Influenza A identification FITC-MAb

For *in vitro* diagnostic use

IFMINFA: Staining Kit for investigation of influenza A by immunofluorescence technique in cell culture and clinical samples. 50 tests.

INTRODUCTION:

Influenza viruses are unique among the viruses with regard to their antigenic variability, seasonality, and impact on the general population. They can cause explosive outbreaks of febrile respiratory illnesses across all age groups and often substantial mortality, particularly in elderly and chronically ill patients.

The influenza A, B and C virus types were defined originally. Subtypes occur only among the influenza A viruses. This subtyping system lies on the variability of the hemagglutinin (HA) and the neuraminidase (NA) genes. Three HA subtypes (H1, H2, and H3) and 2 NA subtypes (N1 and N2) are known to have caused extensive outbreaks in humans.

The onset of influenza activity ranges from October to April in the northern hemisphere but usually peaks between December and March. Influenza typically peaks between May and August in the southern hemisphere.

Successive or overlapping waves of infection by different influenza A subtypes and influenza B virus may cause prolonged influenza activity. Influenza B viruses cause widespread epidemics every 3 to 4 years, but co-circulation of two or three influenza A viruses (H3 and H1) may occur within a single season. Influenza C virus usually represents <1% of viruses.

Influenza viruses can be readily isolated early in illness from a variety of respiratory specimens, including nose and throat swabs, nasal aspirates or washes, sputum, and tracheal aspirates. Throat swabs or washings contain lower virus concentration and are usually less sensitive.

Influenza infection can be diagnosed in the laboratory by direct methods (antigen direct detection in clinical samples, culture techniques or nucleic acid tests) or by serological tests to measure the presence of IgM or the increase of IgG titers. Several continuous epithelial cell lines, particularly MDCK are useful for primary isolation. MDCK cells that are stably transfected to overexpress α-2,6-linked sialic acid receptors appear to be useful for both in vitro susceptibility testing and isolation of virus from clinical specimens.

PRINCIPLE OF THE TEST:

The inmunofluorescence method is based upon the reaction of specific antibodies (MAb) contained in the kit with the antigen in the sample. The antibodies react with the antigen, and the unbound immunoglobulins are removed in the washing step. The antigen-antibody complexes are visualized with the aid of the fluorescein conjugated to the antibody.

KIT FEATURES:

All reagents are supplied ready to use. All the reagents have a number assigned for an easy identification. In the Assay Procedure, the numbers of the reagents to be used in each step are indicated.

KIT CONTENTS:

12 VIRCELL InfA IDENTIFICATION FITC-MAb (ref. IFMINFA): One 2 ml dropper vial of FITC-labelled monoclonal antibody specific for influenza A nucleoprotein diluted in phosphate buffered saline containing bovine albumin, sodium azide and Evans blue as counterstain.

Store at 2-8ºC and check expiration date.

Materials required but not included in the kit.

-Adequate precision micropipettes.
-Centrifuge with swinging rotor giving at least 1500 g.
-Thermostatized incubator.
-Deionized water.
-Vortex.
-Acetone.
-Humid chamber.
-Fluorescence microscope and suitable filters according to the manufacturer´s recommendations.

In the case of performing cell culture samples, biological reagents, chemicals and equipment for inoculation and staining of cell cultures are required.

CONSERVATION:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. Kits are stable through the expiration date when stored closed and at the temperature indicated.

STORAGE OF REAGENTS ONCE OPENED:

Reagents	Stability
MAb	Refer to package label for expiration date (at 2-8ºC)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Use only the amount of reagents required for the test. Do not return the excess solution into the bottles.

VIRCELL SL does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For in vitro diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the VIRCELL PBS and VIRCELL MOUNTING MEDIUM solutions are compatible with the equivalents from other VIRCELL MAb references and lots. The rest of the components are compatible with other kits when the lot is the same.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.

7. The VIRCELL FITC-MAb in this kit includes substances of animal origin. VIRCELL CONTROL contains fixed antigens. The samples processed may contain infectious microorganisms; VIRCELL CONTROL and patient specimens should be handled as potentially infectious. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed of as potentially infectious. Observe the local regulations for clinical waste disposal.
8. VIRCELL FITC-MAb and VIRCELL MOUNTING MEDIUM contain sodium azide (concentration <0.1%). Avoid contact with acids and heavy metals.
9. VIRCELL MOUNTING MEDIUM contains glycerol. Avoid contact with acids and keep away from high temperatures.
10. Evan´s blue (concentration <0.1%) is a carcinogen. Avoid contact with skin or eyes. In case of contact with this solution, rinse thoroughly with water and seek medical attention.
11. Use only protocols described in this insert. Incubation times and temperatures other than specified may give erroneous results.
12. Cross-contamination of patient specimens on a slide can cause erroneous results. Take precautions to avoid it.
13. Microscope optics, light source condition and type will affect the fluorescence quality.
14. Do not leave the reagents at room temperature longer than absolutely necessary.
15. Each slide can be use only once. Do not break it, and do not reuse the wells not used.
16. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents are supplied ready to use. Only the VIRCELL PBS 2 included in the kit Respiratory viral screening & identification MAb must be prepared in advance. Add the contents of the vial 2 to 1.5 litres of deionized water. Shake it until the complete dissolution. Once diluted, store at 2-8ºC.

METHOD OF PREPARING CELL CULTURES FOR STAINING:

- **TRADITIONAL CULTURE TECHNIQUE**

1. Incubate at 37ºC until scheduled for use. Examine cell cultures immediately before inoculating the samples to verify that the morphology is adequate.
2. Remove the culture medium.
3. Add 100-200 µl of the sample investigated.
4. Incubate at 37ºC for 1 hour.
5. Remove the inoculums and add 2 ml of MEM containing 2% of FBS and incubate at 37ºC, examining from time to time.
6. In the event of appearance of CPE or after 7 days incubation, break the monolayer with a Pasteur pipette.
7. Wash twice with PBS centrifuge at 1000 g.
8. Prepare smears with 25 µl of sediment in multi-well slides.
9. Air-dry the slides completely at room temperature.
10. Fix the cells by immersing the slides in acetone for 10 minutes at 2-8ºC.
11. Remove the slides from acetone and allow them to air-dry completely at room temperature.
12. Slides may be stored for 2 to 3 days at 2-8ºC in an airtight container with desiccant or at temperature below –20ºC for longer storage. When removing the slide from storage, allow them to equilibrate at room temperature before opening the storage container.

- **SHELL VIAL TECHNIQUE**

1. Incubate at 37ºC until scheduled for use. Examine cell cultures immediately before inoculating the samples to verify that the morphology is adequate.
2. Remove the culture medium and
3. Add 200 µl of the sample investigated.
4. Centrifuge the tube at 700 g for 45 minutes.
5. Incubate at 37ºC for 1 hour.
6. Remove the inoculums and add 1 ml of MEM containing 2% of FBS.
7. Incubate24-48 hours at 37ºC.
8. Remove the culture medium.
9. Remove the cover slip by punching the bottom of the vial with a red-hot needle. Pick the cover slip with forceps (be careful not to damage the cell monolayer) and air-dry it with a dryer.
10. Adhere to a slide with DPX with the cell monolayer facing upwards (the cell-containing side appears opaque under light). Press slightly the cover slip against the slide with the help of a pipette tip to avoid bubbles.
11. Fix the cells by immersing the slides in acetone for 10 minutes at 2-8ºC.
12. Remove the slides from acetone and allow them to air-dry completely at room temperature.
13. Slides may be stored for 2 to 3 days at 2-8ºC in an airtight container with desiccant or at temperature below –20ºC for longer storage. When removing the slide from storage, allow them to equilibrate to room temperature before opening the storage container.

METHOD OF PREPARING SAMPLES FOR STAINING:

The samples for direct detection may be nasopharyngeal swabs, pharyngeal swabs, bronchoalveolar lavage, bronchial wash and nasopharyngeal aspirate.
Swabs specimen should be placed into 1 to 2 ml of transport medium. Do not prepare slides from dried swabs.
Fluid specimen should be placed into a sterile container.
If the samples are not processed immediately, store at 2 ° C to 8 ° C up to 48 hours. For longer periods store at -70 º C and -90 º C.

- **ASPIRATES OR FLUIDS CONTAINING CELLS**

1. Transfer specimen to a conical centrifuge tube and centrifuge at 300 to 500 g for 10 minutes at 2-8ºC.
2. Remove and discard the supernatant, including the mucus that may be overlaying the cell pellet.
3. Tap the tube gently to loosen the cells, add PBS, and suspend the cells by gently pipetting up and down.
4. Repeat the centrifugation step.
5. Remove and discard the supernatant
6. Tap the tube and add enough PBS to yield an opalescent cell suspension.
7. Deliver approximately 25 µl of the cell suspension in one well of a multi-well slide and look under the microscope to ensure that the cell concentration is adequate.
8. Air-dry the slides completely at room temperature.
9. Fix the cells by immersing the slides in acetone for 10 minutes at 2-8ºC.
10. Remove the slides from acetone and allow them to air dry completely at room temperature.
11. Slides may be stored for 2 to 3 days at 2-8ºC in an airtight container with desiccant or at temperature below –20ºC for longer storage. When removing the slide from storage, allow

them to equilibrate to room temperature before opening the storage container.

• **SWAB SPECIMENS**

1. These samples should be transported in transport medium. Vortex the tube containing the swab in order to release cells.
2. Transfer specimen to a conical centrifuge tube and centrifuge at 300 to 500 g for 10 minutes at 2-8ºC.
3. Remove and discard the supernatant, including the mucus that may be overlaying the cell pellet.
4. Tap the tube gently to loosen the cells, add PBS, and suspend the cells by gently pipetting up and down.
5. Repeat the centrifugation step.
6. Remove and discard the supernatant
7. Tap the tube and add enough PBS to yield an opalescent cell suspension.
8. Deliver approximately 25 µl of the cell suspension in one well of a multi-well slide and look under the microscope to ensure that the cell concentration is adequate.
9. Air-dry the slides completely at room temperature.
10. Fix the cells by immersing the slide in acetone for 10 minutes at 2-8ºC.
11. Remove the slides from acetone and allow them to air dry completely at room temperature.
12. Slides may be stored for 2 to 3 days at 2-8ºC in an airtight container with desiccant or at temperature below –20ºC for longer storage. When removing the slide from storage, allow them to equilibrate to room temperature before opening the storage container.

STAINING PROCEDURE

1. Add a drop of VIRCELL FITC-MAb **11**, **12**, **13**, **14**, **15**, **16** or **17** for identification of each well or coverslips in the shell-vials technique. Ensure that the reagent covers the entire cell surface.
2. Incubate for 30 minutes at 36-38ºC in a humid chamber.
3. Rinse slide briefly with a gentle stream of PBS (avoid directing PBS at wells) and immerse for ten minutes in PBS. Dip wash the slide briefly in distilled water.
4. Dry and add a drop of VIRCELL MOUNTING MEDIUM **6**.
5. Place a coverslip and press slightly to avoid bubbles.
6. View under the fluorescence microscope at 400 x.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal Quality Control testing before release.

VALIDATION PROTOCOL FOR USERS:

Positive and negative controls should be included to test the correct performance each time a new kit is opened. It allows the validation of the assay and kit.
The observed fluorescence pattern should be:
Positive control: characteristic apple-green fluorescence in the whole cell.
Negative control: Red cellular pattern.

INTERPRETATION OF RESULTS:

A positive sample is one in which two or more intact cells display a characteristic apple-green fluorescence pattern with an intensity equal or over 2+.
A negative sample is one which contains an appropriate number of typical cells (at least 20) and all of them display minimum fluorescence (<1) or absence of fluorescence (0).
Those samples with weak staining and/or a single positive cell should be considered uncertain.
An invalid sample is one which presents a strong non-specific staining or has not enough intact cells.
The samples must contain several cells in various 100x fields.

LIMITATIONS:

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
2. A negative result does not exclude an infection.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.
4. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

• **SENSITIVITY AND SPECIFICITY**

48 samples characterized against another commercial available kit and 52 strains from Collections of microorganisms were assayed with VIRCELL VIRAL SCREENING FITC-MAb .The results were as follows:

Samples no.	Sensitivity	Specificity
100	96%	100%

52 strains from Collections of microorganisms were assayed with each one of the identification reagents included in the kit Respiratory viral screening & identification MAb. The results were as follows:

VIRCELL IDENTIFICATION REAGENTS	Sensitivity	Specificity
AdV	100%	100%
InfA	100%	100%
InfB	100%	97%
PIV1	100%	100%
PIV2	100%	100%
PIV3	100%	100%
RSV	100%	100%

27 samples characterized against another commercial available kit were assayed with Respiratory viral screening & identification MAb in an external laboratory. The results were as follows:

Sensitivity	Specificity	Concordance
100%	100%	100%

• **INTRA-ASSAY PRECISION**

9 samples were tested 5 times in a single assay performed by the same operator in essentially unchanged conditions.
Equivalent results were observed in all the assays.

• **INTER-ASSAY PRECISION**

9 samples were individually tested on 5 consecutive days by 2 different operators.
Equivalent results were observed in all the assays.

• **CROSS REACTIVITY AND INTERFERENCES**

16 samples known to be positive for other members of the syndromic group (*Bordetella pertussis, Bordetella parapertussis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium fortuitum, Mycobacterium intracellulare, Mycobacterium kansasii, Mycobacterium tuberculosis, Chlamydophila pneumoniae, Chlamydophila psittaci, Chlamydia trachomatis, Coxiella burnetii, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Streptococcus pneumoniae*) were assayed.

The negative results of the test demonstrate the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
Y°C / X°C	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

LITERATURE:

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