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Comparison of Latex Agglutination and Counterimmunoelectrophoresis for the Detection of Pneumococcal Antigen in Elderly Pneumonia Patients

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A *Streptococcus pneumoniae* latex agglutination (LA) test (Bactigen; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) and counterimmunoelectrophoresis (CIE) were compared for the detection of pneumococcal antigen in serum and urine specimens from 68 elderly patients with pneumococcal pneumonia. The cases were categorized according to the presumptive role of *S. pneumoniae*: definite, putative, questionable (poor score), or questionable (mixed flora). Serum and urine samples were collected on days 1 to 3, 4 to 6, and 7 to 9 of illness and screened in parallel by LA and CIE. LA detected pneumococcal antigen in the serum or urine or both from 31 (46%) of the 68 pneumococcal pneumonia cases compared with 10 (15%) of cases detected by CIE. The highest rates of detection were noted in the 17 definite (bacteremic) cases: 88% by LA and 38% by CIE. The detection rates for both tests were lower in the other nonbacteremic pneumonia categories. Pneumococcal antigen was detected more often in urine specimens than in serum specimens by LA and CIE and was detected in the urine of 92 and 46% of definite cases, respectively, after 7 to 9 days of illness despite antibiotic therapy. Both tests were specific when tested with nonpneumococcal pneumonia cases, but LA detected pneumococcal antigen in two of seven chronic bronchitis cases. This study suggests that LA is as specific and more sensitive than CIE and is useful for detecting antigen in the elderly with proven bacteremic pneumococcal pneumonia. LA is less sensitive for detecting nonbacteremic pneumococcal pneumonia and, therefore, would be of limited value in the care and study of the institutionalized elderly.

The incidence of pneumococcal pneumonia in the elderly is reported to be approximately 9 to 46 cases per 1,000 per annum (3, 19) and 13 to 16 cases per 1,000 per annum (4) for the elderly residing in communities and institutions, respectively, compared with an incidence of 1 to 2 cases per 1,000 per annum for the general population (1, 12). The incidence figures for the elderly may be spuriously low, however, because of the difficulty in obtaining optimum sputum specimens from elderly patients and the infrequent use of routine diagnostic studies.

Alternate methods of diagnosing pneumococcal disease have been implemented in acute-care settings in recent years as adjuncts to routine microbiological workup. The detection of pneumococcal capsular antigen by counterimmunoelectrophoresis (CIE), latex agglutination (LA), and staphylococcal coagglutination has proven useful in diagnosing pneumococcal pneumonia and bacteremia in community-residing populations (9, 11, 16). There are no reports, however, outlining trials of antigen detection tests in community- and institutional-residing elderly populations with pneumococcal pneumonia.

The purpose of this study was to compare CIE with a commercially available pneumococcal LA kit (Bactigen; Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) for the detection of pneumococcal antigen in serum and urine of community- and institutional-residing elderly patients with pneumococcal pneumonia.

MATERIALS AND METHODS

Patients. A surveillance system was established in an acute tertiary care hospital and two long-term care facilities to identify cases of community-acquired and institutional-acquired pneumococcal pneumonia, respecitvely. Sixty-eight patients (mean age, 70 ± 13 years) with roentgenographic evidence of a new pneumonia and a positive blood culture or respiratory secretion culture for *Streptococcus pneumoniae* were enrolled in the study.

Fifteen additional patients were enrolled in the study as controls. Seven patients, with an exacerbation of chronic bronchitis characterized by a positive respiratory secretion for *S. pneumoniae* but no roentgenographic evidence of pneumonia, were included to assess the diagnostic discrimination of CIE and LA. To evaluate test specificity, eight patients were studied who had roentgenographically proven pneumonia associated with respiratory secretion cultures demonstrating organisms other than *S. pneumoniae*: *Staphylococcus aureus* (four patients), *Klebsiella* sp. (one patient), *Haemophilus* sp. and *Enterobacter sakazakii* (one patient), and upper respiratory tract flora only (two patients).

Microbiological studies. *S. pneumoniae* isolates were presumptively identified by ethyl hydrocuprein hydrochloride sensitivity and bile solubility. Isolates were typed by the Quellung reaction by the method of Austrian (2), using type-specific pneumococcal antisera (Statens Seruminstitut, Copenhagen, Denmark) for definitive identification.

Gram-stained smears of respiratory secretions positive for *S. pneumoniae* were examined under ×100 magnification, and oropharyngeal contamination was assessed according to the number of squamous epithelial cells (SECs) and polymorphonuclear leukocytes (PMNs) per low power field, using a modification of the method of Murray and Washington (18). Smears were scored as follows: 0 = <10 PMNs, <10 SECs; 1 = <10 PMNs, >25 SECs; 2 = 10 to 25 PMNs,

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TABLE 1. Detection rates of pneumococcal antigen by CIE and LA in serum and urine specimens from patients with pneumococcal pneumonia, chronic bronchitis, and nonpneumococcal pneumonia

Category (*n*)	No. positive cases/no. tested (%)					
	CIE			LA		
	Serum	Urine	Serum or urine or both[a]	Serum	Urine	Serum or urine or both[a]
Definite (17)	3/17 (18)	6/16 (38)	6/17 (35)	4/17 (24)	14/16 (88)	15/17 (88)
Putative (28)	0/26	2/21 (10)	2/28 (7)	2/26 (8)	8/21 (38)	10/28 (36)
Questionable (poor score) (15)	0/15	2/14 (14)	2/15 (13)	1/15 (7)	2/14 (14)	3/15 (20)
Questionable (mixed flora) (8)	0/8	0/6	0/8	1/8 (12)	2/6 (33)	3/8 (38)
Chronic bronchitis (7)	0/6	0/4	0/7	0/6	2/4 (50)	2/7 (29)
Nonpneumococcal pneumonia (8)	0/8	0/8	0/8	0/8	0/8	0/8

[a] Data represent the total number of cases/number of cases tested for that particular category. Each case was counted once, even if antigen was detected in both serum and urine of that individual.

>25 SECs; 3 = >25 PMNs, >25 SECs; 4 = >25 PMNs, 10 to 25 SECs; 5 = >25 PMNs, <10 SECs; 6 = 10 to 25 PMNs, <10 SECs; 7 = 10 to 25 PMNs, 10 to 25 SECs; and 8 = <10 PMNs, 10 to 25 SECs.

Each case of pneumococcal pneumonia was subsequently categorized according to the likely role of *S. pneumoniae* as the etiological agent. Definite (17 cases) = *S. pneumoniae* was the sole respiratory pathogen isolated from blood. Of these, 7 of 13 (54%) also had *S. pneumoniae* recovered from an accompanying respiratory secretion culture. Putative (28 cases) = *S. pneumoniae* was the sole respiratory pathogen isolated from a sputum or nasopharyngeal aspirate only which showed minimal oropharyngeal contamination (scores 4 to 7). Questionable (poor score) (15 cases) = *S. pneumoniae* was the sole respiratory pathogen isolated from a sputum or nasopharyngeal aspirate only which showed considerable oropharyngeal contamination (scores 0 to 3, 8). Questionable (mixed flora) (8 cases) = *S. pneumoniae* was isolated along with one or more other respiratory pathogens from a sputum or nasopharyngeal aspirate only regardless of the degree of oropharyngeal contamination. Organisms considered as other respiratory pathogens included *Staphylococcus aureus*, *Haemophilus influenzae*, *Haemophilus* sp., and any aerobic gram-negative bacilli.

Specimens for antigen detection. Whenever possible, paired serum and urine specimens were obtained within 72 h of the onset of illness. Day 1 of illness was the date of the first diagnostic chest roentgenogram or positive culture, whichever was earlier. To assess the short-term persistence of antigen, urine and serum specimens were also obtained on days 4 to 6 and 7 to 9. All specimens, with the exception of three sera, were obtained after antibiotics were started. Specimens were stored at −20°C from 2 months to 1.5 years before testing.

All serum and urine specimens collected from the same patient were thawed and tested in parallel by CIE and LA. Serum samples were centrifuged if turbid. Urine samples were cultured quantitatively to screen for contaminants, centrifuged if turbid, and filtered through 0.45-μm MF-Millipore filters (Millipore Corp., Bedford, Mass.). Urine samples were initially tested unconcentrated. If no antigen was detected, urine samples were retested following a 25-fold concentration, using Amicon concentrators (Amicon Corp., Lexington, Mass.). To rule out antigen excess, negative urine specimens collected from patients with definite pneumococcal pneumonia were also diluted 1:10 with Bactigen specimen buffer and were retested.

Antigen detection tests. A discontinuous CIE system was performed, using a modification of the procedures of Ingram et al. (13) and Rytel (20). Briefly, lantern slides (82 by 102 mm) were coated with 10.5-ml aliquots of a 1% agarose solution (Calbiochem-Behring, La Jolla, Calif.) in sodium barbital buffer (pH 8.6) (ionic strength, 0.05) (Fisher Scientific, Rochester, N.Y.). Paired wells (diameter, 3 mm) were punched 5 mm apart in the prepared slide and filled with 10 μl of specimen or pneumococcal antisera. All specimens were tested against pneumococcal omniserum (Statens Seruminstitut). Electrophoresis was performed at 15 mA per slide, constant current, for 45 min with sodium barbital buffer (pH 8.6) (ionic strength, 0.075) (Fisher Scientific) in the electrophoresis chamber reservoirs. Slides were refrigerated after electrophoresis in a humid chamber and read at 20 min and overnight with a dark-ground viewer. Positive and negative controls were included on each slide.

Bactigen LA (Wampole Laboratories) was performed according to the instructions of the manufacturer.

The sensitivities of CIE and LA to detect purified pneumococcal capsular polysaccharide were evaluated. Type 3 polysaccharide (Merck Sharp & Dohme, West Point, Pa.) was selected as a representative polysaccharide because it is a common cause of pneumococcal disease and antibodies to type 3 are present in an adequate titer in pneumococcal antiserum. Twofold dilutions of the antigen were made in Bactigen specimen buffer, fetal bovine serum, and urine, beginning at 1:2 and continuing to 1:128 for CIE and 1:2,048 for LA. Dilutions were tested in parallel by both tests. The sensitivity was calculated as the concentration of antigen in the last positive well. For CIE, this was the last well demonstrating a visible precipitin line, and for LA, this was the last well demonstrating ≥1+ agglutination.

RESULTS

Detection rates of pneumococcal antigen. The results of pneumococcal antigen detection in serum and urine specimens by CIE and LA are summarized in Table 1. Of the 68 elderly patients with pneumococcal pneumonia, LA detected antigen in serum or urine or both from 31 (46%) cases compared with 10 (15%) cases detected by CIE. The highest rates of detection were noted in the 17 definite cases, 88% by LA and 35% by CIE. The detection rates for both tests were

markedly lower in the putative, questionable (poor score), and questionable (mixed flora) categories. LA alone detected pneumococcal antigen in urine from two of the four chronic bronchitis cases with urine available for testing. Regardless of the category, LA detected pneumococcal antigen more frequently than CIE. Despite the lower detection rates, however, CIE did detect antigenemia in one definite case and antigenuria in two questionable (poor score) cases when pneumococcal antigen was not detected by LA.

Both CIE and LA detected antigen more often in urine than in serum (Table 1). In only three cases did LA detect antigen in the serum and not in urine: one definite, one questionable (poor score), and one questionable (mixed flora) case. Detection of pneumococcal antigen in urine by CIE was more dependent on 25-fold concentration of the antigen than detection by LA. Of the 10 cases with pneumococcal antigen detected in urine by CIE, 5 were detected in unconcentrated urine and 5 after a 25-fold concentration. In contrast, of the 28 urine-positive cases detected by LA, 22 were detected in unconcentrated urine, 2 after a 25-fold concentration, and 4 definite cases after 1:10 dilution.

Duration of pneumococcal antigen detection. The duration of pneumococcal antigen detection in serum and urine from the 17 definite cases is illustrated in Fig. 1. Both CIE and LA detected antigen in serum and urine at days 1 to 3, 4 to 6, and 7 to 9 despite concomitant antibiotic therapy. In fact, LA detected pneumococcal antigen in the urine of two putative cases studied at days 10 and 12.

The detection rates in serum and urine varied between days 1 to 3, 4 to 6, and 7 to 9 primarily due to incomplete specimen collection during the periods. An analysis of positive cases which had complete specimen collection, however, showed that once pneumococcal antigen was detected, it persisted throughout the sampling period. The only exception to this pattern was noted in two cases which were positive for antigen in serum by LA at days 4 to 6 but were negative at days 7 to 9. CIE, however, continued to detect pneumococcal antigen in one of these cases at days 7 to 9, indicating that the increased sensitivity of CIE over LA in serum at days 7 to 9 (25 versus 8%) was a reflection of the tests rather than of specimen collection.

Antigen detection and pneumococcal serotypes. The relationships between infecting pneumococcal serotypes and the results of antigen detection in definite and putative pneumococcal pneumonia cases by CIE versus LA are summarized in Tables 2 and 3, respectively. The 17 bacteremic cases, representing primarily community-acquired pneumococcal pneumonia, were caused by 12 different serotypes. All were represented in the current 23-valent vaccine. CIE or LA or both detected pneumococcal antigen in the serum or urine from each of the serotypes, except the case caused by serotype 22.

Of the 28 putative cases, representing primarily institution-acquired pneumococcal pneumonia, 14 different serotypes were isolated, including 5 (39%) nonvaccine types (Table 3). Pneumococcal antigen was detected in serum or urine or both from 10 cases caused by serotypes 8, 9, 11, 15, 17, 20, 28, and 31.

Sensitivity and specificity of antigen detection tests. Both

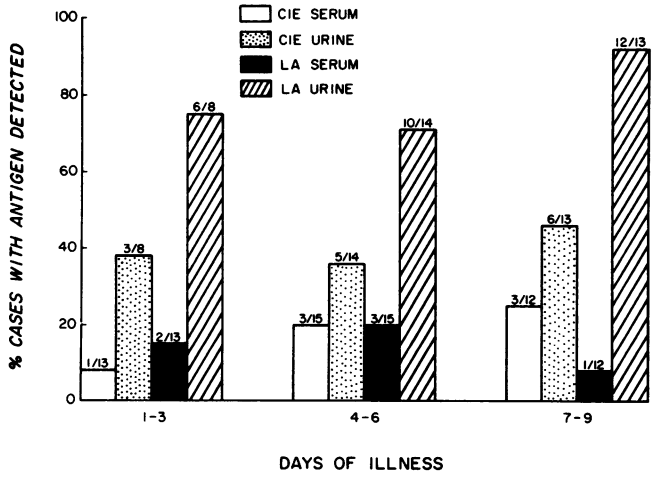

FIG. 1. Duration of pneumococcal antigen detection by CIE and LA in serum and urine from 17 definite pneumococcal pneumonia cases. Values indicate the number of positive cases/number of cases tested.

TABLE 2. Pneumococcal serotypes isolated from 17 definite (bacteremic) cases of pneumonia and detection of pneumococcal antigen

Pneumococcal serotype isolated	No. of patients	No. of patients with antigen detected					
		Serum			Urine		
		CIE	LA	Neither	CIE	LA	Neither
3	1	1	1	0	1	1	0
4	2	0	0	2	1	2	0
5	1	0	0	1	0	1	0
6	3	0	0	3	0	2	0[a]
8	2	1	2	0	1	1	1
10	1	0	0	1	1	1	0
12	1	0	0	1	0	1	0
18	1	0	1	0	0	1	0
19	2	1	0	1	1	2	0
22	1	0	0	1	0	0	1
23	1	0	0	1	1	1	0
33	1	0	0	1	0	1	0

[a] No urine collected for one type 6 case.

TABLE 3. Pneumococcal serotypes isolated from 28 putative (nonbacteremic) cases of pneumonia and detection of pneumococcal antigen

Pneumococcal serotype isolated	No. of patients	No. of patients with antigen detected					
		Serum			Urine		
		CIE	LA	Neither	CIE	LA	Neither
6	2	0	0	2	0	0	2
8	3	0	1	2	0	0	2[a]
9	2	0	0	1[b]	0	2	0
11	1	0	0	1	0	1	0
15	1	0	0	1	1	1	0
16[c]	1	0	0	1	0	0	1
17	3	0	0	3	0	1	0[d]
19	2	0	0	1[b]	0	0	2
20	1	0	0	1	1	1	0
23	1	0	0	1	0	0	1
25[c]	2	0	0	2	0	0	2
28[c]	3	0	1	2	0	0	1[d]
31[c]	3	0	0	3	0	2	0[a]
34[c]	2	0	0	2	0	0	2
Not available	1	0	0	1	0	0	0[a]

[a] No urine collected from one case.
[b] No serum collected from one case.
[c] Serotypes not represented in 23-valent pneumococcal vaccine.
[d] No urine collected from two cases.

TABLE 4. Incidence of inconclusive reactions in serum and urine with LA[a]

Category	Incidence in[b]:			
	Serum	Unconcentrated urine	Concentrated urine[c]	Diluted urine
Definite	3/17 (18)	1/16 (6)	1/8 (12)	2/11 (18)
Putative	3/26 (12)	4/21 (19)	4/13 (31)	NA[d]
Questionable (poor score)	2/15 (13)	3/14 (21)	7/9 (78)	NA
Questionable (mixed flora)	1/8 (12)	0/6	0/3	NA
Chronic bronchitis	1/6 (17)	2/4 (50)	2/4 (50)	NA
Nonpneumococcal pneumonia	0/8	0/8	NA	NA

[a] No inconclusive reactions were noted with CIE.
[b] Values indicate the number of cases with at least one inconclusive reaction/number of cases tested. Numbers in parentheses are percentages.
[c] 25-fold concentration was performed only if unconcentrated urine was negative.
[d] NA, Test not performed.

CIE and LA were found to be highly sensitive and specific. The lower limits for detecting type 3 purified capsular polysaccharide were 15.6 and 0.975 ng/ml for CIE and LA, respectively. Equal sensitivity was demonstrated in buffer, fetal bovine serum, and urine; however, LA was consistently 16 times more sensitive than CIE regardless of the diluent. Both CIE and LA were specific when tested with urine and serum specimens from eight cases of nonpneumococcal pneumonia (Table 1).

Although LA was more sensitive and as specific as CIE in detecting pneumococcal antigen, the interpretation of LA results was more difficult. Inconclusive reactions, granularity in both test and control wells, were recorded in the serum of 10 of 80 (12%) cases tested by LA and in the unconcentrated urine of 10 of 69 (14%) cases despite the use of specimen buffer-absorbant before testing (Table 4). The number of inconclusive reactions observed with LA was higher in urine concentrated 25-fold. The manufacturers do not advocate concentration of urine for this reason. No inconclusive reactions were observed with CIE. The inconclusive reactions recorded with LA were frequently observed in multiple specimens from a single patient, indicating the presence of an interfering factor responsible for the reaction, and were not restricted to a single pneumonia category. There was no relationship between urine contaminants and inconclusive reactions.

DISCUSSION

This is the first report comparing the usefulness of the Bactigen LA *S. pneumoniae* test with CIE for detecting pneumococcal antigen in patients with pneumococcal pneumonia. Our findings indicate that LA is superior to CIE for detecting pneumococcal antigen in elderly patients with pneumococcal pneumonia. Urine specimens obtained from elderly pneumonia patients up to 9 to 12 days after the onset of illness proved to be most useful for antigen detection purposes. The advantage of LA over CIE in the present investigation is probably due to the extreme sensitivity of the Bactigen LA test coupled with the use of the Bactigen specimen buffer-absorbant to remove interfering factors. This advantage, however, was limited primarily to bacteremic pneumonia cases.

Several investigators have reported trials of LA versus CIE for detecting antigen in pneumococcal disease. In a series of cases of meningitis, Bactigen LA detected pneumococcal antigen in 100% of cerebrospinal fluid specimens, whereas CIE detected pneumococcal antigen in 71% of specimens (22). Likewise, the commercially available Burroughs Wellcome *S. pneumoniae* LA test was also found to be more sensitive than CIE in detecting pneumococcal antigen in cerebrospinal fluid and urine from patients with pneumococcal meningitis and septicemia but less sensitive than CIE for detecting antigen in serum (14). Previous studies with noncommercial LA tests have shown LA to be either more or less sensitive than CIE for detecting antigen in cases of pneumococcal disease (8, 21).

The superiority of urine as an antigen detection specimen, as noted in this study, has previously been documented. Coonrod and Rytel (9) detected pneumococcal antigen by CIE in serum, untreated urine, or an ethanol-precipitated fraction of urine (20-fold concentration) in 20, 30, and 47% of cases of pneumococcal pneumonia, respectively. The increased detection of pneumococcal antigen in urine versus serum can be attributed to the lack of confounding serum antibodies, natural concentration of the antigen by the kidneys, and the ease with which urine can be further concentrated 20- to 50-fold (6).

Prolonged detection of pneumococcal antigen in the serum and urine of patients with pneumococcal pneumonia and bacteremia, ranging from 10 to 100 days, has been reported by several investigators (7, 9, 10). The manner in which pneumococcal polysaccharides are handled by the immune system is largely unknown. Investigations with rodent models suggest that pneumococcal polysaccharides are highly resistant to degradation. Therefore, metabolism occurs slowly, resulting in chronic urinary excretion of the degraded polysaccharide (5, 15). Prolonged detection of pneumococcal antigen occurred in our study despite concomitant antibiotic therapy and has been reported by other investigators as well (9, 23). These results substantiate the usefulness of pneumococcal antigen detection as a diagnostic tool after antibiotics have been started, when body fluid cultures may be negative, and suggest that urine specimens, in particular, may be obtained as late as 9 to 12 days after the onset of pneumococcal pneumonia for antigen detection.

Increased rates of antigen detection in bacteremic pneumococcal pneumonia compared with nonbacteremic pneumonia have been documented (9, 17). Presumably, the increased detection is associated with more advanced infection and larger numbers of *S. pneumoniae* which exceed the clearance capacity of the reticuloendothelial system. In addition, pneumococcal serotypes causing bacteremic versus nonbacteremic disease may differ as noted in our series and in a previous investigation (4). The relative success of detecting different type-specific pneumococcal polysaccharides may vary depending on the molecular structure of the polysaccharide, the sensitivity of the antigen detection test for the particular pneumococcal types, including the relative concentration and avidity of the type-specific antibodies used in the test, and the presence or level of preexisting pneumococcal antibodies in the serum (6, 8, 9). Thus, differences in serotype distribution and detection may also contribute to increased antigen detection in bacteremic versus nonbacteremic cases. To increase the rates of pneumococcal antigen detection in the institutionalized elderly, who characteristically have a low rate of bacteremia-associated pneumococcal pneumonia, it may be necessary to enrich the source of type-specific pneumococcal antibody, e.g., omniserum, with globulin to those particular pneumo-

coccal serotypes prevalent in this high-risk population. Alternatively, latex beads coated with higher-titered pool antisera may be useful in this setting.

The detection of pneumococcal antigen in culture-positive chronic bronchitis cases has been reported by other investigators. Edwards and Coonrod (11) detected pneumococcal antigen in 75% of sputum samples by CIE and coagglutination. Eng et al. (R. H. K Eng, S. Suwangool, R. Joshi, and H. Chmel, Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 501 1983) detected pneumococcal antigen in 50% of concentrated urine specimens from chronic bronchitic patients with a coagglutination assay. In this investigation, antigenuria was detected by LA alone in two of the four cases with urine available for testing. Both patients appeared to have had more serious exacerbations than the other five cases as noted by leukocytosis and tachypnea. These findings suggest that the detection of pneumococcal antigen with more sensitive assays may require that the results be reported semiquantitatively when attempting to distinguish pneumococcal pneumonia from chronic bronchitis associated with *S. pneumoniae*.

Although inconclusive reactions were encountered with LA in the present study, LA was a much simpler and less time-consuming test to perform than CIE. In addition, LA does not require specialized equipment and advanced technical expertise for performance. LA is a specific and more sensitive test than CIE for detecting pneumococcal antigen in urine from elderly patients with pneumococcal pneumonia and should prove useful as a diagnostic tool in the care and study of community-residing elderly populations. The low rates of antigen detection in cases of nonbacteremic pneumococcal pneumonia, however, limit the use of LA in institutional-residing elderly. Further studies of institution-acquired pneumonia with latex beads coated with higher-titered antisera should be performed to improve the sensitivity and usefulness in this high-risk population.

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