

## Isolation of Influenza Virus in Human Lung Embryonated Fibroblast Cells (MRC-5) from Clinical Samples

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**Ninety-four pharyngeal swab samples corresponding to 94 patients with suspected influenza virus infection were inoculated in Madin-Darby canine kidney (MDCK) cells, the conventional cell system for the isolation of influenza virus, and in fibroblastic human embryo lung (MRC-5) cells, a cell system less commonly used for this purpose but one frequently used in clinical virology laboratories. Both cell preparations were treated with trypsin. Influenza virus was recovered from 15% of the samples inoculated in MDCK cells and from 18% of those inoculated in MRC-5 cells. The use of MRC-5 cells can simplify the search for respiratory viruses and would assist in the rapid detection of influenza virus during new epidemics.**

Laboratory diagnosis of patients with influenza virus infection early in the season of influenza virus infection provides advance warning that influenza virus is spreading within the community and may foreshadow an epidemic. Diagnosis is thus important for supporting continuing vaccination activities as well as permitting the rational use of antiviral agents such as amantadine and rimantadine. In addition, specific laboratory diagnosis of influenza virus by isolation and identification both during and outside of epidemic periods contributes to international surveillance efforts and improves the prospect of incorporating the correct strains of virus into vaccines developed in the future.

Characterization and isolation of the causal agents of human influenza A virus infection were achieved by Smith et al. (12) by isolation of the virus in ferrets in 1933. Influenza B virus was isolated by Francis (3) in 1936, and influenza C virus was isolated by Taylor (15) in 1950.

The discovery by Burnet (1), in 1936, that influenza virus could grow in embryonated hens' eggs allowed study of the properties of the virus and permitted the development of inactivated vaccines. The egg procedure is still used in virology reference laboratories, but it is slow and troublesome.

Rapid techniques used for the diagnosis of influenza virus infection are based on the direct detection of viral antigen in clinical specimens (16). Direct immunofluorescence on respiratory cells is the rapid test most frequently used (7, 14); Directigen Flu-A (Becton-Dickinson, Cockeysville, Md.) is an enzyme immunoassay which allows, in about 15 min, the detection of the influenza A virus nucleoprotein antigen directly from specimens after passive adsorption on a cellulose membrane (2, 4). However, these methods, although more rapid than culture, may have limited sensitivity and their results may be difficult to interpret, depending on the quality of the specimen (5, 10). Molecular techniques for the rapid detection and amplification of the viral genome can be used, but their routine application for the diagnosis of influenza virus infection requires further development (9, 13).

The isolation of influenza virus strains in tissue culture is still

required for an accurate diagnosis and to recognize recent antigenic variations which may direct future influenza virus vaccine compositions.

A variety of cell lines have proved to be useful for the recovery of influenza viruses from clinical specimens (11). The cells most commonly used include primary monkey kidney, primary human embryo kidney, primary chicken kidney, and continuous Madin-Darby canine kidney (MDCK) cells plus embryonated hens' eggs.

During the last epidemiologic yearly cycle, we tried to isolate influenza virus in our laboratory using fibroblastic human embryo lung (MRC-5) cells (Biomérieux, Lyon, France), the most common cell line routinely used in clinical virology laboratories. From November 1993 to February 1994, 94 pharyngeal swab samples corresponding to 94 patients (54 females and 40 males; average age,  $32.43 \pm 16.61$  years) who presented to a primary health care center with fever, myalgia, and/or respiratory symptoms were sent to the Virology Laboratory of Hospital Covadonga.

The pharyngeal swab specimens were collected in 2 ml of viral transport medium (Viral Transport Medium; Cells Matic; Difco) and were sent quickly to the laboratory where they were stored at 4°C. All specimens were processed during the next 24 h. The specimens were decontaminated before their inoculation by adding penicillin (200 U/ml), streptomycin (200 µg/ml), and amphotericin B (200 µg/ml). After decontamination, 200 µl of each sample was inoculated onto the following cell monolayers: one conventional culture (CC) tube with MDCK cells, one CC tube with MRC-5 cells, and two shell vial cultures (SVCs) with MRC-5 cells. The MDCK cells were obtained from the Spanish Center of the World Health Organization (WHO) (Majadahonda, Madrid) and were harvested as a monolayer in our laboratory. The MRC-5 cells were provided by Biomérieux in suspension, and conventional tubes and shell vial monolayers were prepared. The samples were allowed to adsorb for 1 h at 37°C and 5% CO<sub>2</sub> for CCs and underwent 45 min of centrifugation at  $300 \times g$  at 22°C for SVCs. Later, the samples were removed and inoculation medium was added. This medium was minimum essential medium with Earle's balanced salt solution (Biowhittaker, Verviers, Belgium) supplemented with trypsin (0.25 µg/ml), L-glutamine (2 mM), penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml) without bovine fetal serum. The

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TABLE 1. Comparison of recoveries of influenza virus from MDCK and MRC-5 cell lines

Result on MRC-5 cells	MDCK CC result (no. of specimens)	
	Positive	Negative
CC positive, SVC positive	3	2
CC negative, SVC positive	6	6
CC positive, SVC negative	2	
CC negative, SVC negative	3	72

trypsin is a proteolytic enzyme used to enhance the attachment of influenza virus to a cell monolayer (11).

MDCK cell cultures were incubated for 4 days at 37°C in a 5% CO<sub>2</sub> environment; 4 days is the time necessary for the isolation of influenza virus according to previous experience (8). MRC-5 cells in CCs were incubated for 7 days to allow an extended observation period. MRC-5 cells in SVCs were examined at 3 days, the time used in our laboratory for the recovery of other myxoviruses. After these times, the CCs were scraped and put into two different wells of a slide. Monoclonal antibody against influenza A virus (clone IA52) was used to stain one well of the slide and shell vial, while antibody against influenza B virus (clone IB82) (Sanofi-Pasteur, Marnes-La Coquette, France) was used to stain the second well and shell vial of each culture.

Influenza A virus was recovered from 22 samples (23.4%), obtained in two outbreaks that had occurred at the end of November (12 samples) and the end of December (10 samples). Influenza A virus was recovered from 17 of 22 positive samples (77.3%) on MRC-5 cell SVCs, from 14 samples (63.6%) on MDCK cell CCs, and from 7 samples (33.9%) on MRC-5 cell CCs. Virus in eight of the specimens was recovered only from MRC-5 cells, and virus in three specimens was recovered only from MDCK cells (Table 1).

The isolated viruses were sent to the Spanish Center of WHO, where they were serotyped as influenza virus A/X/Oviedo/93 (H3N2). These strains were also sent to the National WHO Collaboration Center for Influenza in London. They were found to be similar to influenza virus A/Beijing/32/92 and influenza virus A/Shandong/9/93.

The present study demonstrated that influenza virus can be detected and isolated in MRC-5 cell monolayers treated with trypsin. These mammalian cells had been used before for the study of the amino acid sequence of HA1 in influenza A virus (H3N2) grown in mammalian and primary chicken kidney cells, but not for the routine primary isolation of influenza virus from clinical samples (6).

Our results suggest that SVCs with MRC-5 cells complement CC for the rapid detection of influenza virus and allows detection of infections that might otherwise be missed in conventional tubes. However, we do not imply that SVC with MRC-5 cells should replace CC. Other investigators who used SVCs with LLC-MK2 cells found advantages similar to those

that we found when they compared the SVC technique with conventional cell culture (5). The MRC-5 SVC allows the recovery of influenza virus from respiratory samples submitted for the isolation of respiratory viruses in general. This cell line thus provides the advantage of being able to detect influenza virus during the early stages of possible epidemic spread.

We would like to emphasize here that the technique described above could be implemented in most hospital virology laboratories, because MRC-5 cells are the cell line most widely used in the laboratory for the isolation of clinically important viruses. The use of MDCK and MRC-5 cells appears to be adequate for the recovery of the most clinically important respiratory viruses (including parainfluenza viruses, adenovirus, respiratory syncytial virus, and influenza viruses) and could simplify the search for those viruses in cases in which different kind of cells (HEp-2, Vero, etc.) would have been required.

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