

**Detection of Specific Bacterial Antigen by
Counterimmunoelectrophoresis (CEP)**

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OBJECTIVE: To detect specific bacterial antigen in infected body fluids by CEP and to compare the presence of antigen with bacteriological studies of the same specimens.

BACKGROUND: Pneumococcal and *Hemophilus influenzae* b capsular antigens have been detected in the cerebrospinal fluid (CSF) of patients with purulent meningitis at Children's Hospital. *H. influenzae* b antigen has also been detected in subdural effusions from patients with *H. influenzae* b meningitis and in the pericardial fluid from a patient with a pericardial effusion, while pneumococcal antigen has been detected in pleural fluid obtained from patients with pneumococcal empyema.

Bacterial antigen has been detected when Gram's stain and culture results have been negative, thus providing an etiologic diagnosis despite negative bacteriological studies.

DESCRIPTION: CEP was carried out on a variety of body fluids, CSF, subdural fluid, pleural fluid and serum. CEP using commercial antisera against *H. influenzae* b, pneumococcus and meningococcus type A-D; and antisera prepared in rabbits against *Staphylococcus aureus* was carried out by a previously described method (1).

PROGRESS: *H. influenzae* b antigen was detected in the initial CSF of 16 patients with purulent meningitis (Table 1). Eight patients had positive Gram's stain and cultures for *H. influenzae* b. Four patients had negative Gram's stains.

Table 1. Detection of *H. influenzae* b Antigen

Fluid Source	Positive specimens			Antigen duration (mean days)
	Gram's stain	Culture	CEP	
CSF	8	12	16	3.8
Subdural	1	1	6	9.1
Pleural	0*	0*	4	Not done
Serum	Not done	Not done	4	4.6

*1 Specimen: *Escherichia coli*

stain and positive cultures, and four patients had both negative Gram's stain and cultures. In eleven patients repeat CSF samples were studied and the mean duration of *H. influenza b* antigen was 3.8 days (range 0–11 days). Subdural effusions were present in six patients with *H. influenza b* meningitis. One was Gram's stain and culture positive for *H. influenza b*, the remainder were negative. The mean duration of antigen in subdural fluid, from the day of admission, was 9.1 days (range 1–16 days).

H. influenza b antigen was detected in pleural fluid from four patients. One patient had *Escherichia coli* on Gram's stain and culture of the pleural fluid while the remaining patients had negative Gram's stains and cultures. One of these remaining patients had purulent meningitis and a subdural effusion also; *H. influenza b* antigen was detected in these fluids as well.

H. influenza b antigen was found in the sera of four meningitis patients with a mean duration of 4.6 days (range 2–6 days) from the day of admission.

Table 2. Detection of Pneumococcal Antigen

Fluid source	Positive specimens			Antigen duration (mean days)
	Gram's stain	Culture	CEP	
CSF	3	5	11	4.3
Subdural	2	2	2	3.5
Pleural	6	7	15	15.2
Serum	Not done	Not done	3	10.7
Pericardial	1	1	1	1

Pneumococcal antigen was detected in the initial CSF fluid of 11 patients with purulent meningitis (Table 2). Three patients had positive Gram's stain and five patients had positive cultures for pneumococci. In nine patients repeat CSF samples were studied and the mean duration of pneumococcal antigen was 4.3 days (range 0–11 days). Subdural effusions were present in two patients with pneumococcal meningitis; both were culture positive and Gram's stain positive. Antigen was detected three and four days after admission.

Pneumococcal antigen was detected in the pleural fluid from 15 patients. Six had positive Gram's stain and seven had positive cultures for pneumococci. The mean duration of antigen in pleural fluid was 15.2 days (range 0–44 days).

Pneumococcal antigen was present in the serum of two patients with meningitis and one patient with empyema for an average of 10.7 days (range 4–15 days) after admission.

One patient with a pyopericardium had pneumococcal antigen detected in the pericardial fluid, which was also Gram's stain and culture positive for pneumococci.

Staphylococcal antigen was detected in the pleural fluid from 13 patients. Only one had a positive Gram's stain and nine had positive cultures. Staphylococcal antigen was detected in fluid from other sites in individual patients as well (Table 3).

Table 3. Detection of Staphylococcal Antigen

Fluid source	Positive specimens		
	Gram's stain	Culture	CEP
Pleural	1	9	13
CSF	0	0	1
Subdural	Not done	0	1
Abscess	1	1	1
Pericardial	1	1	1

DISCUSSION: Employing specific antisera to detect *H. influenza b*, pneumococcal and staphylococcal antigen by counterimmunoelectrophoresis has been useful in providing a rapid etiologic bacterial diagnosis of cases of purulent meningitis and empyema. This technique detects bacterial antigen when Gram's stains and cultures are negative, thus it appears to be a useful adjunct to routine bacteriologic methods. One cross reaction of *H. influenza b* antisera with *Escherichia coli* from pleural fluid was noted, and this may limit the usefulness of the procedure should more frequent cross reactions be observed.

REFERENCES:

Coonrod, J. D. and Rytel, M. W.: Determination of Etiology of Bacterial Meningitis by Counter-Immuno-electrophoresis. *Lancet* 1: 1154, 1972.