

Comparison of Phadebact coagglutination tests with counterimmunoelectrophoresis for the detection of bacterial antigens in cerebrospinal fluid

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SUMMARY One hundred and seventeen specimens of cerebrospinal fluid from 94 patients were examined for the presence of pneumococcal and *Haemophilus influenzae* type b antigens using counterimmunoelectrophoresis and coagglutination tests. The coagglutination method using Phadebact reagents was as sensitive as counterimmunoelectrophoresis, but culture was a more sensitive diagnostic procedure than either test. A meningococcus coagglutination reagent, included in a prototype meningitis diagnostic kit, was also found to be as sensitive as counterimmunoelectrophoresis when tested on culture-positive cerebrospinal fluid specimens. Coagglutination tests for the detection of bacterial antigen are useful supportive tests when used in conjunction with direct microscopy and culture for bacterial pathogens.

Several techniques have been described which increase the speed and sensitivity of the diagnosis of bacterial meningitis in comparison with a Gram-stained film and culture of cerebrospinal fluid (CSF). There are non-specific indicators of bacterial infection such as the detection of Gram-negative endotoxin and the measurement of lactate in CSF,^{1,2} and the measurement of C-reactive protein in serum.³ Specific techniques include the detection of bacterial antigen in CSF by counterimmunoelectrophoresis (CIE). Early studies suggested that the sensitivity of CIE was comparable to that of Gram stain and culture of CSF combined,⁵ but this has not been confirmed subsequently.⁶⁻⁹ Many variations in technique have been described.¹⁰

Slide agglutination techniques are as sensitive and specific as CIE for the detection of bacterial antigens in CSF.^{6,8} Coagglutination (COA) reagents are suspensions of staphylococci treated to expose superficial protein A coated with specific antisera. Commercially prepared reagents are available as the Phadebact Pneumococcus Test and Phadebact Haemophilus Test (Pharmacia Diagnostics) for the identification of *Streptococcus pneumoniae* and capsulated *Haemophilus influenzae*. The purpose of this study was to compare these two kits with CIE for the detection of bacterial antigen in CSF. At the end of

the study a prototype meningitis kit incorporating a meningococcus reagent was also compared with CIE on culture-positive CSF specimens.

Material and methods

CSF SPECIMENS

Cell count, Gram stain and bacterial culture on blood agar and heated blood agar in 5% CO₂ were performed on all specimens of CSF submitted to the diagnostic laboratory of the Department of Microbiology, University of Leeds. Further investigations for *Mycobacterium tuberculosis*, fungi, viruses, etc were carried out when appropriate. COA tests for *Haemophilus influenzae* and pneumococcus antigen were performed on all CSF specimens with a neutrophil polymorph count of greater than 10 × 10⁹/l, and on the supernatant of centrifuged, bloodstained CSF specimens with a ratio of white blood cells to red cells of greater than 1:500. Specimens were similarly cultured and tested by COA in the Bacteriology Laboratories of the Bradford Royal Infirmary, St Luke's Hospital, Bradford and Staincliffe General Hospital, Dewsbury; any remaining CSF was stored at -20°C and later submitted to the Department of Microbiology, University of Leeds where CIE and repeat COA reactions were tested. A small number of specimens were submitted from other laboratories in West Yorkshire.

COUNTERIMMUNOELECTROPHORESIS

CIE was performed on unheated CSF using Shandon-Vokam CIE equipment. Barbiturate buffer (pH 8.2, ionic strength 0.05) was used to prepare the 1% agarose gel and in the buffer tanks.⁴ A constant current of 2 mA/cm width of gel was applied, as recommended for the equipment used. Slides were examined after 30 min and one hour for lines of precipitation, and then placed in a wet chamber at 4°C and re-examined after overnight cooling. Four antisera were used for CIE; *Pneumococcus omniserum* (Statens Serum Institut, Copenhagen, Denmark), *Haemophilus influenzae* type b (Difco), meningococcus polyvalent groups ABCD, and meningococcus polyvalent groups XYZ and W135 (Wellcome). Three antigen controls were prepared from laboratory cultures of *N meningitidis* (group A), *Strep pneumoniae* and *Haemophilus influenzae* type b and were included in each electrophoresis.¹¹

COAGGLUTINATION TESTS

Initially COA reagents were supplied in a lyophilised form and were reconstituted with buffer and used in accordance with the manufacturer's instructions. Reagents are now supplied ready for use, as staphylococcal suspensions coated with rabbit antibody and stained with methylene blue. The *Pneumococcus* kit comprises two reagents; one is coated with anti-pneumococcal antibodies and the other with gamma globulin from non-immunised rabbits, as the control reagent. The *Haemophilus* kit

also comprises two reagents, one coated with rabbit antibody to *Haemophilus influenzae* type b, and the other antibodies to *Haemophilus influenzae* types a c d e f.

Approximately one ml of CSF was heated for 10 min, in a test tube placed in a water bath at 80°C, to eliminate non-specific agglutinations. Four separate drops of CSF were then placed on one of the white cards provided, and one drop of each reagent added. Each reagent and CSF were mixed and the card rocked slowly and observed for agglutination for two minutes. Reactions were graded visually (+ to +++++).⁶ Instructions provided with the latest reagents suggest that 30 seconds should be the limit for observation of reactions, but some of the weaker agglutinations were not observed until one to two minutes had elapsed.

MENINGITIS KIT

A prototype meningitis COA kit was available for evaluation at the end of the study. This comprised polyvalent pneumococcus reagent, *Haemophilus influenzae* (type b) and (types a, c-f) reagents and a new meningococcus reagent, all ready for use. The kit was tested on CSF specimens which had been stored at -20°C using the method described above.

Results

One hundred and seventeen specimens of CSF from 94 patients were tested for pneumococcal, meningococcal and *Haemophilus influenzae* type b antigens by CIE, and pneumococcal and *Haemophilus influenzae* type b antigens by COA. Forty-six of these specimens, obtained at presentation, were positive on culture for bacteria, and antigen was detected as shown in Table 1. One specimen was positive for pneumococcal antigen by CIE and negative by COA; one was positive by COA and negative by CIE. Two CSFs, from which *Haemophilus influenzae* was cultured, contained no antigen detectable by CIE or COA when tested after storage

Table 1 CIE and COA reactions of 117 specimens of CSF

| Bacteria isolated | Number of CSFs | Positive by CIE | Positive by COA |
|-------------------------|----------------|-----------------|-----------------|
| <i>Strep pneumoniae</i> | 14 | 13 | 13 |
| <i>H influenzae</i> | 10 | 8 | 8 |
| <i>N meningitidis</i> | 13 | 6 | NT |
| Other species† | 9 | 0 | 0 |
| No bacterial growth | 71 | 4* | 4* |

†See Results.

*See Table 2.

NT = not tested.

Table 2 Persistence of pneumococcal antigen in CSF detected by CIE and COA

| Patient | Days after initial specimen | Gram film | Bacterial culture | CIE | COA |
|---------|-----------------------------|-----------|-------------------|-----|-----|
| A | 0* | + | + | + | + |
| A | 3 | + | - | + | + |
| A | 5 | - | - | + | + |
| A | 6 | - | - | - | + |
| A | 9 | - | - | - | - |
| B | 0 | + | + | + | + |
| B | 8 | + | - | - | - |
| C | 0 | + | + | + | + |
| C | 2 | + | - | + | + |
| D | 0 | + | + | + | + |
| D | 2 | + | - | + | - |
| D | 4 | - | - | - | - |

*Initial specimen taken on day of presentation.

antigen available for detection.¹³ A threshold concentration of bacteria is required for antigen detection by CIE and COA^{7,8} and this is higher than the threshold for detection of bacteria by direct microscopy.⁸

Although bacterial antigen in CSF is reported to be stable at -20°C for up to six months^{6,9} one specimen of CSF initially positive by COA was negative by CIE and COA for *Haemophilus influenzae* type b antigen after transport to the laboratory and storage at -20°C for two months. Two specimens positive on culture for *Haemophilus influenzae* were posted to the laboratory and on receipt were negative for antigen by CIE and COA, and were not included in the results. Specimens should be tested for antigen as soon as possible after receipt in the laboratory. One cross-reaction was observed with the prototype meningococcus COA reagent, but instructions supplied with the meningitis kit directed that CSF specimens should be tested with all four reagents, and the false-positive reaction was easily detected.

The meningococcus COA reagent appears to be less sensitive than the pneumococcus or *Haemophilus influenzae* type b reagents (Table 4), a phenomenon previously reported with "home-made" reagents.^{6,8} Although one study⁸ found the COA technique equally sensitive for the detection of meningococcal antigen of groups A, B and C, it is known that the commercially available group B meningococcal antisera are less sensitive than those of other groups.^{11,12,14} For various reasons involved in the collection of specimens from several laboratories, and storage over a period of 18 months, the groups on only four of the eleven meningococcal CSFs tested are known; two group C specimens were positive on CIE and COA, two group B specimens were negative on CIE and COA. The meningococcus COA reagent is prepared with antisera to groups A, B, C, Y and W135, and further studies on CSFs containing the antigens of these groups are indicated.

A coagglutination kit of ready-for-use reagents will provide all laboratories with the facility for bacterial antigen detection in CSF specimens. COA has been shown to be as sensitive as CIE and is a useful test in support of established methods of direct microscopy and culture for the diagnosis of bacterial meningitis.

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