

Latex Agglutination in the Diagnosis of Pneumococcal Infection

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A latex agglutination (LA) method for detection of pneumococcal antigens was evaluated and compared with counterimmunoelectrophoresis (CIE). LA was 2 to 10 times more sensitive than CIE for the detection of purified capsular polysaccharides in defined media, but only when a 1+ or 2+ agglutination reaction was interpreted as positive. LA was much less sensitive than CIE with clinical samples. In 50 cases of pneumococcal pneumonia, antigen was detected in the serum almost twice as often with CIE (40%) as with LA (22%). LA was positive in six cases of pneumonia where CIE was negative; however, in three of these cases, antigen was detected only in undiluted sera, which raised some question about the specificity of the result. With 18 samples of cerebrospinal fluid (CSF) from 11 patients with pneumococcal meningitis, the CIE test was positive more frequently (14 samples) than was LA (11 samples). Moreover, antigen was detected in CSF by LA in only one additional patient than was positive by CIE alone. There was one false-positive LA reaction among 45 samples of CSF from patients without pneumococcal infection. Although LA is a less complicated method than CIE, it is not a sensitive test for pneumococcal antigens and would be of little value as a routine diagnostic method.

Latex agglutination (LA) has been used with considerable success for establishing an etiological diagnosis in infections caused by cryptococci, meningococci, and haemophilus organisms (2, 4, 10, 11). There is relatively little information on the efficacy of LA for diagnosis of pneumococcal infection. However, Whittle and associates reported recently (12) that they could detect pneumococcal antigens by LA in the cerebrospinal fluid (CSF) of a large percentage of patients with pneumococcal meningitis. LA appears to be a less elaborate method than counterimmunoelectrophoresis (CIE), which has also been employed for rapid diagnosis of pneumococcal infections (1, 5, 7, 9). Unlike CIE, LA does not require specialized equipment, and the necessary reagents remain stable for prolonged periods. Perhaps more importantly, LA has been reported in some studies to be more sensitive than CIE, for example, in detection of haemophilus or meningococcal antigens in the CSF of patients with meningitis (11, 12). Because of these observations, we were prompted to evaluate the sensitivity and specificity of LA in the diagnosis of pneumococcal meningitis and pneumonia. Our results indicate that LA is a much less satisfactory method than CIE for the detection of pneumococcal antigens.

MATERIALS AND METHODS

Patients and test samples. Bacterial pneumonia was diagnosed on the basis of fever, leukocytosis, production of purulent sputum, and clinical and roentgenographic evidence of a pulmonary infiltrate. Sera were obtained within 2 days of admission of patients to the hospital in all cases, but usually after initiation of antimicrobial therapy. Pneumococci were typed by previously described methods (6). Some of the sera employed in the present studies were obtained originally for studies dealing with the kinetics of capsular polysaccharide antigenemia (J. D. Coonrod and D. P. Drennan, *Ann. Intern. Med.*, in press).

CSF was obtained from patients with clinical evidence of meningitis. Except as otherwise indicated, CSF samples were obtained before antimicrobial therapy was instituted, and the infecting agent was isolated from cultures of the CSF.

Sera and CSF were stored at -20°C and were not heat inactivated prior to use, except as indicated. Test samples were assigned a code number, and the LA test was performed without knowledge of the bacteriological or clinical findings.

Purified polysaccharides. Highly purified, type-specific pneumococcal capsular polysaccharides were provided by Robert Baker, Eli Lilly & Co., Indianapolis, Ind. Purified type b polysaccharide from *Haemophilus influenzae* was kindly provided by John Robbins.

CIE. CIE was performed as described elsewhere (6), using a modified buffer system that contained

2.3 g of barbital, 5.15 g of barbital-sodium, and 1.46 g of NaCl per liter. Pneumococcal antigen was detected in serum and CSF with a polyvalent anti-pneumococcal rabbit serum (Omniserum, with antibody against 83 pneumococcal types; Statens Seruminstitut, Copenhagen, Denmark). The volume of antiserum used in CIE was 0.02 ml/well, and the volume of sample added to the antigen-containing wells was also 0.02 ml.

Rheumatoid factor. Sera were tested for rheumatoid factor by using the RA test (Hyland Laboratories, Costa Mesa, Calif.) according to the directions of the manufacturer.

LA. For LA, the method of Whittle and associates (12) was used, with several modifications. Polystyrene latex particles (0.81 μ m; Dow Chemical Co., Midland, Mich.) were employed. An optimal dilution of the stock latex suspension for detection of the purified capsular polysaccharides was determined from preliminary experiments. Optimal results were obtained with unwashed latex particles that were diluted 1:85 in glycine-buffered saline (GBS). The GBS was prepared by dissolving 7.3 g of glycine and 10.0 g of NaCl in 900 ml of distilled water; the pH was then adjusted to 8.2 with 1.0 N NaOH, and the solution was brought to a final volume of 1 liter with distilled water.

Rabbit antipneumococcal globulin (RAPG) was precipitated from omniserum (Statens Seruminstitut) in 27% sodium sulfate. The mixture was incubated for 90 min at room temperature, and the precipitate was collected and resuspended in 0.15 M NaCl (to five times the volume of the original serum). The suspension was dialyzed at 4°C against multiple exchanges of normal saline. The same methods were used to prepare a globulin fraction from normal rabbit serum (Grand Island Biological Co., Grand Island, N. Y.).

Latex particles were sensitized with RAPG by mixing an equal volume of the optimal dilution of latex particles with an optimal dilution of RAPG in GBS. The optimal dilution of RAPG consisted of one part RAPG and 39 parts of GBS (see below). Enough sodium azide was added to the latex-RAPG suspension to give a final concentration of 0.1% for the preservative. The suspension of latex particles and RAPG was incubated for 1 h at room temperature to sensitize the particles. Latex particles coated with normal rabbit globulin (NRG) were prepared by the same methods employed for sensitization with RAPG.

The LA test was performed using transparent glass plates (8 by 10 cm), with 20 rings (15-mm diameter) per plate. Test samples of 20 μ l were placed in the rings using disposable micropipettes (Dade, Miami, Fla.), and 10 μ l of latex suspension was added. The samples were thoroughly mixed with wood applicator sticks, and the glass plate was gently agitated manually for 3 min. The agglutination reaction was read against a black background with indirect illumination (Immuno-illuminator; Hyland, Laboratories, Costa Mesa, Calif.). The intensity of agglutination was graded on a scale of 0 to 4+, using criteria described by Newman and associates (10).

Unless stated otherwise, test samples consisted of undiluted CSF or serum. GBS with 0.1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) was also tested as a control for the stability of the latex particle suspension. Test samples were evaluated with specifically sensitized (RAPG) latex particles and particles coated with NRG. The sample was considered to contain pneumococcal antigen when agglutination of specifically sensitized latex particle was one grade higher than the agglutination observed with particles coated with NRG. This criterion of positivity was employed since preliminary experiments (described below) showed that differences in agglutination of less than one grade were not reproducible.

RESULTS

Detection of purified capsular polysaccharide. Figure 1 illustrates the pattern of agglutination obtained when graded amounts of purified capsular polysaccharide in GBS-BSA were mixed with latex particles sensitized with RAPG. GBS-BSA alone produced no change in the granularity of the latex suspension. The addition of increasing amounts of purified capsular polysaccharide caused dose-related effects in the latex suspension, ranging from a slight increase in granularity to the formation of large clumps of latex. The criteria indicated in Fig. 1 were employed for scoring the intensity of agglutination. Initially, dark glass plates and direct illumination were used in reading the agglutination reaction. Subsequently we found that grading of the agglutination reaction was greatly facilitated by performing the test with transparent glass slides and using indirect lighting against a black background.

To determine the optimal concentration of RAPG for the latex test, graded amounts of purified capsular polysaccharide (dissolved in GBS-BSA) were reacted with latex particles that had been sensitized with various dilutions of RAPG in GBS. Particles sensitized with dilutions of RAPG ranging from 1:2 to 1:100 were tested with 0.05 to 6.4 μ g of purified capsular polysaccharide in GBS-BSA per ml. Capsular types 1, 3, 4, 7, 8, 12, 14, and 23 were evaluated. A dilution of RAPG of 1:30 to 1:50 was optimal for detection of each of the antigens. A dilution of RAPG of 1:40 was, therefore, employed for routine use.

The sensitivity of latex coated with a 1:40 dilution of RAPG for detection of various types of purified capsular polysaccharide in GBS-BSA is given in Table 1. The sensitivity of CIE for these antigens is given for comparison. The volume of sample tested was 0.02 ml for both the CIE and LA tests. For most of the polysaccharides, minimal (1+) agglutination reactions were obtained with less antigen than could be

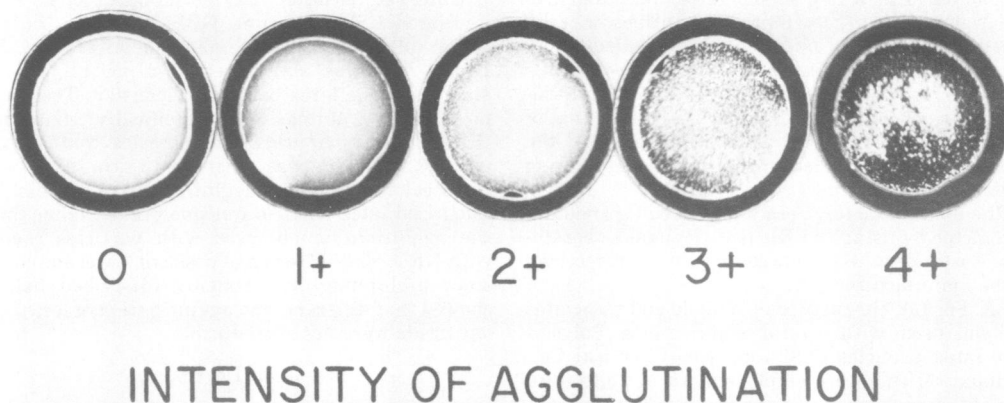


FIG. 1. Criteria for scoring the intensity of the latex agglutination reaction. Latex particles sensitized with RAPG were mixed for 3 min with various quantities of purified type 7 capsular polysaccharide. Criteria for scoring the reactions were as follows: 0, milky, nongranular suspension; 1+, fine granularity; 2+, coarse granularity with a cloudy background; 3+, coarse granularity with a clear background; 4+, complete agglutination and clearing.

TABLE 1. Comparison of the sensitivity of LA and CIE for the detection of purified pneumococcal capsular polysaccharides

Polysaccharide type	Minimum quantity of antigen detected ($\mu\text{g/ml}$)			CIE
	Intensity of LA			
	1+	2+	$\geq 3+$	
1	0.20	1.60	6.40	0.40
3	0.006	0.012	0.05	0.05
4	0.025	0.05	0.10	0.10
7	0.012	0.025	0.10	— ^a
8	0.025	0.05	0.10	0.10
12	0.025	0.05	0.20	0.05
14	0.006	0.025	0.05	— ^a
23	0.012	0.10	0.20	0.025

^a —, Antigen was not detected consistently at any concentration up to 50 $\mu\text{g/ml}$.

detected by CIE. However, the amount of capsular polysaccharide needed to give strong (3 or 4+) agglutination reactions was appreciably more than the minimal amount detectable by CIE for polysaccharide types 1, 12, and 23. Types 7 and 14, which were not detected by CIE, were detected by LA at concentrations of 0.012–0.1 $\mu\text{g/ml}$.

LA in pneumonia. The sensitivity and specificity of LA for detection of pneumococcal antigen in the serum of patients with pneumonia were studied. Preliminary experiments indicated that undiluted normal human serum increased the granularity of latex sensitized with

RAPG: 20 of 25 normal human sera gave a 1+ reaction, and 5 sera gave a 2+ reaction. Similar reactions were obtained with 1:2 or 1:4 dilutions of the sera and with sera heated at 56°C for 30 min. To control for this nonspecific agglutination, it was necessary to compare each test serum with a control consisting of latex particles coated with 1:40 dilution of NRG. Agglutination of particles coated with NRG by normal sera was comparable to, or in some cases exceeded slightly, the degree of agglutination observed with specifically sensitized particles. To evaluate the reproducibility of the test, portions of serum from 25 patients and 15 normals were studied by the same operator on 2 different days. For 22 of the 40 samples, the results of both runs were identical. In 18 samples, there was a difference in the degree of agglutination in the two runs. In eight of these, the NRG control and the test samples were changed by a proportionate amount (a difference of one grade for six samples and less than one grade for two samples). In 10 samples, unilateral changes in agglutination in the NRG control or test samples were found on repeat runs. The changes were of small degree (less than one grade) in each case. Based on these findings, test sera were considered to contain antigen when they agglutinated the specifically sensitized particles by at least one grade more than the NRG control.

Sera taken within 2 days of hospitalization from 50 patients with pneumococcal pneumonia were studied. Controls consisted of sera from 45

individuals with no infection or with nonpneumococcal infection. All sera were tested for pneumococcal antigen by the LA and CIE tests to provide data for comparison of these methods. The results are indicated in Table 2. CIE was superior to LA for detection of pneumococcal antigen in serum; indeed, pneumococcal antigen was detected almost twice as often by CIE as by LA. The possibility that excess antigen in some of the sera might have led to negative results in the LA test was evaluated. Eleven sera that had given a positive result with CIE and a negative result with LA were serially diluted with GBS-BSA. The dilutions were tested by LA, and none gave positive agglutination reactions.

To determine whether the large amount of protein present in serum might have interfered in some manner with the LA test, graded amounts of purified type 3 and type 12 pneumococcal polysaccharide were added to serum obtained from a healthy adult. The serum samples were tested by LA and CIE to determine the minimum quantity of antigen detectable. Purified polysaccharides dissolved in GBS-BSA were tested in parallel with the antigens dissolved in sera to define the effects of serum constituents on the tests. Less antigen was detected by LA when the antigen was dissolved in serum than when it was dissolved in GBS-BSA (fourfold reduction for type 3 and eightfold reduction for type 12). However, less antigen was also detected by CIE when the antigen was dissolved in serum rather than GBS-BSA (eightfold decrease for type 3 and eightfold re-

duction for type 12). Thus, the effects of serum on detection of the antigens were comparable in both CIE and LA. The decreases in detectability of antigen in serum may have been due to binding of the antigen by type-specific antibody; unfortunately, agamma human serum was not available to evaluate this point.

Despite the low incidence of positive results with the LA test, LA was positive with sera from six patients with pneumococcal pneumonia whose CIE test was negative (three cases with bacteremia and three cases without bacteremia). Two of these sera were from patients with type 7 pneumonia, where CIE is known to give poor results. Antigen titers by LA in these cases were 4 and 64. One of the patients had type 16 infection, and the antigen titer in his serum was 2. In the three remaining patients (one case each of infection due to types 6, 12, and 18) antigen was detected only in undiluted sera, which raised a question of the specificity of the reaction. Tests for serum rheumatoid factor on all patients with pneumococcal pneumonia were negative, however, and heating of the sera at 56°C for 30 min did not decrease their reactivity in the LA test. As indicated in Table 2, there were no false-positive results for antigen by either LA or CIE when sera from 45 patients without pneumococcal infection were tested.

The relationship of the infecting pneumococcal serotypes to the results of LA and CIE is indicated in Table 3. An interpretation of the results is limited by the small number of patients infected by any given serotype. However,

TABLE 2. Comparison of LA and CIE for the detection of pneumococcal antigen in serum

Subject	No. studied	No. of patients with antigen in serum detected by:		
		LA	CIE	LA or CIE
Pneumococcal pneumonia				
Bacteremic	28	6 (21.4) ^a	17 (60.7)	20 (71.4)
Nonbacteremic	22	5 (22.7)	3 (13.6)	6 (27.3)
All	50	11 (22.0)	20 (40.0)	26 (52.0)
Controls				
Nonpneumococcal infection ^b	17	0	0	0
No infection	28	0	0	0
All	45	0	0	0

^a Numbers in parentheses indicate percentages.

^b Includes eight patients with *Staphylococcus aureus* bacteremia (three with an associated pneumonia), two with urinary tract infection and bacteremia due to *Escherichia coli*, two with *Klebsiella pneumoniae* pneumonia, four with tuberculosis, and one with an unspecified viral illness.

TABLE 3. Pneumococcal serotypes isolated in pneumococcal pneumonia and the detection of pneumococcal antigen by LA and CIE

Pneumococcal serotype isolated ^a	No. of patients studied	No. of patients with antigen in serum detected by:		
		LA	CIE	LA or CIE
3	8	3	3	3
4	6	0	4	4
9	5	1	3	3
1	4	0	4	4
6	3	2	1	2
7	3	2	0	2
8	3	0	1	1
19	3	0	0	0
23	3	0	0	0
12	2	1	1	2
16	2	1	0	1
Others	8	1	3	4

^a Pneumococci were isolated from blood cultures in 28 patients and from cultures of sputum in 22 patients.

it appears noteworthy that four cases of type 1 infection were detected by CIE, and none of these was antigen-positive by the LA test. Similarly, sera from four patients with type 4 pneumonia were positive for antigen in the CIE test and were negative in the LA test. As mentioned above, two patients with type 7 infection had serum antigen demonstrable by LA, but not by CIE.

LA in meningitis. Pretreatment CSF from 11 patients with culture-positive pneumococcal meningitis, as well as follow-up samples of CSF from 7 of these subjects, was tested for pneumococcal antigen by LA and CIE. The results are indicated in Table 4. In two patients who did not have antigen demonstrable in pretreatment CSF by either CIE or LA, no organisms were visible on Gram-stained smears of the CSF. The CSF of one patient with type 7 pneumococcal infection gave negative results in the CIE test; organisms were visible on a Gram-stained smear of the fluid and antigen was detected by LA. Of the remaining individuals, two patients (with meningitis due to type 1 and type 8 pneumococci) gave negative results in the LA test, but antigen was detected by CIE. These two

patients, as well as the remaining patients, had organisms visible on Gram-stained smears of the CSF. Follow-up CSF samples were obtained from 24 to 96 h after the onset of specific antimicrobial therapy from seven of the patients whose pretreatment CSF had detectable pneumococcal antigen. Cultures and Gram-stained smears of these fluids did not show organisms, but many of the samples did have antigen demonstrable by LA or CIE.

CSF from 38 patients with meningitis due to organisms other than pneumococci and CSF from 7 uninfected controls were tested by LA and CIE. As indicated in Table 4, the specificity of both LA and CIE was good. However, CSF from one patient with type b haemophilus meningitis gave a false-positive reaction in the LA test. This did not appear to be due to cross-reaction of the type b capsular polysaccharide with antipneumococcal antibody. The concentration of type b polysaccharide in the CSF in question was only 2 $\mu\text{g/ml}$, whereas purified type b polysaccharide in a concentration as high as 25 $\mu\text{g/ml}$ did not cause agglutination in the LA test.

Stability of LA reagents. The sensitized latex suspension containing 0.1% sodium azide was stable for up to 2 months at 5°C. We did note a fourfold loss of sensitivity for detection of several types of purified capsular polysaccharides with latex suspensions stored at 5°C for 4 months or longer.

TABLE 4. Comparison of LA and CIE for the detection of pneumococcal antigen in the CSF^a

Subject	No. studied	No. of patients with antigen in CSF detected by:		
		LA	CIE	LA or CIE
Pneumococcal meningitis				
Before antibiotics	11	7 (63.6) ^b	8 (72.7)	9 (81.8)
During antibiotic therapy	7	4 (57.1)	6 (85.7)	6 (85.7)
All	18	11 (61.1)	14 (77.8)	15 (83.3)
Other meningitis				
Haemophilus, type B	23	1 (4.3)	0	1 (4.3)
Meningococcal ^c	5	0	0	0
Miscellaneous ^d	10	0	0	0
All	38	1 (2.6)	0	1 (2.6)
No meningitis	7	0	0	0

^a Except as indicated, CSF was obtained before therapy with antimicrobials; the diagnosis was based on isolation of the organisms indicated.

^b Numbers in parentheses indicate percentages.

^c Isolates were group C in three cases, group B in one case, and group Y in one case.

^d Meningitis was caused in one case each by *S. aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, group B *Streptococcus*, *Enterobacter* species, *K. pneumoniae*, *Mycobacterium tuberculosis*, *Candida albicans*, and *Herpesvirus hominis*.

DISCUSSION

Recently, Whittle and associates (12) reported that latex particles sensitized with globulin from polyvalent antipneumococcal serum (omniserum) provided a useful test for detection of pneumococcal antigen in CSF. They observed that pooled CSF from patients with pneumococcal meningitis reacted in as high a dilution in the LA test as it did in CIE. However, when CSF from individual patients with pneumococcal meningitis was studied, a smaller percentage of cases were positive with LA (82%) than were positive with CIE (98%). In the present studies, LA was more sensitive than CIE for the detection of purified capsular polysaccharides in defined media, but only when a 1+ or 2+ agglutination reaction was interpreted as positive. LA was much less sensitive than CIE for detection of pneumococcal antigens in clinical samples. The difference in the sensitivity of LA and CIE was particularly evident with sera from patients with pneumococcal pneumonia. Antigen was detected in the serum almost twice as frequently by CIE, and zone phenomena did not appear to account for

the negative results obtained with LA. We were not able to show a selective depressant effect of normal human serum on the sensitivity of the LA test, and the reason for the lesser sensitivity of LA with biological samples remains unclear.

We used a method of LA which was very similar to that used by Whittle et al. (12), except we found that a less concentrated suspension of latex particles provided better results. Working with eight different capsular types of purified polysaccharide, we observed that a 1:30 to 1:50 dilution of omniserum globulin was optimal for sensitization of the latex particles. This globulin concentration is similar to the 1:20 to 1:40 dilution of omniserum globulin employed by Whittle and associates. Since omniserum has a lower titer of antibody for type 3 than for other pneumococcal types (by quellung tests), the previous investigators (12) added latex particles sensitized to type 3 to the suspension sensitized with omniserum globulin. We did not do this since we had no particular difficulty in detecting purified type 3 polysaccharide with particles sensitized only with omniserum globulin. It is certainly possible, however, that globulin prepared from omniserum is relatively deficient in antibody to particular capsular types, especially in terms of detection of these antigens in biological fluids by LA. We obtained some evidence of this possibility with the type 1 and type 4 antigens. Four sera and one CSF from patients with type 1 pneumococcal infection were antigen-negative by LA, but contained antigen demonstrable by CIE. Similarly, sera from four patients with type 4 pneumococcal pneumonia were antigen-negative by LA, but contained antigen demonstrable by CIE. Had LA detected type 1 and type 4 pneumococcal antigen in biological fluids to the same extent that CIE did, the incidence of positive results with the two tests would have been similar. We did not explore the possibility of enriching our omniserum globulin with globulin to particular pneumococcal types. Although this approach might have improved the results of the LA test in the present studies, it appeared somewhat impractical for routine use. The incidence of particular pneumococcal types may vary widely with different patient populations, and the advantage accruing from improvements in the sensitivity of the test for a few types would vary with the incidence of those types in the patient population. Furthermore, it is far from certain that different lots of omniserum would behave identically in the test. Omniserum is standardized by the manufacturer for use in the quellung test, and its content of precipitating or agglutinating anti-

bodies may vary from one lot to another. For example, the lot of omniserum used in the present studies was found to be fourfold less sensitive for the detection of purified type 1 polysaccharide by CIE than was a lot that we employed previously (6). Obviously, until carefully standardized antisera are available, there may be variations in results obtained with either LA or CIE due to differences in the antisera employed.

We encountered several difficulties in developing a method of LA that was specific for pneumococcal antigens in biological fluids. Many normal human sera produced low-grade (1 to 2+) agglutination of latex particles sensitized with omniserum globulin. This problem was not eliminated by prior heating of the samples and was frequently present in serum samples diluted 1:2 or 1:4 in GBS-BSA. The problem was resolved by testing samples with latex particles coated with NRG in parallel with specifically sensitized particles. Using this comparative approach, we were able to obtain consistently reproducible results when differences in agglutination of one grade or more were observed between the test sample and the NRG control. The specificity achieved using this approach appeared generally good. However, one false-positive reaction with a CSF sample was encountered, and in three cases of pneumococcal pneumonia, we obtained positive LA tests with undiluted sera where the 1:2 dilutions of the sera were negative for antigen. Although there was no evidence that these latter reactions were nonspecific, there remains some question as to whether LA reactions obtained with undiluted sera should ever be considered specific reactions (2, 3, 8).

Difficulties with nonspecific agglutination of latex particles coated with omniserum globulin may be greater than those observed with rabbit globulin prepared from type- or group-specific rabbit antisera as, for example, in the LA test for haemophilus or meningococcal antigens (10, 11). With the latter tests, optimal sensitization of latex particles is achieved at a much higher dilution of globulin than is true for the polyvalent omniserum preparation. Consequently, in those tests purification of the globulin fraction by dilution is proportionately greater than is the case for omniserum.

Although LA is simpler to perform than CIE, the present studies indicate that it is not an efficacious method for diagnosis of pneumococcal infection. Although difficulties with the specificity of the test can largely be overcome by the use of suitable controls, the method appears too insensitive to have usefulness as a routine diagnostic test.

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