Test time (incubation time after specimens preparation): 15 min
- Shelf life: 2 years
- Storage: 4-30°C

1. Description

Rapid Test FLU A_B is a qualitative, lateral flow immunoassay designed to detect the presence or absence of Influenza virus antigens in nasal or nasopharyngeal swab specimens directly collected.

2. Principal of the assay

In this test, antibodies specific to Influenza type A nucleoproteins are coated on the test line region T_A of the nitrocellulose membrane. Antibodies specific to Influenza type B nucleoproteins are coated on the test line region T_B of the nitrocellulose membrane. During testing, antigens in the specimen react with the antibodies that are coated onto gold nanoparticles. The mixture migrates up the membrane to react with the antibodies immobilized on the membrane and generate a colored line in the test region T_A and or T_B. The presence of one of these colored lines 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.

3. Introduction

Influenza, commonly knows as "the flu", is an infectious respiratory disease caused by influenza viruses type A,B or C. It spreads from person to person through the air via coughs or sneezes, is highly contagious and most common symptoms are fever or chills, cough, sore throat, runny nose, headaches and fatigue. According to World Health Organization (WHO) influenza viruses cause seasonal flu epidemics each year during autumn and winter and can infect up to 20% of the population but once in a few decades a new flu strain could lead to pandemic. Seasonal influenza causes illnesses that range in severity and sometimes lead to hospitalization and death. Most people recover from fever and other symptoms within a week without requiring medical attention. However, influenza can cause severe illness or death, particularly among high risk groups including the very young, the elderly, pregnant women, health workers, immunocompromised people and people with chronic underlying medical conditions.

4. Reagents Provided

	V1701	V1705	V1720
Reaction device	1	5	20
Prefilled extraction tube with Running Buffer	1	5	20
Sterile swabs	1	5	20
Carton case	-	-	1
Positive control*	-	-	1
Negative control*	-	-	1
Instruction of use	1	1	1

5. Materials required but not provided

- Clock or Timer
- Gloves and face protection shield
- Container for biohazardous waste

*Note: positive and negative control in prefilled tube with purple screw cap.

6. Storage Instructions

Store kit components between 4 and 30°C (39.2 - 96°F). Do not freeze any components provided. Expiry of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated prior handling.

7. Safety and Precautions for use

7.1 Health and safety precautions

- Use gloves, protective clothing and eye/face protection and handle appropriately with the requisite Good Laboratory Practices. The product must only be used by qualified personnel.
- WASTE MANAGEMENT: Dispose of all specimens and materials used to perform the test as biohazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
- During the specimen collection human source material spills should be also treated as potentially infectious. Spills should be immediately decontaminated, including the spill area, materials and any contaminated surfaces, with an appropriate chemical disinfectant and should be wiped away.
- Do not re-use any of the kit components as they are single-use only.
- Sterile swabs must be used only for nasal or nasopharyngeal specimen. Avoid to touch the tip of the swab.
- Do not eat, drink or smoke in the area where the specimens and the kit are stored and handled.
- All positive results should be processed following local news and regulations.

7.2 Precautions related to the procedure

- In accordance with Article 1, Paragraph 2b of European Directive 98/79/EC, the use of in-vitro diagnostic medical devices is envisaged by the manufacturer to ensure the suitability, performance, and safety of these products. Consequently, the testing procedure, information, precautions, and warnings in the instructions for use must be followed rigorously. No changes to the test procedure are permitted, nor is any use in combination with other products not approved by the manufacturer. The user is solely responsible for any such changes. The manufacturer is not responsible for false results nor incidents arising as a result of these.
- Do not use the kit if the packaging of components is damaged, if there is an expired reagent or if the desiccant bag is absent inside the vial containing the sticks.
- All reagents should be warmed in room temperature before use.
- Cover or cap all reagents when not in use.
- Do not mix and interchange different specimens.
- Do not interchange individual reagents between kits of different lot numbers.

8. Specimen collection

8.1 Nasal Mid-Turbinate specimen collection

Tilt the patient's head back 70 degrees. Remove a sterile swab from the pouch. While gently rotating it, insert the swab less than one inch (about 2 cm) into patient's nostril (until resistance is met at the turbinates). Rotate the swab five times against the nasal wall then slowly remove from the nostril. Using the same swab repeat the collection procedure with the second nostril. (Figure 1.)

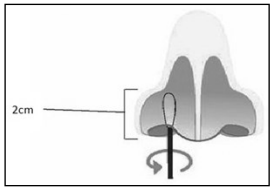


Figure 2. Nasal swab procedure

8.2 Nasopharyngeal specimen collection

Tilt the patient's head back 70 degrees. Remove a sterile swab from the pouch. Place the swab into one of the patient's nostrils. When it reaches the posterior nasopharynx rotate 3 to 5 times over the surface and then remove it slowly while rotating it (Figure 2.)

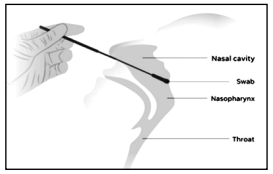


Figure 3. Nasopharyngeal swab procedure

9. Method Procedure

9.1 Calculate the number of swabbing sticks and tubes needed, according to the number of samples to collect.

9.2 After the specimen collection (see Chapter 8), place the swab in the extraction tube, rotate the swab forcefully against the side of the tube for 1min. Best results are obtained when the specimen is vigorously extracted in the solution.

9.3 Remove the swab, squeezing the sides of the tube to extract as much liquid as possible.

9.4 Discard the swab.

9.5 Close the extraction tube with the dropper cup. Add 3 drops in the circular window of the cassette.

9.6 After 15 minutes, the test stick can be visually read and interpreted according to the corresponding figure.

Note: The test result should not be read and interpreted after 30 minutes.

POSITIVE/ NEGATIVE CONTROL: add 2 drops directly form the prefilled tube in the circular window of the cassette

10. Interpretation of results

Note*: *For internal procedure purposes three colored lines are present on the result window of the Rapid Test FLU A_B. The colored lines have no effect on the product's performance since they are washed away during the experiment.*

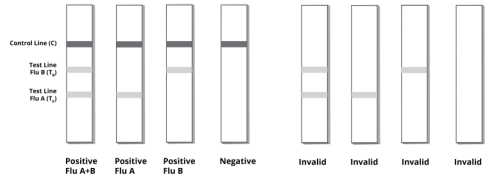
FLU A Positive: Two visible colored bands appear at both Test A (T_A) and Control (C) line. It indicates a positive result for the Influenza virus type A nucleoprotein antigen in the specimen.

FLU B Positive: Two visible colored bands appear at both Test B (T_B) and Control (C) line. It indicates a positive result for the Influenza virus type B nucleoprotein antigen in the specimen.

FLU A/B Positive: Three visible colored bands appear at both Test Lines T_A T_B and Control (C) line. It indicates a positive result for the Influenza virus type A and B antigens in the specimen.

Negative: One visible colored band appears at Control line. It indicates that the concentration of the Influenza antigen is zero or below the detection limit of the test.

Invalid: No colored band appears at Control line no matter whether it appears at Test lines or not.



Interpretation of results

11. Limitations

- The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
- After specimen collection the swab must be extracted as soon as possible. Otherwise they may be stored dry in their original packaging for up to 2 hours at RT. When using a viral transport medium, extract AGAIN the swab in the extraction tube that contains Prognosis Running Buffer. When using the transport media, the test sensitivity can be reduced due to excessive dilution of specimen.

  Note: the transport medium should not contain chaotropic substances such as Guanidinium thiocyanate.
- After the extraction specimens should be tested as soon as possible. Otherwise they can be stored at room temperature 20-25°C (68-77°F) for two hours.
- The test should be used for the detection of INFLUENZA A/B antigen ONLY in nasal or nasopharyngeal swab specimens.
- USE ONLY the sterile swabs that are provided in the kit for the specimen collection.
- During specimen collection avoid contact with bleeding areas and excess of mucus as both of them may give a false positive result due to interference with the test performance.
- Positive results indicate the presence of Influenza A/B antigens but a diagnosis of an infection should only be made by a physician evaluating all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- A negative test result may occur if the level of extracted antigen in a specimen is below the sensitivity of the test or if a poor quality specimen is obtained.
- Positive test results do not rule out co-infection with other pathogens.
- The Rapid Test FLU A_B will indicate the presence of Influenza A/B in the specimen from both viable and non-viable virus.

12. Immunoassay Performance

12.1 Cross-reactivity

In order to determine the cross reactivity of Rapid Test FLU A_B, an evaluation was performed; no cross reactivity against organism, pathogens that could cause infections was observed.

Microorganism	Concentration	Result
Adenovirus Type 1 (Species C)	2.57 x 10^8 $TCID_{50}$/mL	Negative
Adenovirus Type 3 (Species B)	3.39 x 10^7 $TCID_{50}$/mL	Negative
Adenovirus Type 7A (Species B)	1.02 x 10^8 $TCID_{50}$/mL	Negative
Alpha coronavirus 229E	4.68 x 10^4 $TCID_{50}$/mL	Negative
Alpha coronavirus NL63	1.70 x 10^5 $TCID_{50}$/mL	Negative
Beta coronavirus OC43	5.01 x 10^5 $TCID_{50}$/mL	Negative
Escherichia Coli O157	6.4x10^6 CFU/ml	Negative
Influenza A virus*	1.51 x 10^6 $TCID_{50}$/mL	Negative
Influenza B virus*	5.01 x 10^5 $TCID_{50}$/mL	Negative
Listeria monocytogenes	2.5x10^6 CFU/ml	Negative
Salmonella enteritidis	3.6x10^6 CFU/ml	Negative
SARS-Cov-2	1.15 x 10^7 $TCID_{50}$/mL	Negative
Streptococcus pneumococcal	4.2x10^6 CFU/ml	Negative
Streptococcus pyogenes	3.6x10^6 CFU/ml	Negative

Note No cross reaction was observed in Test line T_B when Influenza A virus was evaluated,

No cross reaction was observed in Test line T_A when Influenza B virus was evaluated

12.2 Interference Data

The following substances showed no significant interference on the test results of Rapid Test FLU A_B.

No	Interfering Substances	Final Test
1	Azithromycin	84 mg/ml
2	Amoxicillin	54 mg/L
3	Albuterol	0.05 mg/L
4	Acarbose	0.3 mg/L
5	Chlorpheniramine	0.8 mg/L
6	Chlorothiazide	27 mg/L
7	Rheumatoid factor	200 IU/ml
8	Triglycerides	1.5 mg/L
9	Hemoglobin	100 mg/L
10	Human Chorionic Gonadotropin Hormone (pregnancy)	10-fold dilution
11	Ibuprofen	219 mg/L
12	Xylometazoline (Otriven)	10%
13	Acetylsalicylic Acid	3 mg/ml
14	Mucin	0.5%

12.3 High Dose Hook Effect

No high dose hook effect was observed up to 1.51 x 10^6 $TCID_{50}$/mL of heat inactivated Influenza A virus and 5.01 x 10^5 $TCID_{50}$/mL of heat inactivated Influenza B virus, with the Rapid Test FLU A_B.

12.4 Limit of Detection

The lowest detectable concentration of an analyte in a method is known as LOD. In this case, serial dilution of heat inactivated viruses were used in order to determine the limit of detection in Rapid Test FLU A_B. The LOD is the level at which 95% of the replicates are characterized as positive. The results of 20 replicates are shown below.

LOD for Influenza A virus : 75.5 $TCID_{50}$/mL

LOD for Influenza B virus : 12.5*10^2 $TCID_{50}$/mL

INFLUENZA A

Concentration (TCID50 /ml)	Positive Replicates	Visual Interpretation of results
1.51 x 10^4	20 / 20	Strong positive
1.51 x 10^3	20 / 20	Strong positive
1.51 x 10^2	20 / 20	Positive
75.5	20 / 20	Positive
37.75	5 / 20	Negative

INFLUENZA B

Concentration (TCID50 /ml)	Positive Replicates	Visual Interpretation of results
5.01 x 10^4	20 / 20	Strong positive
5.01 x 10^3	20 / 20	Strong positive
2.5 x 10^3	20 / 20	Positive
12.5 x 10^2	20 / 20	Positive
6.25 x 10^2	1 / 20	Negative

12.5 Clinical performance characteristics
In order to determine the clinical performance of the Rapid Test FLU A_B, a total amount of 956 specimens both with nasopharyngeal and nasal specimen collection were tested.

12.5.1 Nasopharyngeal specimens
Influenza A
878 negative and 78 positive specimens for Influenza A (total amount 956) confirmed with RT-PCR assay [**SARS-CoV-2/Influenza A/B multiplex Real-TM (Sacace)]** were tested. The results are presented below.

Rapid Test FLU A_B	Real-time PCR		
	Positive A	*Negative*	*Total*
Positive	76	2	78
Negative	2	876	878
Total	78	878	956

Clinical Diagnostic Specificity: 99.77%
Clinical Diagnostic Sensitivity for Influenza A: 97.44%

Influenza B
943 negative and 13 positive specimens for Influenza B (total amount 956) confirmed with RT-PCR assay [**SARS-CoV-2/Influenza A/B multiplex Real-TM (Sacace)]** were tested. The results are presented below.

Rapid Test FLU A_B	Real-time PCR		
	Positive B	*Negative*	*Total*
Positive	13	3	16
Negative	0	940	940
Total	13	943	956

Clinical Diagnostic Specificity: 99.68%
Clinical Diagnostic Sensitivity for Influenza B: 100%

12.5.2 Nasal specimens
Influenza A
878 negative and 78 positive specimens for Influenza A (total amount 956) confirmed with RT-PCR assay [**SARS-CoV-2/Influenza A/B multiplex Real-TM (Sacace)]** were tested. The results are presented below.

Rapid Test FLU A_B	Real-time PCR		
	Positive A	*Negative*	*Total*
Positive	75	2	78
Negative	3	876	878
Total	78	878	956

Clinical Diagnostic Specificity: 99.77%
Clinical Diagnostic Sensitivity for Influenza A: 96.15%

Influenza B
943 negative and 13 positive specimens for Influenza B (total amount 956) confirmed with RT-PCR assay [**SARS-CoV-2/Influenza A/B multiplex Real-TM (Sacace)]** were tested. The results are presented below.

Rapid Test FLU A_B	Real-time PCR		
	Positive B	*Negative*	*Total*
Positive	13	3	16
Negative	0	940	940
Total	13	943	956

Clinical Diagnostic Specificity: 99.68%
Clinical Diagnostic Sensitivity for Influenza B: 100%

13. Method Summary
Total procedure time (after specimens preparation): 15 min.

Specimen collection (nasal or nasopharyngeal) according to specific instruction (chapter 8)

⇩

Insert the swab into the extraction tube and rotate it forcefully against the side of the tube for 1min

⇩

Remove the swab, squeezing the sides of the tube to extract as much liquid as possible

⇩

Close the extraction tube with the dropper cup. Add 3 drops in the circular window of the device.

⇩

(Wait 15 mins)
FLU A positive: Formation of 2 lines T_A and Control
FLU B positive: Formation of 2 lines T_B and Control
FLU A/B Positive: Formation of 3 lines T_A T_B and Control
Negative: Formation of Control line ONLY
Invalid: No Control line even if there is a line in T_A or T_B

14. References

1. Centers for Disease Control and Prevention. https://www.cdc.gov/flu/about/index.html
2. https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm.
3. Meng-Yi Han , et al. Evaluation of Lateral-Flow Assay for Rapid Detection of Influenza Virus./ BioMed Research International 2020;3969868 .
4. WHO recommendations on the use of rapid testing for influenza diagnosis, World Health Organisation, July 2005
5. L. Rainen, Collection, transport, preparation, and storage of specimens for molecular methods, 2005, August 2020, https://clsi.org/standards/products/molecular-diagnostics/documents/mm13/.

IVD In Vitro Diagnostics Medical Device
Σ_n Content sufficient for tests
REF Catalog number
Manufacturer
4° 30° Storage Conditions
Instructions for use
EXP Expiration Date

CE

VERSION 6/ 2023-04-13

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For the detection of Influenza A/B antigens in human nasal or nasopharyngeal specimen.

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The Lateral flow kit contains all reagents required for the immunoassay method.

Specimen: Nasal or nasopharyngeal swab.

The extracted specimen can be used with the kit codes V15XX, V16XX, V17XX, V18XX

- For professional use only.
- For in vitro diagnostic use only
- Rapid Test FLU A_B is a sensitive screening assay for the detection of Influenza A/B. Results should not be used as the only source to diagnose or to determine infection status.
- Negative result do not rule out INFLUENZA A/B infection
- Test should only be conducted by medical personnel
- Test time (incubation time after specimens preparation): 15 min
- Shelf life: 2 years
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www.prognosis-biotech.com
e: info@prognosis-biotech.com
t: +30 2410 623922
f: +30 700 700 6262
Farsalon 153 | 41335 Larissa, Greece

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