INFLU-A+B-RESPI-DIPSTICK

For *in Vitro* diagnostic use only

Immunochromatographic test strip for the detection of Respiratory Influenza A and B Viruses in nasopharyngeal secretions (swabs, washing or aspirates)

I. INTRODUCTION AND INTENDED USE

Influenza is a highly contagious viral infection of the upper respiratory tract that is characterized by the antigen variability, the seasonality and the impact on the general population.
Of the two main (A and B) types of influenza viruses, Influenza A subtypes are differentiated by antigens variability of the surface glycoproteins (haemagglutinin, H and neuraminidase, N). Influenza A virus is the most prevalent and is associated with the most serious epidemics. Influenza A has 3 subtypes which are important for humans: A(H3N2), A(H1N2) and A(H1N1), of which the former is currently associated with most deaths.
Influenza can cause severe complications such as bronchitis or pneumonia, particularly in children, elderly people or those with chronic respiratory disease. It is most often a mild viral infection transmitted by respiratory secretions through sneezing or coughing. There are many other viral infections that can mimic influenza, making laboratory tests necessary to distinguish it from other acute respiratory infections.
Virus isolation is still considered as the gold standard method for the Influenza diagnosis, with a sensitivity of nearly 100% after 3 days. Patients health care and economic costs can be greatly improved by the use of rapid, specific and sensitive antigen detection method in order to allow the use new antiviral treatments.
Influ-A+B-Respi-Dipstick is a non-invasive lateral flow assay for the detection of Respiratory Influenza A and B viruses in nasopharyngeal secretions.

II. PRINCIPLE

This is a ready-to-use qualitative immunochromatographic test based on lateral flow principle for detection of Influenza A and B viruses.The membrane is pre-coated with monoclonal antibodies against *Influenza A and B* antigens on the test line region. During testing, the sample reacts with the particle coated with anti-Influenza antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugate and generate coloured lines (one (A/B) or two (A or B) lines). A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS

Each kit contain:
1. Influ-A+B-Respi-Dipstick (25 test): tube containing 25 reactive strips and dissecant.
2. Dilution buffer (1 x 12,5 mL): saline solution buffered to pH 7,5, containing NaN_3 (< 0.1%), a detergent and charged proteins..
3. Instruction for use (1)

Required materials (not supplied)

Specimen collection container, Disposable gloves, Plastic pipettes, testing tubes or vials, Timer or clock.

IV. PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- The kit is for in vitro diagnosis only.
- Avoid touching the nitrocellulose with your fingers
- Wear gloves when handling the samples.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP
- Never use reagents from another lot.
- The tube containing the sensitized strips must be recapped as soon as the necessary number of strips for the operation has been removed, since the strips are sensitive to humidity. Make sure that the desiccant is present.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- 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.

V. STORAGE

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SAMPLES AND PREPARATION

Specimens to be tested should be obtained and handled by standard methods. The use of transport media has not been validated on the kit.

NASOPHARYNGEAL SWAB METHOD:

- Bend shaft to follow curve of nasopharynx. Insert swab through nostril to posterior nasopharynx.
- Rotate swab a few times to obtain infected cells.
- For an optimal sample, repeat procedure using other nostril.

NASOPHARYNGEAL ASPIRATE METHOD (SUCTION APPARATUS, STERILE SUCTION CATHETER):

- Instill several drops of solution saline into each nostril.
- Place catheter through nostril to posterior nasopharynx. Apply gentle suction. Using rotating motion, slowly withdraw catheter.
- For an optimal sample, repeat procedure using other nostril.

Send specimen to lab immediately (testing sensitivity decrease over time). Cool specimen to 2º-4ºC (36º-40ºF) during storage and transport.

VII. PROCEDURE

Allow the tests, samples and buffers to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open the pack until ready to perform the assay.

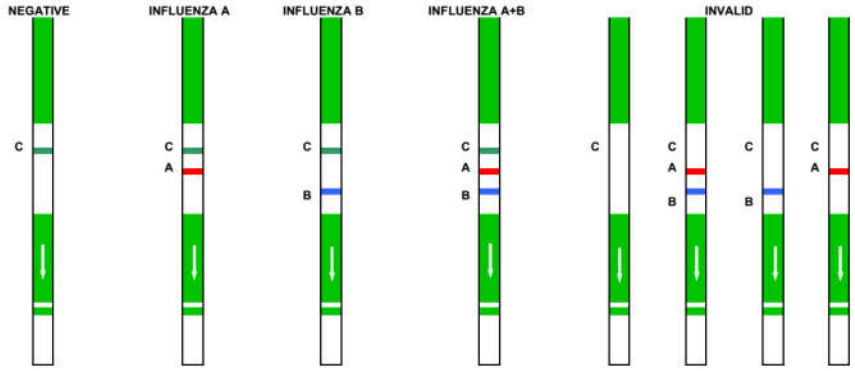
To process the collected nasopharyngeal wash or aspirate samples:

Use a separate pipette and testing tube for each sample. Add the nasopharyngeal wash or aspirate sample (6 drops or 300uL) in a testing tube or vial. Add the diluent Buffer (3 drops or 150uL) and mix. Extract some of the liquid and dispense 150uL in a new testing tube. Remove the Influenza A+B Strip from its sealed pack and use it as soon as possible. Leave the test strip to stand vertically taking care of not surpassing the limit of immersion indicated with the arrows. Start the timer. Read the result at 10 minutes after dispensing the sample.

To process the collected nasopharyngeal swab :
Use a separate testing tube or vial for each sample (swab). Add the diluent Buffer (10 drops or 500uL) into the testing tube or vial, put the nasopharyngeal swab, mix and extract as much liquid possible from the swab. Extract some of the liquid and dispense 150uL in a new testing tube. Remove the *Influenza A+B* Strip from its sealed pack and use it as soon as possible. Leave the test strip to stand vertically taking care of not surpassing the limit of immersion indicated with the arrows. Start the timer. Read the result at 10 minutes
To avoid diluting the latex conjugate in the solution, take care not to immerse the strip above the line placed under the arrows.

VIII. INTERPRETING THE RESULTS

Negative test: In the case only one line appears in the control line region (green control line).
Positive test: In the case two or three lines appears across the central window, in the Result Line Region (red/blue test line) and in the control line region (green control line).
Invalid test: The absence of the migration control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.



IX. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. A GREEN line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

X. PERFORMANCE CHARACTERISTICS

A. Sensitivity – Specificity - Correlation
Different virus extract preparation:
Influenza A/New Caledonia/20/99 (**H1N1) strain** (15 µg/mL hemagglutinin) Influenza A/ (**H2N2) strain** (15 µg/mL hemagglutinin)
Influenza A/Fujian/411/2002 (**H3N2) strain** (15 µg/mL hemagglutinin) Influenza A/ (**H7N7) strain** (15 µg/mL hemagglutinin)
Influenza B/Shanghai/**361/2002 strain (**15 µg/mL hemagglutinin)) Influenza A/ (**H9N2) strain** (15 µg/mL hemagglutinin)
was diluted in the sample diluent and tested (with 4 different lots) in accordance with the kit instructions for use.
We found that, under such conditions, the detection limit using the reference antigen preparation of Influenza A and B is **4.7 ng/mL HA for Influenza A and 18.75 ng/mL HA for Influenza B.**
The **correlation** has been conducted on 115 NPS swab samples in comparison with commercial rapid test (Quidel and Binax Now Influenza A&B) shows a 99% **Sensitivity** and 99 % **Specificity for** both Influ Aand B
An evaluation compared with *RT-PCR technique *resulted:
Sensitivity **(novel H1N1)** 67% Sensitivity **(seasonal flu)** 84%
B. Reproducibility: To check the intra-lot accuracy, one Influenza A positive sample and one Influenza B positive sample, and a dilution buffer solution (as negative control sample) have been tested 10 times on sticks of the same production lot in the same experimental conditions. All observed results were similar as expected.
To check the inter-lot accuracy, same samples (positive in Influenza A and in Influenza B and dilution buffer) were tested on three differents production lots. All results were similar as expected.
C. Interference: Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: Adenovirus, HSV, Parainfluenza, Enterovirus, Rhinovirus, Nocardia asteroides, Respiratory syncytial virus, Streptococcus pneumoniae, Moraxella catarrhalis, Streptococcus pyogenes, Aspergillus niger, Legionella pneumophila, Candida albicans, Haemophilus influenzae.

XI. LIMITS OF THE TEST

- The test must be carried out within 2 hours of opening the sealed pack.
- Samples containing blood or erythrocytes should be avoided for testing since they can lead to false positive.
- A positive result does not rule the possibility that other pathogens may be present.
- Kit results must be compared with all other available clinical and laboratory information.
- The kit is an acute-phase screening test. NPS specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold. If a sample is given a negative result despite the observed symptoms, a culture should be started to check the sample.

XII. BIBLIOGRAPHIC REFERENCES

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IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (25 tests) **Ref. VC1012**
Influ-A+B-Respi-Dipstick 25 items
Diluent buffer 1 x 12,5 mL
Instruction for use 1 item

EDMA (EDMS) CODE 15709090 CE IVD

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