Evaluation of the Rapid Patho Dx Latex Strep Grouping Kit

R. BECK,* R. ETZION, S. SANDLER, AND L. KOHN

Kupat Holim Maccabi Laboratory, Haifa, Israel

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A variety of clinical specimens from throat, nose, ear, eye, wounds, urine, and vagina were collected, cultured, and screened for beta-hemolytic streptococci. The Patho Dx Latex Strep Grouping Kit (Diagnostic Product Corp., Los Angeles, Calif.) technique was applied to colonies taken right from the primary cultures. Isolated strains were sent to the reference laboratory where they were grouped by standard techniques. The kappa coefficient of agreement between the Patho Dx Kit and the standard method was 0.958. We believe that, although better agreement was achieved by others with isolated colonies, a very good agreement is also achieved with primary cultures. The fact that the laboratory is able to report an accurate answer after only 24 h seems most advantageous.

There is a clinical need to differentiate beta-hemolytic streptococci into Lancefield groups. The reference procedures for determining the serological grouping of beta-hemolytic streptococci include the extraction of the carbohydrate antigen and the reaction of this antigen extract with group-specific antisera. These methods require initial isolation of the organism followed by a time-consuming multistep grouping procedure. However, with the development of immunofluorescence coagglutination and latex agglutination techniques, serological grouping of streptococci has become technically simple (3). The Patho Dx Latex Strep Grouping Kit (Diagnostic Product Corp., Los Angeles, Calif.) is a latex agglutination kit for the rapid identification of streptococci serogroups A, B, C, F, and G that involves an immediate room temperature nitrous acid extraction procedure that requires no incubation time. The purpose of this study was to determine the kappa coefficient of agreement between the Patho Dx Kit and a reference method.

**Specimen collection.** A variety of clinical specimens from throat, ear, eye, nose, urine, wounds, and vagina were collected by physicians and nurses. Swabs were placed in a dry, sterile, capped tube or in a Culturette containing transport medium (Amies modified without charcoal). All specimens were delivered to our laboratory within 24 h of specimen collection. The specimens were inoculated into a tryptic soy agar plate containing 5% sheep blood. Plates were incubated in a 5% CO₂ atmosphere for 24 h and were screened for the presence of beta-hemolytic streptococci.

**Comixing with accompanying flora.** The degree of comixing with accompanying flora was approximately 0% in one-tenth of the primary cultures, 70% in one-third of the cultures, and 50% in one-half of the cultures. In another one-tenth, the degree of comixing was almost 95%.

**Patho Dx.** The Patho Dx Latex Strep Grouping Kit includes grouping latex for antigens A, B, C, F, and G, control antigens, and extraction reagents. The test was performed according to the instructions of the manufacturer. Five or more colonies from the primary culture were extracted in test tubes (12 by 75 mm). Extract (50 μl) was dropped on a determination card, and then one drop of specific latex was added. Latex and extracts were subsequently mixed with a stir stick and rocked for 2 to 4 min. Agglutination was either positive (+) or negative (−). In 10% of the tests, a clear-cut result could not be obtained. Isolation was then performed (another 24 h), and the serogrouping was done again by the same procedure.

**Reference method.** We sent isolated strains to the National Reference Center, Government Central Laboratory, Ministry of Health, Jerusalem, Israel, on blood agar plates, where the strains were inoculated onto one quarter of a sheep blood agar plate together with a disk of bacitracin (0.1 U per disk). The plates were incubated at 37°C overnight. Strains with inhibited growth belonged to group A. Identification of streptococci which were not susceptible to bacitracin was performed by a diffusion in gel in Ouchterlony agar (5). Lancefield extract was used as the antigen for the strains (3). Streptococci which were identified by the bacitracin test as group A were again identified by the diffusion method, as described above.

**Grouping sera.** Reference strains were received from the Streptococcus Reference Laboratory, Public Health Laboratory Service, London, England; Richard R. Packlam, Centers for Disease Control, Atlanta, Ga.; and J. Rotha, Institute of Hygiene and Epidemiology, Prague, Czechoslovakia.

**Whole-cell vaccine for group antigen.** A tube of Todd-Hewitt broth was inoculated from a single colony of the reference strain and incubated for 4 h. The culture was then subcultured into 250 ml of broth, which was incubated overnight at 37°C. This culture was plated to test for purity.

After the culture was centrifuged, the deposit was washed once in saline, suspended in 25 ml of buffer (pH 7.8) containing 5% pancreatic extract, and left overnight at room temperature. The cells were then washed six times, suspended in 25 ml of buffer (pH 7.8), and heated in a water bath at 56°C for 30 min. A formaldehyde extract was made from 2 ml of the vaccine, and its group reaction was tested. For some groups, (e.g., groups B and O), the trypsin digestion was omitted.

**Statistical analyses.** The kappa coefficient of agreement as previously described by J. Cohen (1) between Patho Dx and the reference method was calculated. The kappa statistic is widely used in several fields of application in the social and biomedical sciences. The kappa coefficient of agreement is the ratio of the proportion of times that the test agrees (corrected for chance agreement) to the maximum proportion of times that the test could agree (corrected for chance agreement). The maximum value of kappa is 1.0.
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\kappa = \frac{\sum \pi_i - \sum_{i} (\pi_i \times (p - i))}{1 - \sum_{i} (\pi_i \times (p - i))}
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Table 1 displays the frequency of cultures according to their cross-classification by the two diagnostic methods. In 136 primary cultures, colonies of beta-hemolytic streptococci were identified with the Patho Dx Grouping Kit. Of 86 cultures identified as group A, 82 were recovered from the throat, 1 from the nose, and 1 from urine. Of these 86 cultures, 84 were also classified as group A according to the reference method, while 2 were ungroupable (Table 1). Of 29 cultures identified as group B with the Patho Dx Kit, 25 were recovered from the vagina, 2 from the throat, and 2 from urine. Of these 29 cultures, 28 were also classified as group B by the reference method, but 1 culture was misclassified as group A (Table 1). Of 8 cultures identified as group C with the Patho Dx Kit, 7 were recovered from the throat and 1 from the eye. All of these cultures were also classified as group C by the reference method (Table 1). Of 11 cultures classified as group C with the Patho Dx Kit, all were recovered from the throat. The reference method also classified all of these 11 cultures as group G (Table 1). Two of the strains, both group A, were sent to the reference laboratory and did not survive. The kappa coefficient of agreement was then calculated.

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Our results show a good agreement, \(\kappa = 0.958\), between the Patho Dx Grouping Kit and the reference method. Daly and Seskin (2) found 100% agreement between Patho Dx and their reference method.

We suggest that the reasons for these discrepancies are the following. (i) The evaluation of Daly and Seskin was performed on isolated strains, while ours were performed on primary cultures. Interfering cross-reaction caused by a mixed population of organisms was reported (4, 6, 7). (ii) While Daly and Seskin used Wellcome streptococcus grouping sera for their reference method, we used homemade sera for our reference method. From the above, we consider that, although isolated colonies give better agreement with reference methods, a very good agreement is also achieved with primary cultures. This fact enables the laboratory to report an accurate answer after only 24 h with a very rapid and convenient kit.

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**LITERATURE CITED**


