Diagnosis of *Mycoplasma pneumoniae* Pneumonia: Sensitivities and Specificities of Serology with Lipid Antigen and Isolation of the Organism on Soy Peptone Medium for Identification of Infections

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Received 2 April 1990/Accepted 22 June 1990

The sensitivities and specificities of isolation and serology for detection of *Mycoplasma pneumoniae* infections were determined for 3,546 pneumonia patients for whom both isolation and serological data were available. Soy peptone, fresh yeast extract, horse serum-supplemented agar, and diphasic medium were employed for isolation, and the lipid antigen of *M. pneumoniae* was used for serodiagnosis by complement fixation. The number of *M. pneumoniae* colonies most frequently detected was 200 to 600 per throat swab, with a range of ≤ 60 to $\geq 2,000$. The use of diphasic medium increased the number of isolates by 26% compared with direct isolation on agar plates. The organism was isolated from 360 of 525 patients who showed fourfold or greater antibody increases in their paired sera, resulting in a sensitivity of culture of 68%. When persons with titers of ≥ 32 but no fourfold increase were used as the reference, the sensitivity of culture was 58%. The combined sensitivity of the culture method for persons with serological evidence of infection (fourfold increase and titer of ≥ 32) was 64%. The specificity of the culture method was 97% for the 2,527 serologically negative persons. Fourfold antibody increases were found in 360 of 674 persons with isolates of the organism, resulting in a sensitivity of 53%. An additional 247 persons showed titers of ≥ 32 (without a fourfold increase), resulting in a combined sensitivity of 90% for serology with the lipid antigen for the detection of antibodies in culture-positive persons. Fourfold antibody increases were found in 6% of culture-negative persons, resulting in a specificity of 94%. The quantitative culture results provide important base-line data for the development of rapid diagnostic tests for *M. pneumoniae* infection.

Mycoplasma pneumoniae is an important cause of human respiratory disease, accounting for 15 to 20% of total pneumonia (17, 19). Clinical diagnosis of *M. pneumoniae* pneumonia is difficult because many viral and other pneumonias present clinically similar pictures. The agent was first reported in 1962 by Chanock, Hayflick, and Barile (5). It had been recovered on a medium devised by Hayflick (25) in which PPLO agar (34) was supplemented with the fresh yeast extract preparation of Edward (14) and 20% horse serum. The fresh yeast extract turned out to be the critical factor in the growth of *M. pneumoniae* as well as species such as *Mycoplasma orale* (25). However, *M. pneumoniae* grows slowly, with colonies taking a week to more than a month to become visible microscopically. Thus, laboratory results are of little use in planning chemotherapy for the patient. This has resulted in a large interest in rapid tests for diagnosis of *M. pneumoniae* infections.

The purpose of this paper is to present an analysis of our data concerning isolation and serology of *M. pneumoniae* in 3,546 pneumonia patients tested during the course of a 12-year study of pneumonia in a civilian population (19). The sensitivities and specificities of culture and serodiagnosis with the lipid antigen for detection of *M. pneumoniae* infections were determined by using culture-positive persons as the reference for serodiagnosis and serologically positive patients as the reference for the culture method with soy peptone agar. Overall, the sensitivity of either culture or

serology (fourfold antibody increase criterion) was about 66% when the other method was used as the reference. The number of CFU in specimens varied by at least 100-fold.

MATERIALS AND METHODS

Media. The base medium was composed of 20 g of soy peptone (Sheffield Chemical, Norwich, N.Y.) and 5 g of NaCl dissolved in 1 liter of distilled water. The pH was adjusted to 7.3. The agar base was prepared by adding 10 g of Noble agar (Difco Laboratories, Detroit, Mich.) to the base medium. The broth base was prepared by adding 10 g of glucose to the base medium. Both media were sterilized by autoclaving at 121°C for 15 min. Complete agar medium was prepared by combining 65 ml of agar base (molten at 50°C) with 25 ml of horse serum (GIBCO Laboratories, Grand Island, N.Y.), 10 ml of fresh yeast dialysate, 2 ml of penicillin (10,000 U/ml in water), and 1 ml of 3.3% thallium acetate. Portions (5 ml) of complete agar medium were distributed in petri dishes (10 by 35 mm) which were held overnight at room temperature before storage at -4°C. Portions (3 ml) of complete agar medium were allowed to solidify in the bottom of screw-cap diphasic tubes (16 by 125 mm). The diphasic overlay was prepared by supplementing the broth base medium with the same components as for the agar medium. Phenol red (2% solution of the sodium salt in water) was added at 0.1 ml per 100 ml. Portions (3 ml) of complete broth were overlaid over the solidified agar in the 16-by-125-mm tubes. Yeast dialysate was prepared by suspending 450 g of active dry yeast (Fleischman) in 1,250 ml of water at 40°C. The suspension was heated in the autoclave

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at 121°C for 5 min, cooled, placed in a dialysis casing, and dialyzed against 1 liter of water for 48 h at 4°C. The casings and their contents were discarded. The dialysate was autoclaved at 121°C for 15 min and stored frozen. Transport medium was prepared by dissolving 0.5 g of bovine albumin (Sigma Chemical Co., St. Louis, Mo.) in 100 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Transport medium was sterilized by filtration, and penicillin was added to 200 U/ml. Portions (2 ml) were added to 1-dram (ca. 4 ml) vials, and the vials were stored frozen until needed.

Quality control of medium. The most important step in quality control was to evaluate each new lot of soy peptone powder with specimens from persons known to be positive for *M. pneumoniae* (28). Briefly, test agar plates were prepared from several samples of powder obtained from the manufacturer. Only a few lots (about one in three) showed good performance as indicated by colony size, time of appearance of colonies, and relatively high plating efficiency. Such lots were purchased in quantities of 100 to 200 kg and stored for several years at room temperature without loss of activity. Neither the fresh yeast extract nor the horse serum seemed to be responsible for variations in plating efficiencies.

Specimens. The characteristics of the population of patients and incidence of pneumonia have been described previously (19). Briefly, patients having a diagnosis of pneumonia were admitted to the study. Few patients were hospitalized and few produced sputum; consequently, most specimens were throat swabs which were collected and placed into transport medium by breaking the swab in a vial of transport medium. Vials were transported to the laboratory on ice and ordinarily tested the same day that they were collected. Blood specimens (collected at the time of diagnosis and 3 to 6 weeks later) were delivered to the laboratory, held at room temperature for 2 to 3 h, and stored overnight at 4°C. The serum was separated from the clot by centrifugation. Sera were stored at -20°C. Control specimens were collected from a group of families who were being studied for respiratory diseases. The families were recruited from the University of Washington Department of Preventive Medicine, and the study took place between April 1965 and June 1967. Specimens were collected by a public health nurse and tested the same day. A total of 141 persons representing 36 families participated in the study and contributed 1,184 specimens to the study. Specimens were collected at intervals and whenever the participants showed respiratory symptoms.

Serology. Paired sera were tested by complement fixation with the lipid antigen (29). The lipid antigen was prepared from strain AP-164 (29) grown in soy peptone-fresh yeast dialysate broth (27) supplemented with 10% gamma globulin-depleted horse serum (Alpha Gamma Laboratories, Sierra Madre, Calif.). Organisms were concentrated by centrifugation, washed with saline, and extracted with 2:1 chloroform methanol, and the lipid fraction was complexed with bovine albumin exactly as previously described (29). The complement fixation procedure used was a microtitration version of a modified Kolmer technique with overnight fixation. Two full units of complement were used with four units of antigen. The titer is reported as the highest original serum dilution which yielded complete fixation of complement (i.e., no lysis observed). Twofold dilutions ranging from 1:2 to 1:1,028 were tested. A fourfold increase in antibodies between acute- and convalescent-phase specimens was consid-

ered diagnostic, starting with titer increases from <1:2 to 1:4.

Isolation of mycoplasmas. Agar plates were inoculated with 0.1 ml of specimen and incubated in humidified metal containers in air at 37°C. Agar plates were observed twice weekly with a stereoscopic microscope at $\times 20$ to $\times 60$ magnification and oblique lighting. The agar plate was viewed from the bottom of the plate through the agar; thus, plates were not opened during study. The diphasic medium was inoculated with 0.1 ml of sample, observed for pH change and the presence of spherules twice weekly, and subcultured to agar at 7, 21, and 42 days. Cultures were identified as *M. pneumoniae* if the following criteria were fulfilled: typical small colonies were observed on agar plates, acid was produced from glucose in the diphasic medium, typical spherules (3) were observed in the fluid phase of the diphasic cultures, and hemolysis of guinea pig erythrocytes could be demonstrated by the method of Clyde (8). Other mycoplasma isolates were identified by growth inhibition with specific antisera on agar plates (9). All isolates were stored at -70°C and are still viable.

Data analysis. Because *M. pneumoniae* pneumonia cannot be defined on clinical grounds, it was necessary to determine the specificities and sensitivities (40) of diagnostic tests in populations known to be positive by a reference test which was unrelated in principle to the test to be evaluated. The sensitivities of the tests were determined by calculating the percentage of persons positive by a given test (e.g., isolation positive) in the population of persons positive by a reference test (in this example, serologically positive) who had samples successfully tested (i.e., the throat swabs were not overgrown by contaminating bacteria). The specificities of the tests were determined by calculating the percentage of persons negative by a given test in a population of persons known to be negative by the reference test and who also had specimens successfully assayed by the test in question: sensitivity = (number of persons positive by a given test/number of persons with positive reference test who had specimens successfully tested) $\times 100$, and specificity = (number of persons negative by a given test/number of persons negative by the reference test who had specimens successfully tested) $\times 100$.

RESULTS

Comparison of direct agar plating with diphasic medium for recovery of *M. pneumoniae*. Because diphasic media (an agar surface overlaid by broth) are commonly used for recovery of fastidious organisms, a diphasic medium was evaluated for isolation of *M. pneumoniae*. Whereas the direct inoculation of agar plates yielded 951 isolates (Table 1), the addition of a diphasic medium increased the number of isolates to 1,196 (a 26% increase [Table 1]). The time to positivity in the diphasic medium appeared to be directly related to whether or not colonies were observed on the initial plate. All specimens which were positive on the initial plates were also positive in the diphasic medium, and nearly half of these specimens were recognized as positive in the diphasic medium by days 7 to 14. By contrast, specimens negative on the initial plate showed positive reactions in the diphasic medium after 21 days (92% [Table 1]). Three percent of the specimens did not show positive reactions until the day 42 subculture of the diphasic medium, which meant that the cultures could not be reported as negative until some 8 weeks after initial inoculation of cultures.

TABLE 1. Isolation of *M. pneumoniae* with diphasic medium in comparison with direct plating on agar

Initial plate result	No. of specimens ^a	No. of specimens with a time (days) to detection of <i>M. pneumoniae</i> in diphasic medium of ^b :			
		7-14	14-21	21-42	42+
Positive	951	426	256	266	3
Negative	245	8	11	195	31

^a Specimens included were specimens which yielded isolates and which had results recorded for both the diphasic broth and agar plates (cultures which were overgrown by contaminants were excluded). The number of specimens tested included results for more than one specimen from some patients.

^b Incubation time for first observation of a change in pH or presence of spherules at indicated days. The table includes only specimens which were subsequently shown to hemolyze guinea pig erythrocytes when subcultured to agar plates.

Quantitation of the recovery of *M. pneumoniae* on agar plates. Because the specimens had been tested over a 10-year period by the same laboratory staff, the consistency of observations made it possible to estimate the quantities of organisms present in specimens. On the initial plate, the number of colonies most often detected was 10 to 30 in 39% of 1,196 total specimens (Table 2). Since 0.1 ml of specimen was used, the number of CFU was 200 to 600 per specimen in the 2-ml portions of transport media. If we assume that the initial throat swab probably contained 0.1 ml of secretions, the total concentration of CFU in the secretions might be 2,000 or more CFU per ml for some 63% of specimens (Table 2). However, 37% of the specimens contained fewer CFU, and 20% showed no colonies on the initial plate even though they eventually became positive in the diphasic medium (Table 1). Overall, the number of organisms detected showed at least a 100-fold range in CFU (Table 2).

Sensitivity and specificity of the culture method. *M. pneumoniae* was recovered from 674 (19%) patients in a population of 3,546 persons who had a diagnosis of pneumonia, throat swab specimens, and paired sera (Table 3). If fourfold antibody increases between acute- and convalescent-phase sera are considered as the reference standard, the sensitivity of the isolation method was 68% (360 isolates from 525 of those patients with fourfold antibody increases [Table 3]). A significant number of persons showed no antibody increase but had high titers (≥ 32) in both sera because the acute-phase serum frequently had been collected a week or more after onset of symptoms. If these patients who showed high titers (≥ 32) but not fourfold antibody increases are considered as the reference standard, the sensitivity of the isolation method was 58% (247 isolates from 427 persons with ≥ 32 antibody levels). The combined sensitivity of the culture

TABLE 2. Distribution of *M. pneumoniae* colonies on plates directly inoculated with positive specimens

No. of colonies	No. of specimens	%
None ^a	245	20
1-9 ^b	197	16
10-30 ^b	469	39
30-100 ^b	192	16
>100 ^b	93	8

^a Positive diphasic medium only; see Table 1.

^b Ten or fewer colonies were recorded by count; estimates were made for the higher numbers of colonies per plate.

TABLE 3. Comparison of isolation and serology for diagnosis of *M. pneumoniae* pneumonia

Patient isolation result ^a	No. of persons			Total
	4× antibody increase	No 4× antibody increase		
		≥32 ^b	≤16 ^c	
Positive	360	247	67	674
Negative	165	180	2,527	2,872
Total	525	427	2,594	3,546

^a Patients included were those who had pneumonia, serological results for acute- and convalescent-phase sera, and throat swab specimens positive for mycoplasmas. Patients were excluded from this table if either of their sera had anticomplementary titers of ≥ 4 and/or the throat swab specimen was contaminated.

^b No fourfold increase but titers of ≥ 32 in both sera.

^c No fourfold increase but titers of ≤ 16 in both sera.

method for detecting *M. pneumoniae* from persons with serological evidence of recent infection (titer ≥ 32 and/or fourfold antibody increase) was 64%. If we consider that those without fourfold antibody increases or titers of ≤ 16 in both sera are true serological negatives, the specificity of isolation is 97% (2,527 persons of 2,594 persons negative by serology also were negative by isolation [Table 3]).

Sensitivity and specificity of serodiagnosis by complement fixation testing with the lipid antigen. Fourfold antibody increases were found in 53% of persons with isolates of *M. pneumoniae* (360 of 674 isolation-positive persons [Table 3]), and high stationary titers (≥ 32) were observed in another 37%. The combined sensitivity with both serological criteria (fourfold antibody increase and high titers) was 90%. Fourfold antibody increases were found in 6% of persons who were negative for *M. pneumoniae* by culture (165 of 2,872 persons), resulting in a specificity of 94%. If we combine the fourfold antibody increase group with the ≥ 32 titer group, we find that 12% of the isolation-negative group has serological evidence of recent infection (345 of 2,872 isolation-negative persons), for a combined specificity of 89%.

Distribution of antibody titers in pneumonia patients. When convalescent-phase antibody titers were compared with acute-phase serum titers in sera from persons with both fourfold antibody increases and isolates of the organism, the geometric mean titer for acute-phase sera was 1:2.8 and that for convalescent-phase sera was 1:62 (Fig. 1A). If we consider the antibody titers from all persons from whom *M. pneumoniae* had been isolated, the geometric mean titer was 1:11 for acute-phase sera and 1:50 for convalescent-phase

TABLE 4. Use of high titer (≥ 32) for diagnosis of *M. pneumoniae* pneumonia

Test result		Total no. of patients tested	No. of patients with titer of $\geq 32^a$ (%)
Isolation ^b	4× antibody increase		
+	+	360	292 (81)
+	-	314	227 (72)
-	+	165	96 (58)
-	-	2,707	156 (6)

^a Number of persons with a titer of greater than or equal to 1:32 in their convalescent-phase sera.

^b On agar plate or diphasic medium.

FIG. 1. Distribution of complement-fixing antibody titers in patients with *M. pneumoniae* pneumonia. (A) Acute- and convalescent-phase serum titers from patients with isolates of *M. pneumoniae* and fourfold antibody increases. (B) Acute- and convalescent-phase serum titers from all patients with isolates of *M. pneumoniae*.

sera (Fig. 1B). The occurrence of high titers in the first sera probably reflects the delayed collection of some acute-phase sera. For persons with fourfold titer increases but no isolates, the geometric mean acute-phase titer was 1:2.6 and the geometric mean convalescent-phase titer was 1:29. The geometric mean titer of sera from persons without any markers of infection (culture negative, no antibody increase, and serum titer ≥ 32) was 1:2.8, the same as that for the acute-phase sera of persons with fourfold antibody increases.

Use of high titer for diagnosis of *M. pneumoniae* infections. Serum antibody titers of ≥ 32 appeared to be a conservative choice of a titer indicative of recent infection, because that criterion would include most but not all of the convalescent-phase serum titers of persons with isolations and/or fourfold antibody increases (Fig. 1A and B). Titers of ≥ 32 were found in 81% of the convalescent-phase sera of those who had both isolates and fourfold antibody increases and in 72% of the convalescent-phase sera of those who had isolates but no antibody increases (Table 4). Use of the ≥ 32 titer criterion alone would have missed 42% of those who were culture negative but who showed fourfold rises. Of those without markers of recent infection (isolation negative, no fourfold antibody increase), 156 (6%) showed titers of ≥ 32 . Lowering

the criterion to ≥ 16 would include an additional 113 patients, for a total of 269 persons positive only by height of titer.

Detection of other mycoplasmas. Sixty-three mycoplasmas other than *M. pneumoniae* were recovered. Fifty-one of these were typed by disk inhibition (9): 33 were *Mycoplasma hominis*, 16 were *Mycoplasma orale*, 1 was *Mycoplasma buccale*, 1 was *Mycoplasma salivarium*, and 12 were untypable. Two *Acholeplasma* species were recovered in the diphasic medium. Although *Acholeplasma* produce acid in the diphasic medium, they are readily differentiated from *M. pneumoniae* in that large colonies are rapidly produced on agar.

Prevalence of *M. pneumoniae* in healthy persons. In order to determine the prevalence of *M. pneumoniae* in healthy persons, control samples were collected during a family study of respiratory disease over a 19-month period. Paired throat and nasal swabs (1,184) were collected from 141 persons representing all members of 36 families. Half of the persons in the group were children. *M. pneumoniae* was isolated from two children in one family within a 1-month period: once from a boy (throat swab) and three times from his sister (twice from the throat and once from a nasal swab); both had mild respiratory illness. This family had no contact with the laboratories doing mycoplasmal research. No other isolates of *M. pneumoniae* were recovered in this study, indicating that the prevalence of infection was low in our population (two infections in 141 persons over the 19-month period) and that *M. pneumoniae* is not a transient colonist.

DISCUSSION

The soy peptone medium described in this paper differs from that of Hayflick in that it contains a plant (soybean) peptone rather than the beef extract and beef peptone. This medium came about because the pancreatic digest of beef heart medium used in our tissue culture studies of mycoplasmas (35) was difficult to prepare. When we evaluated PPLO agar (the base for Hayflick medium) for plating efficiency with tissue culture *M. hominis* strains, we found that the lot that we tested was much inferior to the pancreatic digest medium. A number of peptones from the Sheffield Chemical Co. were screened, and soy peptone was found to give the best plating efficiency for the *M. hominis* strains found in tissue culture. The basic broth follows the general pattern for bacterial media and contains 2% soy peptone and 0.5% NaCl. For isolation of *M. pneumoniae*, fresh yeast extract was critical, as in the Hayflick medium, and the basic agar or liquid medium was supplemented with 10% fresh yeast dialysate and 20% horse serum.

In this study, *M. pneumoniae* was recovered from 69% of persons with fourfold antibody increases, a result close to that which we reported previously (22) for a smaller sample. The results in the literature are similar for Hayflick-type medium. Chanock et al. (6) recovered *M. pneumoniae* on agar plates with samples from 12 of 13 patients with antibody increases as detected by immunofluorescence. In another study (4), they recovered *M. pneumoniae* from 50% of those who developed a growth-inhibiting antibody response. In studies of college students, Evans et al. (16) recovered *M. pneumoniae* from 72% of 43 persons positive by complement fixation. Edwards et al. (15) found that seroconversion was about twice as sensitive as isolation for the detection of *M. pneumoniae* infections in the military. Griffin and Crawford (23) recovered *M. pneumoniae* from 9 of 25 persons (36%)

with seroconversions. In a more recent study, Sakurai et al. (37) isolated *M. pneumoniae* from 110 of 322 children (34%) with respiratory disease and fourfold antibody increases by either hemagglutination or complement fixation. They recovered *M. pneumoniae* from 24 of 84 children (29%) with high titers. A factor difficult to control is the timing of collection of the specimen, because patients frequently do not seek medical attention until some time after the onset of symptoms. In a prospective study of families with *M. pneumoniae* infections, we found that the highest positivity rate was for specimens collected immediately at onset of illness and that the positivity rate progressively declined thereafter (18). A sensitivity of 68% for the culture method is not unreasonable compared with that for other fastidious bacteria. *Haemophilus ducreyi* is recovered from about 60 to 80% of clinically diagnosed cases of chancroid (33).

Other medium formulations have been evaluated for isolation of *M. pneumoniae*. Tully et al. (39) used SP-4 broth and diphasic media for testing of throat swabs from military recruits which had previously tested negative for *M. pneumoniae* by use of Hayflick media. On retesting, 5 of 50 specimens were positive in Hayflick diphasic medium (with methylene blue), 3 to 7 of the 50 specimens tested in two different experiments were positive on SP-4 with methylene blue, and 23 to 25 of the 50 were positive on SP-4 without methylene blue. The authors concluded that methylene blue was toxic in SP-4 medium, but they did not test Hayflick-type medium in its initial formulation without methylene blue. Their results have been further complicated by the recognition that some of these *M. pneumoniae* isolates recently have been found to be mixtures of *Mycoplasma genitalium* and *M. pneumoniae* (1). Another medium formulation, the modified New York City medium, appeared to show more rapid colony formation than Hayflick-type agar with methylene blue (20), but these results were not confirmed in a study by Dorman et al. (12), in which methylene blue was not employed in the reference Hayflick medium. In a subsequent study, Granato et al. (21) inoculated specimens into Hayflick broth and subcultured the broths on Hayflick agar (without methylene blue) and New York City medium at 3 and 14 days. Colonies were reported to appear 2 to 4 days sooner on the New York City medium. A novel feature of this study was inoculation of the entire swab into the initial isolation medium (we used only 0.1 ml of 2.0 ml of transport medium). The contamination that might have been expected from such a large inoculum apparently was controlled by four antimicrobial agents used in the medium.

It is difficult to compare media because of the variation from lot to lot in the reference medium (frequently the Hayflick medium with PPLO agar base). The results for a new medium may appear favorable if the particular lot of reference medium has a poor plating efficiency. Similarly, a noncultural probe or antigen detection test will appear to be very sensitive if the reference culture method uses a bad batch of medium with poor plating efficiency. Since manufacturers do not evaluate medium components against clinical isolates, it is not surprising that certain batches have poor plating efficiency. Our experience with the soy peptone medium indicates that testing of samples with specimens containing *M. pneumoniae* is the important quality control measure. Only about one in three commercial lots of soy peptone was satisfactory, even though all lots showed some colonies with prototypic strains. Neither the horse serum lot nor the fresh yeast extract lot made much difference in relative plating efficiency. When the soy peptone was purchased in large lots (100 to 200 kg), the quality did not

deteriorate in several years of storage at room temperature. The best way to evaluate a new medium, antigen test, or DNA probe is to test specimens from persons who have been evaluated by culture and serology for evidence of infection, as was done in this study and as has been proposed by Kok et al. (31).

Another confounding factor is *M. genitalium*. This organism resembles *M. pneumoniae* very closely culturally and in fact would be indistinguishable from *M. pneumoniae* by the criteria used in this study or those described elsewhere (10). However, *M. genitalium* is susceptible to thallium acetate, which was included in our medium throughout. In addition, those *M. pneumoniae* isolates from our study which have been compared by immunoblotting and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were typical strains of *M. pneumoniae* (41). The occurrence of *M. genitalium* in the throat (1) indicates that both cultural and noncultural tests must be able to distinguish *M. genitalium* from *M. pneumoniae*.

In our study, 20% of specimens were positive only in the diphasic medium, indicating that those specimens had few CFU. In comparison, Craven et al. (11) found that 47% of 89 positive specimens were positive only in a diphasic medium (Hayflick-type medium with 0.1% methylene blue). A significant proportion of persons with a fourfold antibody rise had cultures negative for *M. pneumoniae* (31% [Table 3]), indicating that those specimens had even fewer viable organisms. In some cases, the organisms may be nonviable, but antigen may be present because Kok et al. (31) demonstrated antigen in some specimens which were culture negative.

The slow growth of *M. pneumoniae* in primary isolation attempts means that a negative report could be released only some 6 to 8 weeks after inoculation of specimens, because some diphasic cultures (3%) did not become positive until the day 42 plating. In addition, the apparent concentration of organisms showed at least a 100-fold range (Table 2). Both of these factors are important in the search for rapid methods for noncultural diagnosis of infections. The cDNA probe for ribosomal RNA of Gen-Probe (San Diego, Calif.), in its ¹²⁵I-labeled form, had a detection limit of 2×10^3 CFU/ml or 3.2×10^5 genomes with cultures of *M. pneumoniae*, "detection levels 10- to 100-fold less sensitive than culture," according to Harris et al. (24). Whereas two studies (13, 38) showed close correlation between culture and probe, Harris et al. (24) found that the probe did not detect *M. pneumoniae* in persons who were culture negative but who showed serological evidence of infection. At a detection limit of 10^3 CFU for the probe, only some 25 to 60% of the throat swabs positive by culture might be positive by probe (Table 2). A ³²P-labeled whole genomic probe used for the detection of *Ureaplasma urealyticum* also showed a detection limit of 10^3 CFU (36). The polymerase chain reaction is more promising because its detection limit was about 10^2 color-changing units (2) or 4×10^1 CFU (26) for cultures of *M. pneumoniae*. The detection limit of the polyclonal rabbit antiserum assay of Kok et al. (31) was 10^4 CFU from culture. Two different monoclonal antibodies against a 43-kilodalton antigen (7, 32) have been used for antigen detection. One test had a detection limit of 10^5 to 10^6 CFU, and thus it was necessary to culture the specimens in medium for 2 to 12 days to amplify the amount of antigen sufficiently for detection (7).

Serodiagnosis with lipid antigen and the fourfold rise criterion showed a sensitivity of 53% in detecting culture-positive persons. When titers of ≥ 32 were included, the sensitivity increased to 90% (Table 3). The specificity of serodiagnosis was 94% for either the fourfold antibody

increase or the ≥ 32 criterion. Combined, the specificity was 89%. Since many patients were not diagnosed as having pneumonia until a week or more after onset of symptoms, it is not surprising that some acute-phase sera already had a high antibody titer. It is likely that such a delay in diagnosis will be a common feature in efforts to diagnose *M. pneumoniae* pneumonia. Overall, the lipid complement fixation test is a sensitive and specific measure of infections in a population of persons with pneumonia; however, it is recognized that persons with bacterial meningitis may show false-positive reactions in the lipid complement fixation test (30).

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grant AI 06720 from the National Institute of Allergy and Infectious Diseases.

We thank D. Grayston for computer analysis.

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