

EVALUATION OF A MICROHAEMAGGLUTINATION TEST FOR THE SEROLOGICAL DIAGNOSIS OF *MYCOPLASMA PNEUMONIAE* INFECTIONS:

N PARASAKTHI, MBBS, MSc.

Department of Medical Microbiology, Faculty of Medicine, University of Malaya

Summary

Seventy-six samples from three categories of patients were tested for *M. pneumoniae* antibodies by the Serodiamyco kit and complement fixation test. Sixty-three of the 76 samples agreed on both assays, 59 being classified as negative and four as positive. In addition, 11 other samples were positive by the kit. With a concordance rate of 83%, the Serodiamyco shows promise as a step towards more convenient assays for the serodiagnosis of *M. pneumoniae* infections.

Key words: *Mycoplasma pneumoniae*, serology, microhaemagglutination test, complement fixation test.

INTRODUCTION

Mycoplasma pneumoniae produces a spectrum of effects varying from asymptomatic infections to mild upper respiratory tract disease and pneumonias. In a serological survey of viral and mycoplasmal respiratory infections amongst Malaysian children, *M. pneumoniae* accounted for six out of 33 laboratory confirmed infections.¹ The laboratory diagnosis consists of isolation of the pathogen from clinical specimens and serology. Isolation procedures are difficult and time consuming. Serological techniques have thus been the mainstay of diagnosis. The immunofluorescence technique (IF), complement fixation test (CF) and the indirect haemagglutination test (IHA) have been used for *M. pneumoniae* antibody detection.^{2,3,4,5,6} This study compares a commercially available micro-IHA kit (SERODIA-MYCO, Fujirebio Inc, Tokyo) with the CF test for the serological diagnosis of *M. pneumoniae* infections.

MATERIALS AND METHODS

Sera from 3 categories of patients were tested by the CF test and the micro IHA. 30 samples were from patients with a clinical diagnosis of atypical pneumonia, 17 from patients with non-respiratory infections and 29 from normal healthy adults. All samples were single specimens and those from patients with suspected atypical pneumonia were collected more than two weeks after onset of illness. All samples were heat inactivated at 56°C for 30 minutes and stored at -20°C prior to testing.

The CF test was a microtechnique using commercial *M. pneumoniae* antigen (Wellcome

Laboratories, U.K.). The sera were titrated at an initial dilution of 1:8 and the titre was read as the reciprocal of the serum dilution at the well with 30% lysis of sheep red blood cells (rbc).

The micro IHA, which utilizes chick rbc sensitized with cell membrane components of *M. pneumoniae*, was performed in accordance with the manufacturer's instructions. All sera were initially examined at a dilution of 1:10 and titrated for end-point determination where necessary. Unsensitized cells were used as controls. The plates were read after incubation for one hour at room temperature, and again after overnight incubation at room temperature. A reactive control serum from rabbits was included in all individual runs. Agglutination patterns were considered as reactive when a smooth mat of cells, sometimes with its edges slightly folded, covered at least 75% of the base of the well. The initial dilution of the serum, rather than the final, was used to determine the IHA antibody titre so that a direct comparison could be made with the CF titre.

RESULTS

In the serological diagnosis of acute *M. pneumoniae* infections by the CF test, a titre of 256 and above was considered significant while a titre of 128 was suggestive of infection and 64 or less was considered insignificant. The manufacturers of Serodiamyco recommended a titre of 10 or greater as significant.

Sixty-three of the 76 samples agreed on both assays; 59 being classified as negative and four as positive. An additional 11 samples

Address for reprint requests: Dr. N. Parasakthi, Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 59100 Kuala Lumpur, Malaysia.

were positive by the IHA only. These results are shown in Table 1.

In the group with suspected atypical pneumonia, three patients were serologically confirmed by the CF whilst one had a probable infection; these four patients were also positive by the Serodiamyco, with titres of 20 to >160. The IHA, in addition, classified four other samples as positive (titres of 20, 40 and >160).

Of the 17 patients whose sera were screened for non-respiratory infections, none were positive by the CF but four were positive by IHA; these four patients had titres below 8 by CF and 10 by IHA. Amongst the 29 normal, healthy adults, all had CF titres below 64 but three were positive by IHA with titres of 10, 40 and 80.

DISCUSSION

The detection of cold agglutinins and antibodies against *Streptococcus MG* for the diagnosis of *M. pneumoniae* infections are of limited value. Specific diagnosis by isolation of the pathogen is difficult and time consuming, thus serological methods have been the mainstay

of diagnosis. The CF test is commonly used for this purpose. It has the advantage of being carried out simultaneously with other CF tests to detect antibodies against respiratory viruses which may also produce atypical pneumonia.

The IHA test has been found to be sensitive and specific,^{4,5,6} but the preparation and standardization of tamed erythrocytes is technically laborious thus limiting its usefulness. The Serodiamyco IHA, however, comes with standardized cells and is thus simple to perform and furthermore, the test being read after an hour's incubation, results are rapidly available. A minor disadvantage of the kit was the presence of non-specific agglutinins in 2 samples. These samples may be retested after absorption with an absorption medium which is available separately from the manufacturer.

In the evaluation of the test, an IHA titre of 10 or greater has been taken as evidence of recent infection. Higher IHA baseline titres of ≥ 128 and ≥ 160 have been used by others.^{4,6} The low positive titre with the Serodiamyco is, however, beneficial since it is possible to perform qualitative screening test at a single dilution. However, it is desirable to determine

TABLE 1
COMPARISON OF THE SERODIAMYCO IHA WITH THE CF TEST

Category of patients	Antibody Assay Method		No. of sera (Total=76)
	CF	IHA	
I. Suspected Atypical Pneumonia (30)	+	+	3
	+	-	0
	+/-	+	1
	+/-	-	0
	-	+	4
	-	-	22
II. Non Respiratory Infections (17)	+	+	0
	+	-	0
	+/-	+	0
	+/-	-	0
	-	+	4
	-	non-specific	11
III. Normal Healthy Adults (29)	+	+	0
	+	-	0
	+/-	+	0
	+/-	-	0
	-	+	3
	-	-	26

the baseline titre for the Malaysian population with larger normal population studies.

Both assays agreed on 63 out of 76 samples, giving a concordance rate of 83%, of which 59 samples were negative and 4 were positive. A further 11 samples were identified as positive by the IHA which the CF failed to identify. These could represent false positives due to the low diagnostic titre used in the Serodiamyco IHA or they could in fact be positives which were falsely considered negative by the CF as the diagnostic titre used for the CF was ≥ 128 although other workers have used CF titres of ≥ 160 as diagnostic titres.' The discordance between the two tests could in addition be due to differences in the chronological sequence of appearance of IHA and CF antibodies and to differences in the classes of antibodies being detected.

Currently there is an underestimation of *M. pneumoniae* infections and this may be partially overcome with the availability of simple assays to detect specific antibodies. The Serodiamyco kit shows promise towards fulfilling this need and further evaluation, in a controlled prospective study with the use of paired sera, is desirable.

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