Detection of Specific Bacterial Antigen by Counter Immunoelectrophoresis (CIE)

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OBJECTIVE: To detect specific bacterial antigen in infected body fluids by CIE and to compare the presence of antigen with gram stain and culture results from the infected body fluid.

BACKGROUND: Bacterial capsular antigens of pneumococcus, *Hemophilus influenza* type b, and meningococcus have been detected in body fluids of patients with disease due to these bacteria. Specific bacterial antigens have been detected in cerebrospinal fluid (CSF), subdural fluid, synovial fluid, serum and urine using CIE and specific antisera. The CIE technique may detect infection even when gram stains and cultures are negative. It has been estimated that CIE gives false negative results in about 10% of CSF specimens (CIE negative but culture positive).

Purulent meningitis is common at Children's Hospital, Bangkok. Eighty to one hundred twenty cases are seen annually. Gram stains and bacterial cultures of the CSF have been negative in over 50% of the cases seen in the past six months. Prior treatment with antibiotics is one reason for the failure to recover organisms in culture.

Empyema cases are also common at Children's Hospital. Eighty to one hundred cases are seen annually. Gram stains and cultures are negative in approximately 50% of the cases.

Hemophilus influenza type b and pneumococcus are the most frequent bacterial isolates from CSF; pneumococcus and staphylococcus are the most frequent isolates from empyema specimens.

DESCRIPTION: Gram stains and cultures were performed on CSF specimens from cases of suspected purulent meningitis and on pleural fluid specimens from cases of empyema using routine bacteriological methods. CIE using antisera against pneumococcus and antisera against *Hemophilus influenza* type b was carried out by a previously described method (1). Follow-up CSF and empyema specimens had cultures and CIE performed. Subdural and pericardial fluids were also studied when available.

PROGRESS: *Hemophilus influenza* type b antigen has been detected in thirteen CSF specimens. Eleven of these were confirmed by positive culture (Table 1). Gram stains were positive on only three occasions. There has been only one false negative (CIE negative but culture positive). *Hemophilus influenza* type b antigen has persisted in the CSF for 2–5 days. Subdural effusions from 2 of these 14 patients have had CIE detectable *H. influenza* type b antigen. One patient with a purulent pericardial effusion has had detectable *H. influenza* type b antigen in pericardial fluid.

Four CSF specimens have had detectable pneumococcal antigen (Table 2). Three were confirmed by gram stain and two have been confirmed by culture. There has been one false negative (CIE negative but culture positive). Pneumococcal antigen persisted in the CSF for 11 days (1 patient) and 13 days (1 patient). Cases of tuberculous meningitis and salmonella meningitis have not cross-reacted with the pneumococcal or *Hemophilus influenza* type b antisera.

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Table 1. Presence of *Hemophilus influenza* type b Antigen in CSF, Subdural Fluid and Pericardial Fluid

Case	Source	Gram Stain	Culture	CIE	Duration
1	CSF	+	+	+	4 days
2	"	+	+	+	3 days
3	"	+	+	+	5 days
4	"	−	+	+	4 days
5	"	−	+	+	
6	"	−	+	+	
7	"	ND	+	+	
8	"	ND	+	+	
9	"	ND	+	+	
10	"	ND	+	+	
11	"	ND	+	+	
12	"	ND	−	+	3 days
13	"	ND	ND	+	2 days
14	"	−	+	−	
15	Subdural	−	−	+	9 days
16	"	−	−	+	
17	Pericardial	ND	ND	+	4 days

ND = no determination

Table 2. Presence of Pneumococcal Antigen in CSF

Case	Gram Stain	Culture	CIE	Duration
1	+	+	+	13 days
2	+	−	+	11 days
3	+	−	+	
4	−	+	+	
5	−	+	−	

Table 3. Presence of Pneumococcal Antigen in Empyema Fluid

Case	Gram Stain	Culture	CIE	Duration
1	+	+	+	
2	+	+	+	
3	+	+	+	
4	+	+	+	
5	+	+	+	
6	+	+	+	
7	+	–	+	
8	+	–	+	10 days
9	–	+	+	11 days
10	–	+	+	
11	–	–	+	13 days
12	–	–	+	
13	–	–	+	
14	–	–	+	
15	–	–	+	

Pneumococcal antigen has been detected by CIE in 15 cases of empyema (Table 3). Of these fifteen, six have had both positive gram stains and positive cultures for pneumococcus; two have had positive gram stains but negative cultures; and two have had negative gram stains and positive cultures. Five have had negative gram stains and negative cultures. CIE detectable antigen has persisted from 10–13 days in 3 patients studied. There have been no cross reactions in empyema cases with isolates of *E. coli* or *Staphylococcus aureus*. There have been no false negatives (CIE negative but culture or gram stain positive).

DISCUSSION: CIE is a useful clinical laboratory procedure for detecting capsular bacterial antigens of pneumococcus and *Hemophilus influenza* type b in infected body fluids. This technique can detect bacterial antigen when gram stains and cultures are negative. Results using CIE are more rapidly available than culture. In the experience at Children's Hospital CIE results are more reliable than gram stains, cultures or the combination of gram stain and culture. This technique is particularly useful in cases of purulent meningitis or empyema that have received prior antibiotic therapy, in which gram stains and cultures are often negative.

REFERENCES:

Coonrod, J.D. and Tytel, M.W.: Determination of Etiology of Bacterial Meningitis by Counter–Immunoelectrophoresis. Lancet 1:1154, 1972.