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Commercial Latex Agglutination Tests for Detection of *Haemophilus influenzae* Type b and *Streptococcus pneumoniae* Antigens in Patients with Bacteremic Pneumonia

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The validity of commercial latex agglutination kits for detection of *Haemophilus influenzae* type b and *Streptococcus pneumoniae* antigens in serum and urine specimens was studied. We tested serum and urine specimens from 44 patients with bacteremic pneumonia (23 *S. pneumoniae*, 13 *H. influenzae* type b, 11 other) with commercial latex agglutination kits (Directigen, Bactigen) for *S. pneumoniae* and *H. influenzae* type b antigens. All specimen samples were randomized and read blindly by two readers. Interreader reproducibility was 100%. The sensitivity and specificity of both kits for *H. influenzae* type b antigens in serum and urine were >90%. None of the 24 urine samples from *S. pneumoniae* bacteremic patients were positive by either kit, although 6 ng of type 3 polysaccharide could be detected in spiked urine. Sensitivity for *S. pneumoniae* antigens in serum was 27% for Directigen and 38% for Bactigen. Specificity for *S. pneumoniae* antigens in serum was 95% for Directigen and 74% for Bactigen. The results suggest that the kits are useful in diagnosing *H. influenzae* type b pneumonia. However, the commercially available *S. pneumoniae* reagents tested appear to have limited utility for diagnosing *S. pneumoniae* pneumonia because both kits lack sensitivity and Bactigen lacks specificity, as well.

Acute lower respiratory tract infections (ALRI) are a major cause of morbidity and mortality in the United States and throughout the world. Better diagnostic methods for defining the causes of ALRI, particularly for disease resulting from bacteria, are needed for planning both treatment and prevention strategies. In general, methods currently available include blood and sputum cultures. Difficulties in using these methods for isolating the pathogenic organism to provide a definitive diagnosis of ALRI are well known. A positive blood culture provides the basis for a specific etiologic diagnosis, but the sensitivity of this method is low, even for pneumococcal pneumonia. The method of direct examination and culture of sputum lacks both sensitivity and specificity. Many healthy individuals will have *Streptococcus pneumoniae* and *Haemophilus influenzae* present in the nasopharynx, so the detection of these organisms on sputum culture does not necessarily imply pathogenic significance. Furthermore, these culture methods are even less sensitive if patients received antimicrobial agents before the clinical specimens were obtained.

As an alternative to culture, antigen detection is a promising noninvasive approach to sensitive and specific diagnosis. Bacterial antigens produced at the site of infection are distributed to body fluids and can be detected by various procedures. Methods currently available for detection of these antigens include counterimmunoelectrophoresis (CIE), radioimmunoassay, enzyme-linked immunosorbent assay, and agglutination tests. Although the radioimmunoassay and enzyme-linked immunosorbent assay are the most sensitive of these methods, they both require sophisticated equipment and expertise and, therefore, have not been evaluated for general clinical use for antigen detection. CIE is the least sensitive method and also requires relatively complex equipment and buffer systems. These drawbacks have limited the use of CIE for diagnosis of bacterial infections.

At this time, the most practical method for detection of bacterial antigens is the agglutination test. Latex-particle agglutination (LA) tests use latex particles coated with antibody specific for the polysaccharide antigen to be detected. These antibody-coated latex particles are mixed with the specimen from the patient, and if the antigen is present, a visible agglutination reaction develops. LA tests for detection of polysaccharide antigens produced by *S. pneumoniae* and *H. influenzae* type b in cerebrospinal fluids are sensitive and specific, but the usefulness of these tests for the detection of antigens in the serum and urine of patients with pneumonia has yet to be proven. This study was designed to evaluate the sensitivity and specificity of two commercial LA kits, Directigen (Hynson, Westcott and Dunning, Baltimore, Md.) and Bactigen (Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) for detection of *S. pneumoniae* and *H. influenzae* type b antigens in the serum and urine of patients with ALRI.

MATERIALS AND METHODS

Antigen detection tests. Individual Bactigen kits for the detection of *S. pneumoniae* and *H. influenzae* type b antigens and Directigen meningitis test kits were supplied by the respective manufacturers. The specific instructions that accompanied the kits for performing the tests on serum and urine were followed. Positive and negative controls supplied with the kits were run as specified. If the quantity was sufficient, negative urine specimens were retested after 25-fold concentration in Minicon B-15 concentrators (Ami-

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TABLE 1. Microbial diagnosis of bacteremic pneumonia in 44 patients from whom specimens were obtained

Etiologic agent isolated	No. of patients
Streptococcus penumoniae (type)	
5	1
7	1
10	1
14	2
16	1
19	2
23	1
34	1
Typing not done	13[a]
Haemophilus influenzae (type)	
a	2
b	13
d	1
Nontypable	1
Streptococcus pyogenes	3
Salmonella cholerae-suis	2
Klebsiella pneumoniae	1
Staphylococcus aureus	1
Total isolates	47[b]

[a] All local patients. One local patient had *H. influenzae* type b disease; the remaining 30 patients were from Papua New Guinea.

[b] Three patients had both *S. penumoniae* and *H. influenzae* cultured from their blood.

con Corp., Danvers, Mass.). Negative urine specimens from patients whose serum specimens were either missing or were reactive with LA were retested at dilutions of 1:2, 1:5, and 1:10 in glycine-buffered saline (0.1 M glycine in 0.9% saline, adjusted to pH 8.2). For the Directigen test, boiled serum and urine specimens were centrifuged at 13,000 × *g* for 3 min in a Fisher model 235B microcentrifuge before testing. For Bactigen tests, urine specimens were filtered through Millex or Swinnex HA 45-μm-pore-size filter units (Millipore Corp., Bedford, Mass.) with high protein-binding, mixed cellulose-ester filters, as suggested by Weinberg and Storch (14). Reactions were read independently by two persons.

Specimens. Specimens were obtained from two sources. One source was local patients with pneumococcal pneumonia. A total of 13 urine specimens and 12 serum specimens were obtained from 14 patients with bacteremia, on admission to a local hospital 4 to 6 days after onset of their illness. All specimens were stored at −70°C until tested. The serum and urine of one patient was tested on the day of collection.

The other source of specimens was pediatric patients with ALRI of diverse etiologies. Urine and serum specimens were obtained from 30 patients in Papua New Guinea within 7 days of onset of clinical pneumonia and bacteremia.The following etiologic agents had been isolated from the blood of these patients: *H. influenzae* type b, *S. pneumoniae*, *H. influenzae* type b and *S. pneumoniae*, *H. influenzae* type a, *H. influenzae* type a and *S. pneumonia*, *H. influenzae* type d, nontypable *H. influenzae*, *Streptococcus pyogenes*, *Salmonella cholerae-suis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Table 1). Specimens had been stored frozen for 0.5 to 2 years before they were shipped frozen from Papua New Guinea to the United States. At the Centers for Disease Control, specimens were stored at −70°C for 1 to 2 months until tested. Two samples sufficient for testing were transferred to vials and coded randomly with two sets of numbers, one set for testing with Bactigen reagents, the other with Directigen. To avoid bias, no culture results were known until testing had been completed. Serum and urine specimens from the same patient were coded and tested separately, and all tests were read blindly by two readers. Blood cultures from patients were processed by the routine methods in use at the participating hospitals.

TABLE 2. Sensitivity of LA kits for detection of *S. pneumoniae* and *H. influenzae* type b antigens

Antigen sought	Specimen	No. of true positives/total no. of positives tested (%)	
		Directigen	Bactigen
S. pneumoniae	Serum	6/22 (27)	8/21 (38)
	Urine	0/23 (0)	0/23 (0)
H. influenzae type b	Serum	11/12 (92)	12/12 (100)
	Urine	13/13 (100)	12/13 (92)

Sensitivity of tests with purified *S. pneumoniae* antigens. Directigen and Bactigen latex reagents for *S. pneumoniae* were tested against Pnu-Immune 23 vaccine (Lederle Laboratories, Pearl River, N.Y.) and type 3 pneumococcal polysaccharide (Merck Sharpe & Dohme, West Point, Pa.) diluted in normal urine and in glycine-buffered saline.

CIE. CIE was performed by standard methods. Equal portions of three pools of rabbit antisera produced at the Centers for Disease Control against 15 prevalent types of *S. pneumoniae* (types 1, 3, 4, 6, 7, 8, 14, 15, 18, 19, 20, 23, 24, 28, 40) were combined and tested opposite dilutions of pneumococcal vaccine in normal urine and specimens from the local group of patients.

RESULTS

LA tests for *H. influenzae* type b. (i) Sensitivity. All 13 urine specimens (100%) and 11 of 12 (92%) serum specimens from patients with confirmed *H. influenzae* type b disease were positive when tested with the Directigen *H. influenzae* type b latex reagent. With the Bactigen test, 12 of 13 (92%) urine specimens and all of 12 (100%) serum specimens were reactive (Table 2).

(ii) Specificity. Directigen tests on serum and urine specimens from patients who did not have *H. influenzae* type b pneumonia were 97% specific; Bactigen tests were 96% specific (Table 3). False-positive reactions occurred with specimens from two patients from whom *S. pneumoniae* and *Salmonella cholerae-suis* had been isolated.

LA tests for *S. pneumoniae*. (i) Sensitivity. Of 22 serum specimens from patients with culture-confirmed pneumococcal pneumonia, 6 (27%) were positive with the Directigen test, whereas 8 of 21 (38%) sera were reactive with the Bactigen reagents. None of 23 undiluted, 8 diluted, or 18 concentrated urine specimens were positive with either test (Table 2).

TABLE 3. Specificity of LA kits for detection of *S. pneumoniae* and *H. influenzae* antigens

Antigen sought	Specimen	No. of true negatives/total no. of negatives tested (%)	
		Directigen	Bactigen
S. pneumoniae	Serum	19/20 (95)	14/19 (74)
	Urine	20/21 (95)	17/21 (81)
H. influenzae type b	Serum	29/30 (97)	27/28 (96)
	Urine	29/30 (97)	27/28 (96)

TABLE 4. Etiologic agent isolated in cases of false-positive reactions

Etiologic agent	Specimen	Reagent giving false-positive LA test[a]
S. pneumoniae (type 5)	Serum	Bactigen (Hib)
	Urine	Directigen (Hib) and Bactigen (Hib)
S. choleraesuis	Serum	Directigen (Hib)
H. influenzae (type b)	Serum	Bactigen (*S. pneumoniae*)
H. influenzae (type b)	Serum	Bactigen (*S. pneumoniae*)
H. influenzae (type b)	Serum	Bactigen (*S. pneumoniae*)
	Urine	Bactigen (*S. pneumoniae*)
H. influenzae (type b)	Urine	Bactigen (*S. pneumoniae*)
H. influenzae (type d)	Serum	Bactigen (*S. pneumoniae*)
	Urine	Bactigen (*S. pneumoniae*)
H. influenzae (nontypeable)	Urine	Directigen (*S. pneumoniae*) and Bactigen (*S. pneumoniae*)
S. aureus	Serum	Bactigen (*S. pneumoniae*)
K. pneumoniae	Serum	Directigen (*S. pneumoniae*)

[a] Hib, *H. influenzae* type b.

Bactigen and Directigen tests detected as little as 25 ng of pneumococcal polysaccharides per ml with Pnu-Imune 23 vaccine (containing 575 μg of pneumococcal polysaccharides per 0.5-ml dose) diluted in glycine-buffered saline or normal urine. Both tests detected 20 ng of type 3 polysaccharide per ml diluted in glycine-buffered saline. With urine as the diluent, 6 ng/ml was detected by Bactigen and 12 ng/ml by Directigen.

(ii) Specificity. A total of 20 of 21 (95%) urine and 19 of 20 (95%) serum specimens from patients whose infections were caused by etiologic agents other than pneumococci gave negative results with the Directigen test (Table 3). The single reactive urine specimen came from a patient with nontypable *H. influenzae* bacteremia, whereas the serum specimen was from a patient with *K. pneumoniae* bacteremia. The Bactigen *S. pneumoniae* test was nonreactive with 17 of 21 (81%) urine specimens and 14 of 19 (74%) serum specimens from patients with bacteremia of different etiologies. The nonspecific reactions occurred with specimens from seven patients with infections caused by *H. influenzae* type b, *H. influenzae* type d, nontypable *H. influenzae*, or *Staphylococcus aureus* (Table 4).

CIE. Of 12 urine specimens from local patients with pneumococcal pneumonia, 5 (42%) were positive when examined by CIE. Three of the positive specimens were detected only after concentration. Of 11 sera, 2 (18%) were positive with CIE and came from patients whose urine specimens were also positive. With Pnu-Imune 23 vaccine, CIE detected 1.0 μg of total polysaccharides, but 100 ng was detected with type 3 polysaccharide alone.

Reader reproducibility. There was 100% agreement between the two readers in differentiating reactive from nonreactive LA tests and in detecting the precipitin lines in CIE gels. Directigen tests produced the most clearly defined positive and negative reactions and required the least time to read.

DISCUSSION

A number of studies have shown that LA tests are a practical and reliable method for detecting bacterial antigens in the cerebrospinal fluid of patients with meningitis (4, 10, 11, 15). The testing of serum and urine specimens by LA has not been recommended for the diagnosis of meningitis because the quantity of antigen circulating in these body fluids is usually less than in cerebrospinal fluid.

However, LA tests can detect *H. influenzae* type b antigen in the serum and urine of patients with meningitis and other systemic *H. influenzae* type b infections at sensitivity and specificity levels >90% (3, 5–7, 9, 13). Our data on the detection of *H. influenzae* type b antigens in serum and urine specimens of patients with bacteremic pneumonia support these findings. In our study, *H. influenzae* type b antigen was detected equally well in serum or urine specimens with either Directigen or Bactigen commercial LA kits.

In contrast to information on *H. influenzae* type b antigen detection, studies evaluating the utility of LA tests for the detection of *S. pneumoniae* polysaccharide antigen in serum and urine from patients with bacteremic, but nonmeningitic, *S. pneumoniae* disease have been inconclusive. In one study evaluating LA tests for *S. pneumoniae* antigen detection (Pneumoslide; BBL Microbiology Systems, Cockeysville, Md.), Van der Auwera et al. (12) found 53% of serum specimens from 15 patients with bacteremic pneumococcal pneumonia positive for *S. pneumoniae* antigen and 87% of concentrated urine specimens from 23 of these patients positive. Cerosaletti et al. (1), using Bactigen kits, found 24% sensitivity in serum specimens and 88% sensitivity in urine specimens from 17 patients with bacteremic pneumococcal pneumonia. On the other hand, Congeni et al. (2), using Wellcogen kits (Burroughs Wellcome Co., Research Triangle Park, N.C.), found that pneumococcal antigen was detected in two of six (33%) serum specimens from bacteremic patients and in none of five (0%) urine specimens. In a recent study in which Bactigen kits were evaluated for the diagnosis of bacteremic pneumococcal pneumonia, 8% sensitivity in serum and 19% sensitivity in urine were reported (S. Lenthe-Eboa, G. Brighouse, R. Auckenthaler, A. Zwahlen, D. Lew, and F. Waldvogel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, V22, p. 392). In a similar study, Bactigen detected *S. pneumoniae* antigen in 29% of urine specimens from patients with bacteremic infection (M. Lukaszewski and M. Simberkoff, personal communication).

In our study, both Directigen and Bactigen kits for *S. pneumoniae* antigen detection in serum and urine lacked sensitivity. Although previously reported studies have demonstrated better sensitivity for *S. pneumoniae* antigen detection in urine specimens compared with serum, we were unable to detect *S. pneumoniae* antigen in any urine specimen by the LA method. Of interest, 42% of urine specimens tested were positive for *S. pneumoniae* antigen by the CIE method. Variation in pneumococcal antigen detection by LA could be due to a number of different factors, such as specimen handling and storage. Also, host factors, such as age and stage of illness at the time of specimen collection, may influence the quantity and antigenic state of pneumococcal polysaccharides in body fluids.

In our study, we found that the Bactigen kit for *S. pneumoniae* antigen detection also lacked specificity. With polyvalent *S. pneumoniae* antiserum, false-positive reactions similar to those we encountered have been noted previously (8). However, it also is possible that these false-positive reactions represent true infections undetected by

routine culture methods. In our study, three patients had two organisms cultured simultaneously from their blood.

We conclude that the Directigen and Bactigen *H. influenzae* type b LA kits may be sensitive and specific methods for diagnosing *H. influenzae* type b pneumonia. We believe that these reagents are worth using at the present time and may improve the diagnosis of *H. influenzae* type b pneumonia. However, because the sensitivity and specificity are not 100%, these reagents should be used along with routine cultures. In contrast, LA with Directigen and Bactigen *S. pneumoniae* kits was not a sensitive method for detecting *S. pneumoniae* antigen in serum or urine specimens. Furthermore, Bactigen *S. pneumoniae* LA reagents also lacked specificity. Additional work with these *S. pneumoniae* reagents is needed if the kits are to be useful in diagnosing pneumococcal pneumonia.

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