Comparison of the Seradyn Color Vue Passive Agglutination Test and Complement Fixation for Detection of Mycoplasma pneumoniae Antibodies

DIANE S. LELAND,† KATHLEEN A. BARTH, AND ELIZABETH B. CUNNINGHAM

Department of Pathology, Indiana University Medical Center, Indianapolis, Indiana 46202-5200

Received 28 September 1992/Accepted 21 January 1993

We compared traditional complement fixation (CF) with a new passive agglutination method, the Seradyn Color Vue (SCV) test (Seradyn, Indianapolis, Ind.), for detection of Mycoplasma pneumoniae antibodies in 170 stored serum samples. The SCV test was 90% sensitive in identifying as positive 27 of 30 CF high-titer (\( \geq 1:64 \)) serum samples and 100% specific in identifying as negative 134 of 134 CF low-titer (\( \leq 1:32 \)) or negative (< 1:8) serum samples. The SCV test was technically undemanding, and it required no expensive equipment.

Mycoplasma pneumoniae, a respiratory pathogen, causes up to 20% of all cases of pneumonia in humans (6). Mycoplasmal and viral pneumonia, pharyngitis, and tracheobronchitis are often clinically indistinguishable. It is important to differentiate mycoplasmal and viral infections, because mycoplasmal infections are readily treatable with tetracycline and its derivatives and with erythromycin, while viral infections do not respond to antibiotics. The clinician is often forced to make treatment decisions based purely on clinical assessment, because laboratory testing for confirmation of mycoplasmal infections does not provide results in a timely manner.

Although M. pneumoniae can be isolated in specialized broth and agar media, the organism routinely requires 14 to 21 days to grow. Because of the specialized and tedious nature of mycoplasma culturing, this service is not offered by many clinical microbiology laboratories.

Serodiagnosis is one of the original serologic tests still in use at present measures antibodies termed cold agglutinins; these antibodies agglutinate human erythrocytes in the cold but not at 37°C. Because only 34 to 68% of patients infected with M. pneumoniae produce cold agglutinins, false-negative results are common; also, false-positive results are seen with diseases not related to M. pneumoniae infections (2).

M. pneumoniae-specific antibodies can be identified by several methods, including indirect immunofluorescence (IF), enzyme immunoassay (EIA), and complement fixation (CF). These methods are usually available only at larger laboratories, and they require highly trained personnel and specialized equipment; results are often not available for several days.

The Seradyn Color Vue (SCV) M. pneumoniae test for the identification of M. pneumoniae antibody has recently become available commercially (Seradyn, Indianapolis, Ind.). It is a passive agglutination test that requires only a 40-min incubation period, it is technically undemanding, and it involves no specialized equipment.

In this study, we compared the SCV test with traditional CF for the detection of M. pneumoniae antibodies in 170 stored serum samples selected at random from freezer storage. The samples, collected from both hospitalized patients and outpatients, were received for mycoplasmal antibody testing in our serology laboratory over the past 3 years. Upon their receipt in the laboratory, the samples were tested by CF. They were then frozen and stored at -20°C, and they remained frozen until the present study was initiated. All sera were thawed and were tested by the SCV test. When the SCV test result agreed with the initial CF result, no further testing was done. When the SCV result did not agree with the initial CF result, the sample was retested in duplicate by both the SCV test and CF; the most frequent result (i.e., two of three determinations) was accepted as the result for that sample. In all calculations, the CF result was accepted as the true result for each sample.

In the M. pneumoniae CF test (7), serial twofold dilutions of each serum sample (1:8 to 1:8,192) were tested in a standard CF microtitration system (16) with mycoplasma antigen purchased from Whittaker M. A. Bioproducts (Walkersville, Md.). Rabbit hyperimmune serum (Whittaker M. A. Bioproducts) was used as the positive control.

The SCV test is marketed as a kit which contains all reagents, including positive control serum. For SCV testing, sera were heat inactivated by incubation in a 56°C water bath for 30 min. For each test serum sample, serial twofold dilutions of 1:20 to 1:10,240, which yielded final dilutions of 1:40 to 1:20,480, were prepared in V-bottom microwell plates. The diluent (supplied in the test kit) was normal rabbit serum in a 0.004 M potassium phosphate buffer. One drop (25 \( \mu L \)) of red high-density particles coated with mycoplasma lipid antigen was added to each well. One additional microwell containing a final serum dilution of 1:20 was prepared for each serum sample, and uncoated red high-density particles were added to this well; this well served as a nonspecific agglutination control. The mixtures were carefully mixed and then incubated for at least 40 min. During this incubation period, the particles settled to the bottom of the microwells. After the mixtures had been incubated for 40 min, results were evaluated if the unagglutinated particles had settled to form a compact center point. If the particles had not settled completely after 40 min, incubation was extended until settling was complete. The manufacturer indicates that the reaction mixtures may stand overnight without any change in results (12).

* Corresponding author.
pneumoniae antibodies, with agglutinated particles spread within the well. All wells with most of their particles settled to form a compact center point with some agglutinated particles on the outer margin (+) are wells 6, row B, and 4, row G.

The settled SCV particles (Fig. 1) were evaluated as follows, as directed by the manufacturer (12): +++, agglutinated particles spread out uniformly; ++, small center point with agglutinated particles spread within the well; ±, most particles settled to form a compact center point, with some particles agglutinated on the outer margin; and −, particles concentrated to form a heavy compact center point. The ++ and + readings were interpreted as positive, while the ± and − readings were interpreted as negative. The endpoint result for each sample was the highest dilution showing a positive (++ or +) reaction. The endpoint titers were reported as recommended by the manufacturer in terms of the total test dilution.

The SCV test, marketed as a qualitative assay for M. pneumoniae antibodies, is reported as positive when the titer is \( \geq 1:320 \) and as negative when the titer is \( \leq 1:160 \) (12). Because the manufacturer of the SCV test compared a 1:320 SCV titer with a CF titer of 1:64, we used these titers as the basis for comparison of the two tests. In qualitative determinations, we considered CF titers of \( \geq 1:64 \) and SCV titers of \( \geq 1:320 \) positive (high titer). CF results of \( \leq 1:32 \) and SCV results of \( \leq 1:160 \) were considered negative. For the purpose of comparison of the methods, we divided the negative qualitative results further into low titer, which included CF titers of 1:8 to 1:32 and SCV titers of 1:40 to 1:160, and negative, which included CF results of \(<1:8\) and SCV results of \(<1:40\).

In qualitative testing of the 170 serum samples (Table 1), the SCV was 100% specific in identifying as negative 134 CF low-titer (1:8 to 1:32) or negative (<1:8) samples. The SCV was 90% sensitive in identifying as positive 27 of 30 CF high-titer (\( \geq 1:64 \)) serum samples; the 3 CF high-titer serum samples that were identified as negative by SCV showed antibody at 1:80 or 1:160 but not at the \( \geq 1:320 \) level specified as the positive cutoff for the SCV test. The SCV positive predictive value was 100%, and the negative predictive value was 97.8%. Our results demonstrate that the SCV test, when used in qualitative determinations, reliably detects clinically significant levels of M. pneumoniae antibodies.

Although the presence of M. pneumoniae antibody cannot be used as an indicator of recent infection because antibody tends to persist following infection (5), it has been previously suggested that high-antibody titers for a single sample may be suggestive of recent infection. High titer has been defined as \( \geq 1:32 \) (8), \( \geq 1:64 \) (4, 10), and \( \geq 1:128 \) (9, 13). This lack of agreement concerning the interpretation of single titers reinforces the importance of encouraging physicians to pursue the traditional approach of comparing antibody levels in acute- and convalescent-phase samples. However, single-sample results have been previously shown to be helpful. Kenny et al. (8) found 90% sensitivity for confirming culture-positive M. pneumoniae infections when either a fourfold increase in titer in paired serum samples or a single CF titer of \( \geq 1:32 \) was considered evidence of infection; these criteria yielded sensitivities of 53 and 37%, respectively, when considered individually.

Although the SCV test package insert (12) indicates that the assay is intended for the qualitative determination of M. pneumoniae antibodies, the test is performed in a traditional quantitative serology format with twofold serial dilutions. With 74 serum samples that demonstrated antibody by both SCV and CF, sera that produced high titers in the CF test also produced high titers in the SCV test and sera that produced low titers in the CF test produced low titers in the SCV test (Table 2). Although we were not able to test paired serum samples to evaluate changes in titer, the favorable correlation of SCV and CF titers suggests to us that the SCV test, like the CF test, may be useful in quantitative determinations.

The SCV test was less sensitive than CF in detecting low levels of antibody (Table 1). It detected antibody in 46 (69%) of 67 CF low-titer samples, and it was negative for 21 (34%) of 64 CF high-titer samples (Table 2).
of these samples. Of the 21 samples that were SCV negative, 15 had very low CF titers of ≤1:16, which are generally considered insignificant.

The sensitivity and specificity of the SCV test relative to those of CF in this study were comparable to those obtained in a study conducted at an independent laboratory and reported by the SCV manufacturer (12). The independent study of 196 samples showed SCV to be 93.2% sensitive and 98.7% specific relative to CF; these values compare favorably to the SCV sensitivity and specificity of 90 and 100%, respectively, reported in the present investigation. Nakamura et al. (11) reported 100% sensitivity for the SCV test in detecting significant levels of antibody in convalescent-phase serum samples from 20 individuals with confirmed M. pneumoniae infections. CF detected significant levels of antibody in only 65% of these patients. These comparison studies of the SCV test and CF confirm that the SCV test is an acceptable replacement for CF in the measurement of M. pneumoniae antibodies.

The sensitivity and specificity of the SCV test relative to those of CF in this study were comparable to those reported in comparison studies of CF and IF (3) and CF and selected EIAs (1, 14). Although both IF and EIA have been reported to give higher titers than CF, the qualitative results (positive or negative for significant levels of M. pneumoniae antibodies) of the three methods usually agree. Because the SCV test, like IF and EIA, has been shown to be comparable to CF, we conclude that it should be as useful as IF or EIA in the clinical laboratory. The SCV test may prove more useful than certain EIAs which have been shown to produce both false-positive and false-negative results (15). The SCV test is quicker and less technically demanding than IF and EIA, and it does not involve specialized or expensive equipment.

The manufacturer indicates that SCV reactions may be read after "at least 40 min" of incubation of the mixture (12). We found that some results were readable at 40 min; however, we preferred a longer incubation period of ≥1 h, usually 2 h, in order to allow particles to settle completely and to produce more easily read reactions.

The SCV test includes testing of each serum sample against uncoated particles; this testing detects nonspecific agglutinins that react with the high-density particles rather than with the test antigen. When nonspecific agglutinins are present, the assay is invalid. Of the 170 samples tested in this study, 1 yielded invalid SCV results because of agglutination of the uncoated particles. The manufacturer of the SCV test predicts that nonspecific agglutinins will be found in 1 in 3,000 samples (12). Two of the 170 serum samples tested in this study yielded invalid results in the CF test because of anticomplementary activity. The low rate (1 of 170) of invalid results due to nonspecific agglutinins in the SCV test was comparable to the low rate (2 of 170) of invalid results due to anticomplementary activity in the CF system. This indicates that the SCV test is a versatile test system that is no more likely than the CF test system to be invalidated by nonspecific interfering substances.

From the results of this study, we conclude that the SCV M. pneumoniae antibody test has good sensitivity and excellent specificity. It should be useful for mycoplasma antibody testing in laboratories in which equipment or technical expertise is limited. Because the SCV test is technically undemanding and because the results are available within approximately 1 h, this assay may provide a convenient alternative for M. pneumoniae antibody testing in any laboratory and may provide the information that a physician needs in order to distinguish mycoplasmal infections from viral infections.

REFERENCES