Direct Use of Counterimmunoelectrophoresis in Detection of Group B Streptococci in Specimens Containing Mixed Flora

LAWRENCE J. FENTON†* AND MARILYN H. HARPER

The Neonatal-Biology Section, Department of Pediatrics, University of Arizona Health Sciences Center, Tucson, Arizona 85724, and the Tucson Medical Center, Tucson, Arizona 85712

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Isolation procedures for the group B streptococci were bypassed by applying counterimmunoelectrophoresis (CIE) directly to the broth containing the initial inoculum. A total of 458 tests were performed on 389 specimens received from 93 mothers and 81 babies. Of all tests, 19.4% were positive group B streptococci by the Lancefield precipitin method. Twenty hours of incubation were required to produce a positive CIE result in 54% of those specimens which were eventually proven to contain group B streptococci by Lancefield technique. There were no false positive CIE results. It is recommended that the initial broth culture be sampled and tested with CIE after an overnight incubation in those cases where another 24 h may be critical in terms of identifying the group B streptococci. A positive result with CIE can be clinically relied upon, but a negative result is indeterminate, and routine isolation procedures would need to be followed in that case.

The group B streptococcus (GBS) is now well recognized as an important pathogen in the newborn period (1). Early infection with the GBS clinically mimics idiopathic respiratory distress syndrome to such an extent that some clinicians have advocated antibiotics for all babies with respiratory distress in hopes that immediate therapy would improve survival (5). Although the value of this approach is unknown, information as to whether the mother was colonized with GBS would be helpful in making decisions in certain clinical situations. Obtaining such data, however, frequently requires a means of rapidly detecting the GBS.

Although counterimmunoelectrophoresis (CIE) has been shown to be quite useful in rapidly identifying the GBS in a pure culture of streptococci (2, 3), 18 to 24 h may elapse before a pure colony is isolated for identification. We therefore attempted to detect the presence of GBS in broth which had been directly inoculated with the clinical sample to see if the isolation procedures could be bypassed, thus greatly shortening the time to positive identification.

MATERIALS AND METHODS

Vaginal, periurethral, and rectal cultures were obtained from all mothers upon admission to the labor room, prior to any vaginal exam or use of antiseptic solution in the perineal area. Throat, ear, nose, and umbilical cultures were obtained from all newborns on

admission to the transitional nursery prior to bathing. The specimens were collected with cotton-tipped applicator sticks and stored in silica gel packets (Dri-Pax; Davison Chemical, Baltimore, Md.) for not more than 24 h. When received in the laboratory, the swabs were placed in 3 ml of Todd-Hewitt broth containing colistin (10 µg/ml) and nalidixic acid (15 µg/ml). A total of 37 of the maternal specimens were run in duplicate using Todd-Hewitt broth containing gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml). These selective media had been previously tested in our laboratory, on known controls, and shown to be identical in terms of CIE results. All tubes were incubated at 37°C in room air.

At 4, 6, and 20 h of incubation, these tubes were visually checked for growth. Tubes that appeared turbid were tested for GBS by both CIE and the Lancefield method (4, 7, 8). Simultaneously, broth from the turbid cultures was plated at 4, 6, and 20 h of age on Trypticase soy agar plates with 5% sheep erythrocytes. Tubes that were turbid at 4 h were also tested at 6 and 20 h, and those turbid at 6 h were tested again at 20 h as a check on internal consistency and the effects of the growth of competing organisms. These plates were incubated anaerobically (Gas-Pak, BBL) overnight. Streptococci (either beta-hemolytic or nonhemolytic) which were bile esculin negative and sodium hippurate positive were grouped and typed using the Lancefield precipitin method. Typing sera were generously donated by Hazel W. Wilkinson, Center for Disease Control, Atlanta, Ga. No typing was done by CIE.

The CIE was performed on clean microscope slides (1 by 3 inches, ca. 2.5 by 7.6 cm) covered with 3 ml of 1% agarose (Sigma) in tri-barbital-sodium barbital buffer (Gelman) at pH 8.8, ionic strength 0.035. Three sets of two 3-mm wells, 2 mm apart, were punched

† Present address: Neonatal-Biology Section, Sioux Valley Hospital, Sioux Falls, SD 57105.

equidistant from one another on the slide. One well was filled with 10 μ l of grouping antiserum (Difco); the other was filled with 10 μ l of broth culture (unspun). The slides were positioned in the electrophoresis chamber (Gelman) with the antigen on the cathode side. Each side of the chamber was filled with 50 ml of barbital buffer. The current was adjusted to provide 2 mA per slide and maintained for 1 h. The slides were observed immediately after electrophoresis for a line of precipitation between the wells. The test was simply graded as positive or negative; i.e., any precipitation, even a weak line, was graded positive.

RESULTS

A total of 458 tests were performed on 389 specimens received from 93 mothers and 81 babies. By the Lancefield method, 19.3% of the mothers and 18.5% of the babies were colonized with GBS. Only 10.2% of the mothers and 11.1% of the babies were colonized by CIE. Of all tests, 89 (19.4%) were positive for GBS by the Lancefield method. These data are summarized in Table 1. It can be seen that although 6 h of incubation produced reasonable turbidity in 56 samples, only 13% of these could be identified as GBS. The majority of tubes required an overnight incubation to produce turbidity. Of the 89 tests which were eventually positive for GBS, 48 (54%) were identified after 20 h of incubation by CIE. There were no false positive CIE results; i.e., all specimens positive for GBS by CIE were also identified as GBS by the Lancefield method. An analysis of the 41 specimens in which CIE failed to identify GBS revealed that all but three specimens contained mixed flora which were not inhibited by the colistin and nalidixic acid. The most common contaminant in the mixed-flora group was alpha-hemolytic streptococci. Of the remaining three specimens which were pure cultures, two were late growers (i.e., turbidity did not develop until 20 h), one was nonhemolytic, two were type III, and one was nontypable. There was no discernible pattern as to site cul-

tured with regard to a positive or negative CIE. The results from the 37 duplicate maternal specimens grown in different media did not differ from the results of the entire sample.

The data were analyzed to see if the positive and negative results of CIE were distributed in any pattern by the organism with regard to organism type. Those specimens which had been duplicates (merely using different broths) were eliminated from this analysis to avoid bias in any group. The type III organism seemed to behave differently from the other types and from the group as a whole, in that CIE was negative much more frequently than it was positive. Statistical analysis was not warranted because of the small numbers in each group.

DISCUSSION

The growth characteristics of a pure culture of GBS in Todd-Hewitt broth, after previous incubation in Todd-Hewitt broth, are such that maximum growth may be reached in 4 to 6 h in 3 to 5 ml of broth. During this time, approximately 10^5 to 10^7 organisms per ml are produced and are sufficient to produce a positive CIE. These time frames are identical to those found by Hill et al. (3). Our data indicate that somewhere between 6 and 20 h is required as a minimum time before the majority of GBS can be identified by CIE or Lancefield technique in a mixed-culture situation. For practical purposes, in most laboratories, this means an overnight incubation. It is also apparent from these data that a mixed culture significantly impedes the identification of GBS. The reasons for this are speculative, but certainly the total concentration of GBS at the end of the log phase in a broth with many other organisms competing for nutrients will be substantially decreased. Whether immunological interference with the grouping antisera also occurs is unknown. There were no false positives, however, so that one can rely on a positive CIE in mixed flora as being accurate. In many clinical laboratories it takes a minimum of 48 h to definitively identify a streptococcus by group.

In summary, the data indicate that at least 50% of those cultures which will eventually yield GBS can be identified as GBS after an overnight incubation. A positive result can be relied upon clinically, whereas a negative result is indeterminate. There are a number of clinical situations in which a positive result obtained in 20 h may influence therapy. We would, therefore, recommend testing the initial broth culture with CIE after an overnight incubation in those cases where another 24 h may be critical. This report has to be considered a very preliminary clinical evaluation of the value of CIE as an early iden-

TABLE 1. Comparison of CIE and standard methods of isolation at various times of incubation of clinical specimen

Determination	No. of tubes at incubation time:			
	4h	6h	20h	Total
Cumulative number of tubes with obvious growth	13	56	458	527
CIE positive ^a	0	0	48	48
Eventually identified as GBS by Lancefield method ^b	0	14	75	89

^a CIE was performed directly on the broth containing mixed flora.

^b Lancefield testing was done only after isolation.

tification test. It is possible that alternative methods of treating the mixed broth or a more potent grouping antiserum would permit even earlier identification with CIE. Other techniques such as fluorescent antibody (6) may be ultimately more advantageous than CIE and need further evaluation in a clinical setting.

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LITERATURE CITED

1. Baker, C. J. 1977. Summary of the workshop on perinatal infections due to group B streptococcus. *J. Infect. Dis.* 136:137-152.
2. Edwards, E. A., and G. L. Laron. 1973. Serological grouping of hemolytic streptococci by counter-immunoelectrophoresis. *Appl. Microbiol.* 26:899-903.
3. Hill, H. R., M. E. Riter, S. K. Menge, D. R. Johnson, and J. M. Matsen. 1975. Rapid identification of group B streptococci by counter-immunoelectrophoresis. *J. Clin. Microbiol.* 1:188-191.
4. Lancefield, R. C. 1934. A serological differentiation of specific types of bovine hemolytic streptococci (group B). *J. Exp. Med.* 59:441-458.
5. Miller, T. C. 1977. Emergency treatment of group B streptococcal disease. *Pediatr. Clin. North Am.* 24:501-508.
6. Romero, R., and H. W. Wilkinson. 1974. Identification of group B streptococci by M precipitin reactions in capillary pipettes. *J. Exp. Med.* 78:127-133.
8. Wilkinson, H. W., R. R. Facklam, and E. C. Wortham. 1973. Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). *Infect. Immun.* 8:228-235.