

# VIRAL DIAGNOSIS IN ACUTE RESPIRATORY INFECTIONS USING THE INDIRECT IMMUNOFLUORESCENCE TECHNIQUE

Pages with reference to book, From 280 To 284

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## Abstract

From September 1984 to February 1985 a total of three hundred and five specimens of nasopharyngeal secretion (NPS) were examined by the indirect immuno-fluorescence technique to determine the frequency of infections caused by the respiratory syncytial virus (RSV), influenza A, influenza B and parainfluenza viruses, 1 and 3. The specimens were collected from infants and children less than 5 years of age visiting the hospital with acute respiratory infections. Standardized techniques and quality controlled reagents were used. At least one of the above viruses was detected in 70 specimens (23.0%); RSV in 50, influenza A in 7, influenza B in 4, parainfluenza type 1 in 3 and parainfluenza type 3 in 6 specimens (JPMA 37: 280 , 1987).

## INTRODUCTION

Acute respiratory diseases are common in all age groups especially in young children thus accounting for large number of admissions to hospitals. Accurate assessment of the responsible organisms in acute respiratory infections can be important for a number of reasons including patient management, understanding of pathogenesis and epidemiology, antibiotic usage and development of appropriate vaccines.

The majority of these infections are of viral etiology<sup>2</sup> and laboratory diagnosis of viral respiratory diseases has been based traditionally on the virus isolation from throat washings or the detection of a significant rise in specific antibodies. Classical laboratory techniques currently used for virus isolation though specific and sensitive are often tedious, time consuming, difficult, expensive and in most laboratories, impossible to perform. Serological diagnosis usually requires two serum samples taken 2-3 weeks apart to allow for detection of immune response. Techniques developed in recent years are based on the demonstration of specific viral antigens within a few hours of specimen collection. These are fluorescent antibody technique, electron microscopy, various enzyme techniques and radio immunoassay. However, for the detection of respiratory viral antigens the most appropriate methods are fluorescent antibody technique FAT<sup>3</sup> and enzyme linked immunosorbant assay, ELISA<sup>4,5</sup>

FAT is the method of choice for the rapid laboratory diagnosis of respiratory viral infections<sup>6</sup> It is sensitive, specific and relatively inexpensive and the reagents have long shelf lives. The test itself does not present many technical difficulties. However, the selection and preparation of specimens for the test are of great importance for obtaining reliable results. Nasopharyngeal secretions offer the best material and the proper collection of these specimens should yield intact cells containing the viral antigens. For older patients nasal or throat washings or even sputum may be used but the throat swabs have been found unsuitable. Elution of cough and nasal swabs yield suitable intact cells but the sensitivity is lower than that of nasopharyngeal secretions.

## MATERIAL AND METHODS

NPS were collected from children under 5 years of age visiting the hospitals (Rawalpindi General Hospital and Central Government Islamabad Poly Clinic) because of an acute respiratory infection. Specimens were collected in the hospital by the medical officer especially trained in this field. They were then transported in cold (+4 C) to National Institute of Health, Islamabad, within two hours of collection where they were processed without delay. The cells from the secretions were washed in buffer (PBS, pH 7.2) and smears were prepared on multiwell slides (Flow) according to a standard technique<sup>7</sup>. Two slides with six smears on each were prepared from each specimen. The smears were air dried and fixed in acetone for 10 minutes at +4°C.

One 6-spots slide was stained immediately while the other was stored at -20°C for reference. The slides were stained by indirect immunofluorescence technique for the following five viruses: RSV, influenza A, influenza B, para influenza 1 and 3. Calf immune sera against RSV, influenza A and para-influenza 3; chick immune sera against influenza B and para-influenza 1 and fluorescence isothiocyanate conjugated antiserum and antichick were obtained from Wellcome Reagents. All these reagents were standardised and quality checked by Dr. Monica Grandien, National Bacteriological Laboratory, Stockholm, Sweden.

For counterstaining, Evan's blue 1:30,000 dilution was used. Final rinsing of the stained slides was done in distilled water and then mounted in buffer glycerol and saline (90% pure glycerol mixed with 10% phosphate buffer saline pH 7.2-7.5). The mounted preparation can be kept for 2-3 days at +4 C.

## **RESULTS**

A total of 305 specimens were examined for RSV, influenza A, influenza B, para-influenza type 1 and para-influenza type 3.

Each slide was viewed using fluorescence microscope by a person trained in this field. An objective of 20x was used for screening and 40x for confirmation. A typical bright fluorescence in the cytoplasm with a dark nucleus of the epithelial cells, in either well treated with antiserum was considered positive. However, in influenza A virus infection both nuclear and cytoplasmic fluorescence was noted. Only intact cells were examined for fluorescence. The positive cells found were predominantly ciliated epithelial cells. It was observed that in RSV infections the number of infective cells were greater while fewer were present in influenza A infections (Figure 1, 2).

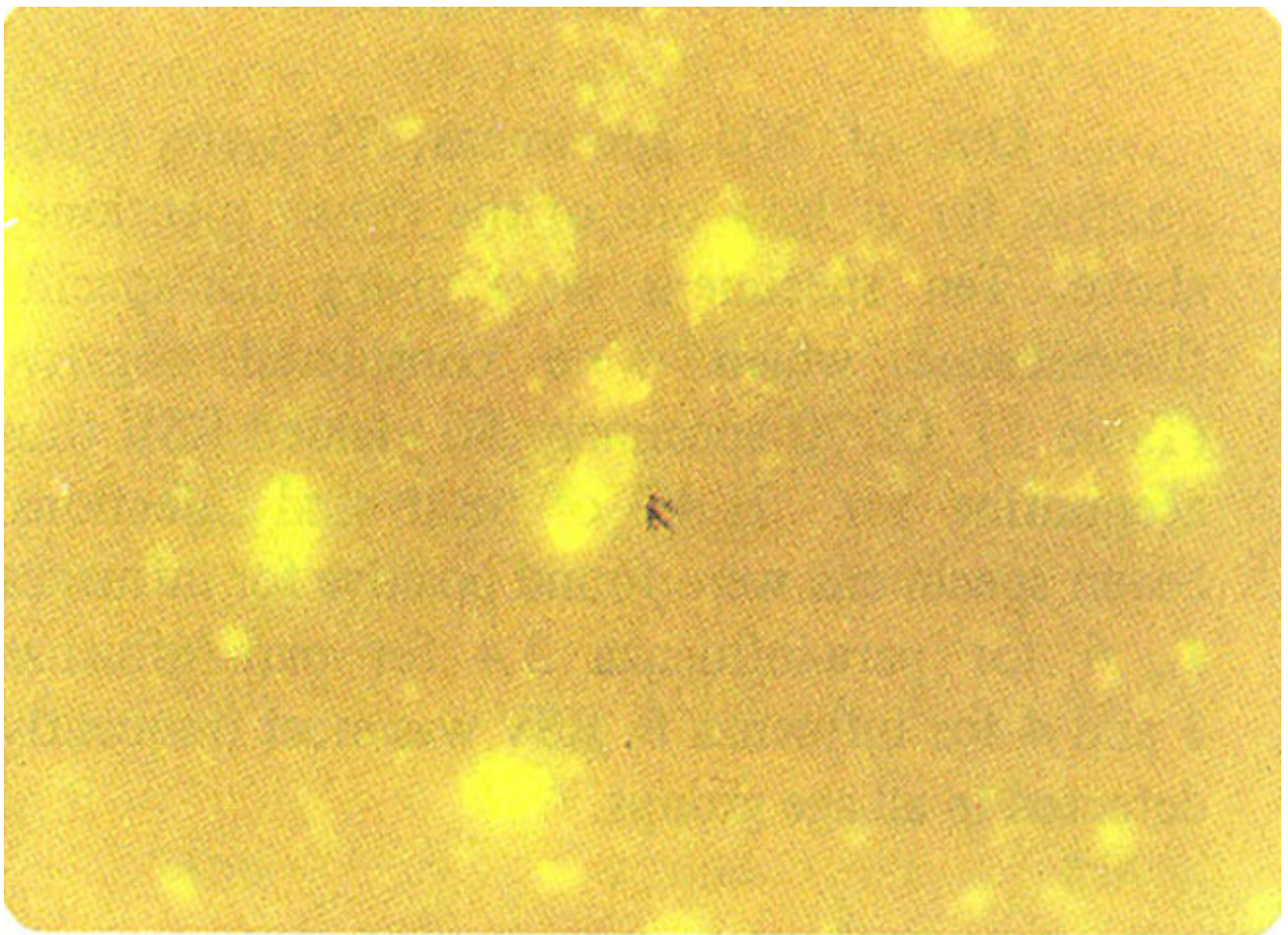
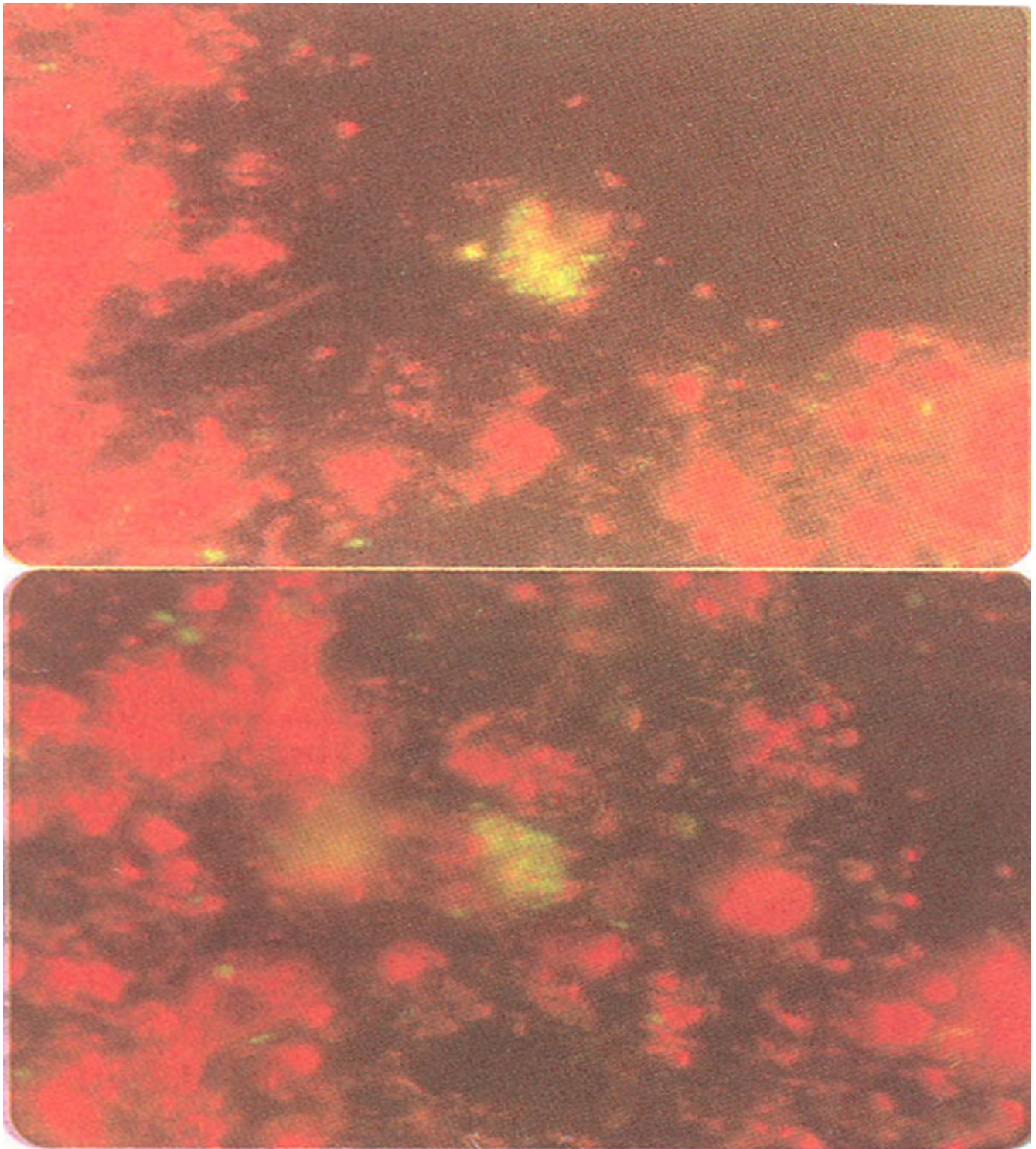


Figure 1. RS Virus antigen in a cell of secretion taken from a child with acute respiratory illness. Note the cytoplasmic fluorescence staining and dark nucleus without stain. Five infective cells can be seen in this field.



**Figure 2. Influenza A antigen in a cell from NPS from a child with acute respiratory illness. Note nuclear and cytoplasmic fluorescence.**

Out of 305 specimens, 75 were found positive for at least one of the viruses mentioned above. Ten specimens were giving non-specific fluorescence which were considered negative (Table 1).

**TABLE – 1**  
**Results of Fluorescent Microscopic Examination**  
**of 305 NPS.\***

Type of Fluorescence observed	No. of Specimens	% age
Specific intracellular fluorescence	70	23%
Non-specific fluorescence	10	3.3%
Negative for fluorescence	225	73.7%
Total	305	100%

**\* Nasopharyngeal Secretions.**

Of 70 specimens 50 yielded RSV thus accounting for 71.4% of total viral infections. Seven specimens were found positive for influenza A, 6 for para-influenza 3, 4 for para-influenza 1 and 4 for influenza B, RSV was most frequently detected in all age groups.

**DISCUSSION**

The fluorescent antibody technique currently may be considered the method of choice for the rapid diagnosis of respiratory virus infections. The results can be sent to the physician on the same day of specimen collection since the average time for slide preparation, staining and reading was not more than 3 hours. Fluorescence antibody technique has been found specific, sensitive and relatively inexpensive and also the results of this technique are reported satisfactory when compared with those of cell culture<sup>8-10</sup>

In our study we preferred indirect immunofluorescence technique since it is more sensitive as compared to the direct one<sup>11,12</sup> and also instead of using several different conjugates which all need repeated standardization and controls, only two fluorescence isothiocyanate (antibovine and antichick) conjugates were used. A direct conjugate may be used if only one virus is to be investigated.

Nasopharyngeal secretions for examination were preferred since other materials from respiratory tract have lower cell content and autolysis may occur earlier thus diminishing the intact mucosal epithelial cells. However, sputum, nasal wash, tracheal and bronchial secretions and lung tissue may all be used for investigation by immunofluorescent technique as recommended by WHO.<sup>6</sup>

In this study the performance of the test did not present any difficulty with the exception of non-specific fluorescence of cellular debris and bacteria adherent to epithelial cells in some of the slides (Table 1). Anyhow, this problem can be overcome by using monoclonal instead of poly clonal antibodies against virus antigens.<sup>13-15</sup>

Accurate assessment of the responsible organisms in acute respiratory infections may be valuable to the physician for a number of reasons including patient management, understanding of pathogenesis and epidemiology, development of vaccines and antibiotic usage. Although cell cultures can provide a

presumptive diagnosis of respiratory viruses within few days, most hospitals lack these facilities of viral laboratory so FAT is the method of choice for rapid laboratory diagnosis.

From this study it is concluded that viruses play a great role in the etiology of acute respiratory diseases in children under five years of age, RSV being predominantly detected in all age groups. These results (Table II)

**TABLE – 1**  
**Results of Fluorescent Microscopic Examination**  
**of 305 NPS.\***

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Non-specific fluorescence	10	3.3%
Negative for fluorescence	225	73.7%
Total	305	100%

**\* Nasopharyngeal Secretions.**

are comparable with studies done in the developed countries<sup>16-18</sup> thus showing the Importance of respiratory syncytial virus as a cause of serious acute respiratory illness in young children. Rapid diagnosis of RSV may eliminate unnecessary antibiotic use and the possibility of treating severe infections with ribovirin<sup>19</sup> or other antiviral agents.

Influenza A virus was positive in only eight specimens. The study period shows that most of these specimens were collected during the winter. A low isolation for influenza A (Table III)

**TABLE – III**  
**The Age Distribution of acute Viral Respiratory Infections.**

Virus	0-3	Months	4-6	Months	7-12	Months	13-24	Months	25-60	Months
	No.	(%age)	No.	(%age)	No.	(%age)	No.	(%age)	No.	(%age)
RSV	15	83.2%	5	83.3%	6	60%	14	61.00%	10	76%
Influenza A	1	5.6%	0	0.00%	2	20%	3	13.00%	1	8.00%
Influenza B	1	5.6%	0	0.00%	1	10%	1	4.00%	1	8.00%
Parainfluenza (type 1,3)	1	5.6%	1	16.7%	1	10%	5	22.00%	1	8.00%
Total	18	100%	6	100%	10	100%	23	100%	13	100%

indicates that during this particular period epidemic of influenza A did not occur. However, it shows that sporadic cases of influenza AB, and para influenza types 1 and 3 were diagnosed in Islamabad and Rawalpindi area during this winter.

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