# EVALUATION OF WHO MONOCLONAL ANTIBODY KIT FOR DIAGNOSIS OF ACUTE RESPIRATORY VIRAL INFECTIONS

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**Abstract.** In 1990 and 1991, six laboratories located in the WHO Western Pacific Region (WPR) and South East Asian Region (SEAR) were selected, based on their experience in the immunofluorescence antibody technique (IFAT), to participate in the evaluation of a WHO monoclonal antibody (MAb) kit to detect respiratory syncytial (RS) virus, influenza A virus, influenza B virus, parainfluenza virus and adenovirus. Despite differences in the initial standardization procedures, the WHO monoclonal antibodies were found to be of high quality, sensitivity and specificity when tested on clinical specimens. The constant supply of affordable high quality reagents from WHO would enable their use in clinical virological laboratories in the developing countries as well as promote the utilization of IFAT as an adjunct to cell culture isolation in the diagnosis of acute respiratory viral infections.

## INTRODUCTION

Acute respiratory infections, a major problem particularly in developing countries, account for a large number of hospital admissions and contribute significantly to morbidity and mortality among infants and children, especially those below one year of age (Wynne and Hull, 1977).

In determining the etiology of these infections, which are mainly viral in origin (Chanock and Parrot, 1965), the use of simple and rapid methods of diagnosis has replaced classical virological laboratory techniques, such as tissue culture and serology, to provide acceptable results in a shorter time and hence allow successful intervention in the management of patients, their contact and control of diseases in the communities (WHO, 1981).

The immunofluorescence antibody technique (IFAT) was advocated by the WHO as a method of choice for the rapid diagnosis of respiratory viral infection. It enables the demonstration of specific viral antigens within a few hours of specimen collection and early in the disease (WHO, 1981). In order to assist the establishment of this method in developing countries, WHO has undertaken to supply reagents for IFAT to collaborating laboratories and has initiated this exercise by distribution kits, comprising of monoclonal antibodies (MAb) developed against specific respiratory viral agents, for evaluation by collaborating centres in Africa, Eastern Mediterranean, Europe, South America, Southeast Asia and the Western Pacific.

This report summarizes the results of the evaluation carried out by the respective laboratories in the Western Pacific Region (WPR) and the Southeast Asian Region (SEAR) in 1990 and 1991, using the WHO MAb kit to detect respiratory syncytial virus (RSV), influenza A virus, influenza B virus, parainfluenza virus and adenovirus.

## MATERIALS AND METHODS

### Laboratories

The evaluation was conducted in six laboratories located in Singapore, Malaysia, Thailand, India, Japan and Australia. These laboratories were selected based on their experience in IFAT.

### Patients and specimens

Specimens were obtained from patients seen in outpatient clinics or admitted to hospitals because of acute respiratory infection. The specimens consisting of nasopharyngeal aspirates, nasal swabs, throat swabs, throat washings or sputum were collected and transported in ice to the virus laboratory. The cells from the secretions/swabs were then washed and smears prepared on microscope slides. The IFAT was then carried out following the method described by Gardner and McQuillan (Gardner and McQuillan, 1980).

**Standardization of reagents**

In a preliminary assessment, a checkerboard titration was carried out by some laboratories on both the monoclonal antibodies and conjugates to determine the end point titrations and working dilutions using positive viral isolates in cell culture and positive nasopharyngeal deposits respectively. Negative cell controls were also included to determine the degree of non-specific staining of the conjugate.

**Screening of specimens**

Clinical specimens were examined by IFAT using previously determined optimal working dilutions of WHO MAb, appropriate dilutions of polyclonal antibodies or commercially obtained MAb. The WHO MAb were directed against respiratory syncytial (RS) virus, influenza A virus, influenza B virus, parainfluenza virus and adenovirus. The polyclonal and monoclonal reagents used in the comparative studies were obtained commercially from Wellcome Diagnostics, England and Denka-Seiken, Japan, respectively.

## RESULTS

The results obtained from the titrations performed by the respective laboratories to determine the end point titrations and working dilutions of the WHO monoclonal antibodies and conjugates are summarized in Table 1.

The end point titrations which were done only by Laboratory 1 for monoclonal lot 1/90 and together with Laboratory 2 for monoclonal lot 1/91 as shown in Table 1, indicate the high quality of the WHO monoclonal antibodies. This was shown by the high dilutions achieved which still allowed adequate fluorescence to be detected. The values ranged from 1/200 for the pooled parainfluenza virus monoclonal antibody to 1/16,000 for the monoclonal antibody to influenza B virus. The optimal working dilutions used in the six laboratories as shown in Table 2 exhibited wide variations ranging from 1/10 for all viruses in Laboratory 3 to 1/10,000 for influenza B virus in Laboratory 1. This was not so apparent in the optimal conjugate dilutions determined by all six laboratories where the range was from 1/5 in Laboratory 3 to 1/150 in Laboratory 1. The wide variation in optimal dilutions used in the different laboratories could be attributed to the differences in the initial standardization protocols.

Laboratories 4, 5 and 6 which only did preliminary testing on small numbers of specimens found the reagents to be satisfactory in their hands. Comparative data from Laboratories 1, 2 and 3 on the performance of the monoclonal antibodies are presented in Table 3a and 3b. In Table 3a, the WHO monoclonal antibodies were compared with polyclonal antisera and commercial monoclonal antibodies in IFAT. In general, the polyclonal antisera faired better than the commercial monoclonal antibodies. Both Laboratories 2 and 3 found that there was high concordance between the results obtained using the polyclonal antisera and the WHO monoclonal anti-

Table 1

Comparison of end point titration of WHO MAb Lot 1/90 and Lot 1/91.

| Virus | Monoclonal lot 1/90 | Monoclonal lot 1/91 | |
|---|---|---|---|
| | Lab 1 | Lab 1 | Lab 2 |
| Respiratory syncytial virus (RSV) | 1/10,000 | 1/4,000 | 1/4,000 |
| Parainfluenza virus | 1/2,000-1/5,000 | 1/2,000-1/4,000 | 1/200** |
| Influenza A | 1/2,000 | 1/1,000 | 1/1,600 |
| Influenza B | 1/16,000 | 1/16,000 | *ND |
| Adenovirus | 1/3,000 | 1/1,000 | 1/1,600 |

* ND Not done

** This was obtained from pooled parainfluenza (1-4) MAb

Table 2
Working dilutions of the reagents used by each Laboratory.

| Laboratory | Mouse monoclonal antibody pools against | | | | | *Antimouse conjugate |
|---|---|---|---|---|---|---|
| | RSV | Influenza A | Influenza B | Parainfluenza | Adenovirus | |
| 1 | 5,000 | 1,000 | 10,000 | 1,000 | 1,230 | 150 |
| 2 | 40 | 40 | 40 | 40 | 40 | 40-80 |
| 3 | 10 | 10 | 10 | 10 | 10 | 5-10 |
| 4 | ND** | 200 | 200 | ND | ND | 40 |
| 5 | 25 | 25 | 25 | ND | 25 | 50 |
| 6 | 50 | 50 | 50 | 50 | 500 | 20 |
| Range | 10-5,000 | 10-1,000 | 10-10,000 | 10-1,000 | 10-1,250 | 5-150 |

* Dako antimouse conjugate
** ND - not done

Table 3
Comparison of IFAT results obtained using WHO MAb (M) on clinical specimens.

a) With polyclonal (P) IF or commercial MAb (A) IF

| Laboratory No. | Virus | | | | |
|---|---|---|---|---|---|
| | RS | Influenza A | Influenza B | Parainfluenza | Adenovirus |
| 2 | *12/0/0/88 | **4/0/3/58 | **1/0/0/64 | **0/0/8/57 | **0/0/2/63 |
| 3 | *18/0/1/119 | *0/0/0/138 | *0/0/1/137 | *2/0/4/132 | *2/0/0/136 |

* The four figures in each cell represent: P+M+/P+M-/P-M+/P-M-
** The four figures in each cell represent: A+M+/A+M-/A-M+/A-M-

b) With isolation in cell culture** (C)

| Laboratory No. | Virus | | | | |
|---|---|---|---|---|---|
| | RS | Influenza A | Influenza B | Parainfluenza | Adenovirus |
| 2 | *0/0/0/1 | 24/0/0/0 | 14/0/0/0 | ***ND | ND |
| 3 | 9/0/21/135 | 7/0/0/58 | 1/0/0/64 | 11/0/0/119 | 1/0/2/121 |

* The four figures in each cell represent: C+M+/C+M-/C-M+/C-M-
** PMK, MDCK with trypsin, vero, HEp-2
*** ND - not done

bodies for RS virus. However, additional positives were obtained by the latter for the other viruses when compared with the commercial monoclonal antibodies.

Similar comparison of the results obtained using WHO monoclonal antibody with cell culture isolations are shown in Table 3b. The variation in cell culture sensitivity to particular viruses has resulted in the WHO monoclonal antibodies picking up more positive specimens. In addition, immunofluorescence may still be positive with specimens taken late in the infection when locally produced antibody may inhibit virus infectivity. Infectivity in the specimen may also have been lost in transit.

During the course of the evaluation , a total of 12,180 clinical specimens were tested by 3 Laboratories using the WHO monoclonal antibodies. Of the 2,389 specimens tested for RSV, 698 (29.2%) were positive (Table 4). Of 2,470 specimens which were tested for influenza A and B, 53 (2.1%) were positive for influenza A and only 14 (0.6%) were positive for influenza B. Parainfluenza was detected in 47 (1.9%) out of 2,430 specimens and adenovirus was found in 29 (1.2%) out of 2,421 specimens. The results suggest that the positivity rates are similar to those obtained elsewhere although the positive rates for adenovirus and parainfluenza appear low.

## DISCUSSION

Complete unbiased analysis of the performance of the WHO monoclonal antibodies was not possible as not all the participating laboratories in the evaluation provided the necessary data or information required. This was partly due to the lack of a standardized protocol in the evaluation exercise which has also contributed to the discrepancy in the results obtained. The end point titration carried out only in Laboratories 1 and 2 on positive viral isolates indicated the high quality of the monoclonal antibodies. The optimal working dilutions for monoclonal antibodies and conjugates used by the various laboratories exhibited wide variations as not all participating laboratories carried out preliminary assessment to determine the appropriate working dilutions. In addition, immunofluorescence results could be influenced by the quality of the fluorescence equipment as well as variations in laboratory procedures and test interpretations. The sensitivity of the immunofluorescence procedure also depended in part on the density of the antigen in the cells and tissues tested which was directly dependent upon proper sampling and specimen preparation technique. The conjugate should also be evaluated on both strongly and weakly reactive substrates using the same substrate as that being examined in routine diagnostic tests.

The use of cell culture preparation which shows fewer non-specific staining reactions than clinical specimens may give misleading high optimal dilutions as obtained by Laboratory 1. Lower working dilutions as obtained by Laboratory 3 and 5 could give unwatnted non-specific reactions while higher dilutions would lead to loss of sensitivity. However, despite these pitfalls, the overall performance of the monoclonal antibodies and conjugates was found to be good and

Table 4
Total number of clinical specimens examined and number of virus positive specimens for each center.

| Laboratory | Total no. of specimens | Mouse monoclonal antibody pools against | | | | |
|---|---|---|---|---|---|---|
| | | RSV | Influenza A | Influenza B | Parainfluenza | Adenovirus |
| 1 | 10,665 | *649/2,086 | 46/2,167 | 46/2,167 | 30/2,127 | 24/2,118 |
| 2 | 825 | 30/165 | 7/165 | 1/165 | 11/165 | 3/165 |
| 3 | 690 | 19/138 | 0/138 | 1/138 | 2/138 | 2/138 |
| Total: (% positive) | 12,180 | 698/2,389 (29.2%) | 53/2,470 (2.1%) | 14/2,470 (0.6%) | 47/2,430 (1.9%) | 29/2,421 (1.2%) |

* No. positive/no. of specimens examined

as previously published (Routledge *et al*, 1986; Walls *et al*, 1986; Waner *et al*, 1985) showed minimal background and non-specific staining. The differences in the optimal dilutions used by the different laboratories did not seem to affect the performance of the monoclonal antibodies but only limited the number of test a particular laboratory might obtain per unit volume used.

The good quality of the monoclonal antibodies was further validated when the monoclonal antibodies performed as well as or better than polyclonal reagents, giving particulate fluorescence as they reacted with specific epitopes. This resulted in ease of reading and interpretation thus minimising the amount of time required to read them. Similar results were reported by other investigators (Routledge *et al*., 1986; Waner *et al*, 1986; Shalit *et al*, 1985; Bell *et al*, 1989).

When compared with commercially available monoclonal antibodies, the WHO monoclonal antibodies were far superior in being able to detect more positive specimens at higher dilutions, therefore lowering the cost per unit test. As shown in Table 3, the WHO monoclonal antibodies seemed to be detecting more positive specimens compared to tissue culture isolation.

Overall, the WHO monoclonal antibodies have been found to be of high quality, sensitivity and specificity. This would contribute to its value as an alternative to cell culture isolation providing definitive diagnosis within a few hours of specimen collection especially in institutions where cell culture or other facilities are not available.

The constant supply of affordable high quality reagents from WHO would benefit the developing countries in the utilization of immunofluorescence in the rapid diagnosis of acute respiratory viral infections. This would directly assist in the management of patients and their contacts in a more rational and economical way. It will also lead to the control of hospital cross infections and antibiotics usage.

The use of a standardized immunofluorescence technique which is applicable widely could also provide epidemiology surveillance world wide, a prerequisite for the success of vaccine development against RS virus and parainfluenza type 3 virus which is currently ongoing in the WHO.

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