

ORIGINAL ARTICLES

Comparison of two monoclonal antibody kits with cell culture isolation in the detection of respiratory virus antigens

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Abstract

Two different preparations of monoclonal antibodies developed against respiratory viruses have been evaluated by the immunofluorescence antibody technique. The Chemicon monoclonal antibodies were found to be more efficient at picking up positive specimens with a high sensitivity and specificity than Imagen monoclonal antibodies. However, the overall concordance rate of the monoclonal antibodies was 92.3%-100%. Generally, when compared with cell culture isolation, the immunofluorescence antibody technique was found to be more sensitive. The high quality of the Chemicon monoclonal antibodies contribute to their value in providing definitive diagnosis, within a few hours of specimen collection, thus allowing early management of patients, their contacts and control of hospital infection.

Key words: Monoclonal antibody, immunofluorescence, respiratory viruses.

INTRODUCTION

Respiratory viruses are responsible for a major proportion of human morbidity and mortality especially among children in the developing countries. Techniques used for identification of such agents include cell culture isolation and confirmation, serology and direct antigen detection. However, cell culture isolation, despite being the gold standard, and serology lack the rapidity that direct antigen detection methods offer.²

With the advent of antiviral therapy against some respiratory viruses, rapid screening to identify the agent is important for early institution of therapy and management.^{3,4} Such techniques include the immunofluorescence antibody techniques (IFAT) and several enzyme immunoassays.^{5,6}

As part of the World Health Organization (WHO) Project on global surveillance of respiratory viruses, the General Virology Laboratory, University Hospital, Kuala Lumpur recently participated in the evaluation of a monoclonal antibody (MAB) kit (Light Diagnostics, Chemicon Int. Inc. USA) developed against respiratory viruses. This kit comprises of MABs for the screening and typing of Respiratory syncytial virus, Influenza A virus, Influenza B virus, Adenovirus and Parainfluenza virus type

1,2,3 by indirect IFAT. We have compared the performance of the Chemicon MAB with a commercial Mab preparation (Dako Diagnostics Ltd. Denmark) and cell culture isolation. The following report summarizes the results of the evaluation which was carried out from November 1994 - February 1995.

MATERIALS

Patients and specimens

Specimens were obtained from patients admitted to the University Hospital, Kuala Lumpur because of acute respiratory infection. These consisted of nasopharyngeal aspirates, nasal swabs, throat swabs, throat washings or sputum. After collection, the specimens were transported on ice to the virus laboratory before processing for IFAT or culture. For IFAT, the cells from the secretions/swabs were washed and smears prepared on microscope slides. The smears were air dried and fixed with ice cold acetone for 10 minutes and stained immediately or stored dry at -20°C until stained.*

For virus isolation, cell secretions in viral transport media were treated with antibiotics for one hour prior to inoculation into MDCK cells, Vero cells and Hep-2 cells. Cells were then incubated at 33°C and checked daily for cytopathic effect.

METHODS

Staining procedures

Chemicon, Light diagnostics (Indirect IFAT)

Briefly, 12 µl of respiratory viral screen/identification monoclonal antibody respectively were applied to appropriate wells of the test slide. Slides were then incubated at 37°C for 30 minutes in a humid chamber before washing thoroughly with phosphate-buffered saline (PBS) for 10-15 seconds. After washing off the excess buffer, 12 µl of Fluorescein isothiocyanate (FITC) conjugated goat antimouse were added to each well and slides were further incubated at 37°C for 30 minutes. Following this, slides were rinsed thoroughly as before and examined under a fluorescence microscope (Leitz) at 400X. Positive staining is represented by the presence of at least two or more intact cells per field at 400X magnification exhibiting the respective type of fluorescence expected. Negative cells were stained red using Evans blue as a counterstain.

Imagen, Dako (Direct IFAT)

25 µl of the respective FITC conjugated MAb were applied to the appropriate fixed cells for 15 minutes and incubated at 37°C in a moist chamber before slides were washed, dried, mounted and read as described above and following the manufacturers recommendation.

RESULTS

A total of 170 specimens obtained from paediatric patients with acute respiratory tract infection were

screened for the presence of respiratory viruses using the Chemicon MAb antibody kit (Light diagnostics, USA) and another commercial MAb kit from Imagen (DAKO Diagnostics Ltd. Denmark) as well as cell culture isolation.

Of these, 69 (40.5%) were positive by either IFAT or cell culture. Table 1 shows the comparative analysis of immunofluorescence obtained with the Chemicon MAbs versus that of Imagen Dakopatts. The overall concordance rate of the two MAbs varies between 92.3%-100% with sensitivity and specificity ranging from 90.4% to 100% respectively. The Chemicon MAbs were found to be more efficient at picking up positive specimens with a high degree of sensitivity and specificity.

Generally there was high predictive value and low error rate with the Chemicon MAbs. However, there was a discrepancy in 14 specimens whereby the Imagen MAbs failed to pick up 13 of the positive samples. In contrast, the Chemicon MAb failed to detect only one specimen. These could be attributed to the MAb preparation and or the immunofluorescence technique as indirect IFAT is expected to be more sensitive than direct IFAT.

A similar comparison was also carried out using the Chemicon MAbs and Imagen MAbs with cell culture isolation (Table 2a and 2b respectively). The Chemicon MAbs performed better than the Imagen MAbs when both were compared with cell culture. The former was able to detect 16 extra positives not isolated by cell culture. The variation in cell culture sensitivity to particular viruses has resulted in the Chemicon

TABLE 1: Comparative analysis of two monoclonal antibodies in IFAT for the detection of respiratory viruses.

Virus	n	Imagen +		Imagen +		Sensitivity %	Specificity %	Predictive value		Error rate	
		Chemicon +	Chemicon -	Chemicon +	Chemicon -			Positive %	Negative %	Positive %	Negative %
RSV	170	43	1	12	114	97.7	90.4	78.1	99.1	9.5	2.2
Influenza A virus	170	1	0	1	168	100	100	100	99.4	0	50
Influenza B virus	170	0	0	0	170	0	100	0	100	0	0
Parainfluenza (1-3)	170	1	0	0	169	100	100	100	100	0	0
Adenovirus	170	1	0	0	169	100	100	100	100	0	0
Measles	170	0	0	0	170	0	100	0	100	0	0

Key: - = Negative; + = positive

TABLE 2: Comparative analysis of virus isolation and IFAT for the detection of respiratory viruses using :

(a) Chemicon MABs

Virus	n	Isolation +		Isolation +		Sensitivity %	Specificity %	Predictive value		Error rate	
		Chemicon +	Chemicon -	Chemicon +	Chemicon -			Positive %	Negative %	False positive %	False Negative %
RSV	170	40	8	15	107	83	88	88	93	12.2	16.6
Influenza A virus	170	1	1	1	167	50	99.4	50	99.4	0.59	50
Influenza B virus	170	0	0	0	170	0	100	0	100	0	0
Parainfluenza (1-3)	170	1	0	0	169	100	100	100	100	0	0
Adenovirus	170	1	0	0	169	100	100	100	100	0	0
Measles	170	0	0	0	170	0	100	0	100	0	0

Key:- = Negative; + = positive

(b) Imagen MABs

Virus	n	Isolation +		Isolation +		Sensitivity %	Specificity %	Predictive value		Error rate	
		Chemicon +	Chemicon -	Chemicon +	Chemicon -			Positive %	Negative %	False positive %	False Negative %
RSV	170	34	11	3	122	75	97.6	91.8	97.6	2.4	24
Influenza A virus	170	1	0	0	169	100	100	100	100	0	0
Influenza B virus	170	0	0	0	170	0	100	0	100	0	0
Parainfluenza (1-3)	170	0	1	0	169	0	100	0	99.4	0	100
Adenovirus	170	1	0	0	169	100	100	100	100	0	0
Measles	170	0	0	0	170	0	100	0	100	0	0

Key:- = Negative; + = positive

MABs picking up more positive specimens. In addition, immunofluorescence may still be positive with specimens taken late in infection when locally produced antibodies may inhibit virus infection. In contrast, the Imagen Mabs failed in detecting 12 specimens when viruses were isolated by cell culture, suggesting a lower sensitivity than the Chemicon Mab preparation.

Even though infectivity in the specimens may

also have been lost in transit, cell culture isolation picked up 8 specimens not detected by either the MABs. This is thought to be due to inadequate sampling of the specimens for IFAT, accounting for a certain degree of false negativity.

In our study population, RSV was the most frequently detected respiratory pathogen, accounting for 92.7% (64/69) of the positive isolates followed by Influenza A (4.3%),

Parainfluenza 1-3 (1.4%) and Adenovirus (1.4%). This observation is in agreement with our previous report.'

DISCUSSION

We have evaluated the use of two different preparations of MAb developed against respiratory viruses using the IFAT. Compared to the cell culture isolation, the Chemicon MAbs has been found to be of high specificity and sensitivity and seemed to be more sensitive than the Imagen preparation. The respiratory screen of the Chemicon MAb kit offers a distinct advantage of eliminating all the negative samples thus directly saving technician time and reducing the cost per test. Although, the Chemicon monoclonal antibodies were advocated by the manufacturers for use for cell culture confirmation only, we have found it to be useful for screening of specimens directly. This is possible as we have found minimal non-specific staining reactions and all our positive screening was confirmed by typing. The high quality of the Chemicon MAbs would contribute to its value in providing definitive diagnosis within a few hours of specimen collection especially in institutions where cell culture facilities are not available or in an outbreak situation. Its use in IFAT would also provide rapid identification of viral respiratory agents thus directly assisting in the management of patients, their contacts, control of hospital infection and antibiotic usage.

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