SEROLOGICAL GROUPING OF HEMOLYTIC STREPTOCOCCI BY COUNTER-IMMUNOELECTROPHORESIS

Earl A. Edwards, et al

Naval Medical Research Unit No. 4
Great Lakes, Illinois

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1. Serological grouping
2. Hemolytic streptococci
3. Counter-immunolectrophoresis

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Serological Grouping of Hemolytic Streptococci by Counter-Immunoelectrophoresis

EARL A. EDWARDS AND GEORGE L. LARSON

Immunology Division, Naval Medical Research Unit No. 4, Great Lakes, Illinois 60088

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The counter-immunoelectrophoresis (CIE) method of grouping streptococci was more sensitive than the capillary precipitin method. The precipitate was easier to read, and the test was simple to perform and required fewer reagents. An autoclave-Pronase-B extraction procedure is described and was found superior to either acid, Pronase-B, or the autoclave extraction procedures for recovering groupable polysaccharide from different strains of streptococcal groups A, B, C, D, F, and G. Data obtained from over 400 strains indicate that the sensitivity of the CIE and a more efficient extraction of groupable polysaccharide provides a simple method for rapid diagnosis of streptococcal infections.

A rapid method for differentiating group A streptococci from other beta-hemolytic streptococci and/or other agents causing upper respiratory infections has been the goal of microbiologists since the relationship between group A streptococci and rheumatic fever was established. A presumptive test using a bacitracin-saturated filter paper disk has been described (11, 12) and is commonly employed in most diagnostic laboratories. Although this method has simplified the approach for rapid differentiation of group A beta-hemolytic streptococci from other streptococci, technical problems may have contributed to improper interpretations resulting in inadequate or needless treatment of patients (1, 8). From the standpoint of providing maximal patient care, as far as the consequences of streptococcal infections are concerned, specific grouping of beta-hemolytic streptococci is the most accurate. Such specific grouping of beta-hemolytic streptococci requires the extraction of group-specific carbohydrates and subsequent precipitin testing (10) or the use of fluorescent antibody techniques with group-specific labeled antiserum (13). The latter test requires expensive equipment and well-trained technicians. In small laboratories, where isolation and grouping of streptococci may occur infrequently, the use of the Lancefield acid extraction method may be too complicated a procedure to be reliable. In addition, the precipitate in the capillary tube may be hard to interpret because of either a dirty capillary tube or a slightly cloudy antiserum. Consequently, errors in grouping streptococci in laboratories with limited capabilities may occur with unacceptable frequency.

The counter-immunoelectrophoresis technique (CIE) has been shown to provide a very sensitive and easily interpreted procedure for detecting Australia (9), meningococcal (6), pneumococcal (4), and Haemophilus influenzae type B antigens (2). It has also been used to group or type meningococci and pneumococci (6, 7). Recent studies indicate that the method may also be useful in grouping streptococci (E. A. Edwards and G. L. Larson, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 76, 1973; reference 3).

The present study was undertaken to determine the sensitivity, specificity, and reliability of CIE compared with the capillary tube precipitin test in differentiating and grouping beta-hemolytic streptococci. In addition, an improved method of extracting groupable polysaccharide from various strains of streptococci is described which was from 4 to 32 times more efficient than the conventional acid extraction procedure.

MATERIALS AND METHODS

Counter-immunoelectrophoresis. The CIE method has been previously described (6), except 0.06 M barbital buffer (pH 8.6) was used in both the agarose and electrode vessels. Glass slides were coated with 1% agarose in barbital buffer (0.06 M, pH 8.6; Kallestad Laboratories, Minneapolis, Minn.). Wells (3-mm diameter) were cut in parallel rows 1.5 mm apart. Streptococcal extract (10 u.liters) was placed in the wells on the cathode (negative) side of the plate, and specific grouping serum (10 u.liters) was added to
the opposite wells. Electrophoresis was carried out for 1 h. by using 12 mA per glass slide (3.25 by 4 inches; Kodak slide) and 3 mA per microscope slide. Visualization of the precipitin bands was made by using a viewer (Kallestad Laboratories) or a 7× hand lens.

Laboratory strains of beta-hemolytic streptococci were used in the preliminary evaluation of the CIE method to detect group-specific streptococcal antigen compared with the capillary precipitin tube test as the accepted standard of reference. The number of strains and their groups were 15 A, 8 B, 4 C, 3 D, 3 F, and 4 G. Each strain was streaked onto a blood agar plate, and the plates were incubated for 18 to 24 h at 37°C under 10% CO₂. A single colony was transferred into 30 ml of Todd-Hewitt (T-H) broth (Difco Laboratories, Detroit, Mich.). The broth culture was incubated from 18 to 24 h at 37°C without CO₂. The growth was sedimented by centrifugation and extracted by the Lancefield method (10).

Comparison of the sensitivity of CIE with the capillary precipitin test. To determine whether the CIE test was more sensitive in detecting group-specific polysaccharide than was the capillary precipitin tube test, the following tests were made. A 5-ml sample of an 18- to 24-h T-H broth culture, each of types 1 and 2, was acid-extracted (10). Twofold serial dilutions of the extracts were made in 0.15 M NaCl (saline). Each dilution of each streptococcal type was tested for antigen by both the precipitin tube method I and the CIE test. The results in Table 1 show the correlation between grouping streptococci by the conventional capillary precipitin test and the CIE method. In 266 attempts using five different

![Fig. 1. Flow diagram showing the procedure of grouping streptococci by the conventional method (right) and a 4-h culture method (left).](image-url)
TABLE 1. Correlation between grouping streptococci by the capillary precipitin tube test and CIE

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<thead>
<tr>
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<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>231</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>231</td>
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*Group-specific polysaccharide was extracted by the Lancefield acid extraction procedure.

groups of beta-hemolytic streptococci, there was 100% correlation. No effort was made to grade the intensity of either the precipitate in the capillaries or of the band formed by CIE. They were recorded as positive or negative. The data in Fig. 2 demonstrate the specificity of the CIE method of grouping streptococci. In grouping over 357 strains of beta-hemolytic streptococci by this method, there were no cross-reactions.

The data from our experiment to determine whether the CIE method would be more sensitive in detecting streptococcal polysaccharide than was the capillary procedure are shown in Fig. 3. Increased sensitivity would allow an earlier identification of streptococcal infections. The results indicate that the CIE method is from 4 to 16 times more sensitive in detecting streptococcal polysaccharide than was the capillary method when using the same extracted materials.

The data in Fig. 4 compare the amount of group-specific polysaccharide recovered from a 5-ml sample of an 18- to 24-h broth culture:
each 5 ml was extracted by one of the four procedures described. These data indicate that
the autoclave-Pronase-B extraction procedure is from 4 to 16 times more efficient in extracting
groupable polysaccharide than are any of the other three procedures.

Figure 5 demonstrates the efficiency of the four extraction procedures in yielding groupable
polysaccharide from various amounts of an 18- to 24-h broth culture of five different group A
streptococcal strains. It is evident that the autoclave-Pronase-B extraction procedure is
consistently more efficient in extracting groupable polysaccharide than are either of the three
other methods used.

The data in Table 2 show the results of the clinical studies for identifying streptococci. Of
183 patients studied, 25 were harboring beta-hemolytic streptococci by the conventional
streak-plate method. By transferring a colony to T-H broth and by subsequent extraction for
group-specific polysaccharide, the extracts were groupable by either CIE or the capillary precipi-
tin tube test. However, in the 4-h culture method, whereas the CIE detected group-
specific polysaccharide in all 25 cases, only 19
out of 25 (76%) gave positive identification with
the precipitin tube technique. This difference is
significant ($P = 0.01$, contingency table, R.
Latscha). These results are consistent with our
previous experiments in demonstrating that the
CIE method is more sensitive in detecting
streptococcal polysaccharide than is the capil-
ary precipitin tube procedure. Such sensitivity
would allow the laboratory to identify strepto-
cocci in approximately 6.5 h if such rapid
identifications were necessary.

**DISCUSSION**

The use of the CIE method of grouping
streptococci was demonstrated to be simple,
rapid, and reliable. By using the capillary precipitin method as the accepted standard of
reference, CIE gave comparable results in all
instances and was easier to interpret.

The simple and efficient procedure of extract-
ing groupable polysaccharide, using a combina-
tion of autoclave-Pronase-B as detailed above,
and the rapid and sensitive method of detecting
antigen by the CIE method make this procedure
applicable to routine diagnostic services in
small, as well as large, laboratories. These
procedures eliminate the use of many chemi-
cals, require no pH determinations, and require
only small volumes of cultures. The apparatus
is currently used routinely in most blood bank
laboratories and, therefore, requires no addi-
tional expensive equipment. The method is
simple, inexpensive (8 cents/extraction), and
does not require highly trained technicians. It is
conceivable that by using the extraction proce-
dure and CIE to group streptococci, the use of
blood agar plates could be eliminated.

**ACKNOWLEDGMENTS**

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