

## Counterimmunoelectrophoresis for Early Detection and Rapid Identification of *Haemophilus influenzae* Type b and *Streptococcus pneumoniae* in Blood Cultures

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Counterimmunoelectrophoresis was shown to be a sensitive method for rapid detection of pneumococcal and *Haemophilus influenzae* type b antigens in blood cultures.

Early detection and rapid identification of bacteria in blood cultures are of utmost importance for the diagnosis and treatment of septicemias.

The predominant organisms isolated from blood cultures of pediatric patients 6 months to 5 years old are *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, accounting for approximately 85% of all isolates from bacteremic children (8, 9).

In recent years, counterimmunoelectrophoresis (CIE) has gained wide acceptance as a sensitive method for the rapid detection of bacterial antigens in infectious diseases (6). CIE has been used to detect bacterial capsular polysaccharides in cerebrospinal fluids and sera of patients with systemic diseases due to *Neisseria meningitidis*, *H. influenzae*, and *S. pneumoniae* (1-3, 5, 7).

Our study was designed to evaluate CIE as a method for the early detection and rapid identification of *S. pneumoniae* and *H. influenzae* type b in blood cultures of bacteremic patients.

Pneumococcal polyvalent antiserum containing antibodies to all 83 pneumococcal polysaccharide types was purchased from the Statens Serum Institut, Copenhagen, Denmark, and antisera to *H. influenzae* type b were obtained from Burroughs Wellcome Co., Research Triangle Park, N.C.

Antigen controls of *H. influenzae* type b and *S. pneumoniae* were prepared as described by Finch and Wilkinson (4).

CIE was carried out in a Cordis electrophoresis apparatus (Cordis Laboratories, Inc., Miami, Fla.) and involved the use of Cordis plates coated with 1% agarose gel in 0.04 M barbital-0.02 M acetate buffer, pH 8.2. A continuous system was used in which the compositions of the buffers in the gel and in the reservoir were equal. The antigens (blood culture samples, antigen controls, and bacterial suspensions of known cell concentration) were placed in the

cathode wells of the plate. Plates with antigen wells of 15- and 30- $\mu$ l capacities were used. The antibody wells (15- $\mu$ l capacity) were on the anodic side of the plate. A constant current of 100 V was applied for 45 to 60 min. At the conclusion of the run, all plates were immediately inspected with the aid of a viewer. The test was scored as positive if a precipitin band located at the interwell space was observed.

The sensitivity of the CIE method for the detection of bacterial antigens in blood cultures was determined by using suspensions containing known concentrations of *S. pneumoniae* and *H. influenzae* type b. The bacteria were grown in blood culture medium (GIBCO Laboratories, Grand Island, N.Y.) containing brain heart infusion or in tryptic soy broth and were serially diluted in the respective media to contain  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ , and  $10^8$  cells per ml. Samples of each dilution were electrophoresed against the corresponding antisera.

The detection limit for *S. pneumoniae* strongly depended on the amount of the antigen applied to the plate (Fig. 1A and B). When plates with antigen wells of 15- $\mu$ l capacity were used (Fig. 1A), the detection limit was  $10^7$  cells of *S. pneumoniae* per ml. A faint precipitin band was observed at the well which had been filled with a sample containing  $10^6$  cells per ml. By using plates with antigen wells of 30- $\mu$ l capacity, the sensitivity of the method increased more than 10-fold. Under these conditions, a strong precipitin band was observed at the well that had contained  $10^6$  cells per ml (Fig. 1B). Very faint precipitin bands were seen at the wells which had been filled with suspensions containing  $10^5$  and even  $10^4$  cells per ml.

Figure 1C shows the detection limit for *H. influenzae* type b, which was similar to that observed for *S. pneumoniae*: there was a strong, clearly visible precipitin band at the well that had been filled with  $10^6$  *H. influenzae* type b cells per ml, and very faint bands were visible at

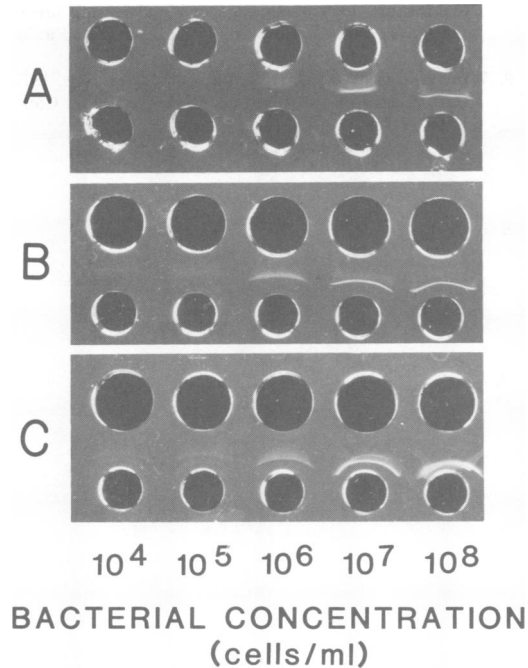


FIG. 1. Use of CIE for the detection of antigens of *S. pneumoniae* (A and B) and *H. influenzae* type b (C) in tryptic soy broth containing the indicated concentration of bacteria.

wells that had contained  $10^5$  and  $10^4$  cells per ml.

It should be mentioned that at the concentration of  $10^6$  cells per ml, the nutrient media remained crystal clear, with no visible evidence of growth. A concentration of  $10^7$  cells per ml was required to produce visible turbidity.

During a 6-month period, all blood cultures received in the microbiology laboratory of the Methodist Hospital were studied by the CIE method by using pneumococcal and *H. influenzae* type b antisera. An inoculum of 2 to 5 ml for each of two blood culture bottles (GIBCO Laboratories) was used. One inoculated bottle was vented by using a sterile, cotton-plugged needle. All bottles had been incubated at  $36^\circ\text{C}$  for 18 to 24 h when they were inspected for evidence of growth. All units of vented bottles, with or without visible evidence of growth, were examined for the presence of capsular antigens of *S. pneumoniae* and *H. influenzae* type b; 1 ml of broth was withdrawn with a sterile syringe, and a Gram stain was made. A  $30\text{-}\mu\text{l}$  amount was used for the detection of bacterial antigens in blood culture by CIE, and 0.5-ml samples were subcultured and used for viable counts. Viable counts were made by plating out known volumes

of the various cell dilutions on chocolate agar plates. Pneumococcal antigen was detected in 22 blood cultures. Sixteen cultures showed visible evidence of growth at the time of sampling (from faint turbidity [viable count of approximately  $10^7$  cells per ml] to heavy turbidity [viable count of more than  $10^8$  cells per ml]). Pneumococcal antigen was detected in six blood cultures with no visible evidence of growth at the time of sampling. Viable counts indicated the presence of  $6 \times 10^6$  to  $10 \times 10^6$  cells per ml; all cultures became turbid after incubation for an additional 18 h. Bacteria from all CIE-positive blood cultures grew in subcultures and were identified as *S. pneumoniae* by the conventional methods used in the microbiology laboratory.

*H. influenzae* type b antigen was detected in seven blood cultures, none of which showed visible evidence of growth at the time of sampling. All subcultures grew gram-negative rods which were identified as *H. influenzae* type b by the conventional bacteriological and serological methods.

Neither false-positive nor false-negative reactions were observed in this study. Blood cultures positive for *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter* sp., and *Pseudomonas aeruginosa* were all negative for the presence of pneumococcal and *H. influenzae* type b antigens.

In conclusion, CIE is a valuable method for the early detection of bacteremia caused by *H. influenzae* type b and *S. pneumoniae*. The data presented herein were obtained from studies of a relatively small number of positive cultures. We feel, therefore, that for the present, CIE results should be confirmed by culture.

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