Novel Color Test for Rapid Detection of Group A Streptococci

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A novel color test for the rapid detection of group A streptococci has been developed. The test, designed to be suitable for use in clinical laboratories as well as by less experienced personnel, incorporates the simplicity of latex tests with a color change to indicate the presence of group A streptococcal antigen. The test, which takes 5 min, was evaluated with 646 throat swabs, with a 15.6% incidence of group A streptococci; for swabs which yielded 10 or more group A streptococcal colonies in cultures, the sensitivity was 96.8%, and the specificity was 99.1%. In addition, the color test was 100% sensitive and specific when used to detect group A streptococcal antigen in beta-hemolytic colonies from culture plates.

Early therapy of streptococcal throat infections can shorten the duration of the illness, provide symptomatic relief, and possibly even reduce the period of infectivity (7, 10). Accurate diagnosis is difficult on clinical grounds alone (12), and conventional bacteriology does not provide information to support a diagnosis for at least 18 h after the patient has been seen by the physician. More than 20 years ago, attempts were being made to detect streptococci more rapidly (9, and recently, a number of commercial kits which are capable of providing an answer while the patient is still in the doctor’s office have become available. These involve rapid extraction of the streptococcal antigen from a throat swab and detection of the antigen in the extract by a latex agglutination test or an enzyme immunoassay procedure (11; S. W. Cooper, M. Flynn, and D. H. Katz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C264, p. 372).

A novel reagent for detection of the antigen which combines the benefits of both of these procedures has now been developed. It is based on latex agglutination and is rapid and simple to perform but provides a color change for clarity and ease of reading, particularly important when relatively inexperienced personnel may be performing the test. The test and control reagents are incorporated in a single suspension, accomplished by mixing a suspension of blue latex particles which have been sensitized with antibody specific for group A streptococcal antigen with a suspension of red latex particles similarly sensitized with immunoglobulin from unimmunized rabbits (S. G. Hadfield, A. Lane, and M. B. McIlmurray, J. Immunol. Methods, in press). The mixture is purple and remains an even purple color in a negative reaction. When group A antigen is added, clumps of aggregated blue particles form, leaving red unagglutinated control particles in the background. If a nonspecific reaction occurs, purple clumps separate, leaving a clear background. These three possible states are easy to distinguish from one another.

This report provides a preliminary evaluation of the new system both for the rapid detection of group A streptococci in throat swabs and for the rapid identification of group A streptococci growing in laboratory cultures.

MATERIALS AND METHODS

Throat swabs. A total of 646 throat swabs were investigated. These were taken by general practice and hospital physicians from patients with pharyngitis. Cotton-tipped, wooden-shafted swabs were placed in Cary-Blair transport medium (CM 519; Oxoid Ltd., Basingstoke, England) for transportation to the laboratory. The majority of the swabs were received in the laboratory on the day on which they were taken, and the remainder were received within 24 h of being taken. On receipt the swabs were kept at room temperature; all were fully processed within 4 h of their arrival in the laboratory.

Cultures. Throat swabs were inoculated onto Columbia agar (CM331; Oxoid Ltd.) supplemented with 5% defibrinated horse blood. After overnight incubation at 37°C in an atmosphere of 5% CO2 in air, the numbers of beta-hemolytic streptococcal colonies present on each plate were estimated as <10, 10 to 19, 20 to 50, and >50. The beta-hemolytic streptococci isolated were grouped with Streptex (Wellcome Diagnostics, Dartford, England).

The same swabs were subsequently subjected to the direct testing procedure, the results of which were therefore recorded at least 1 day before the culture results were known.

Stock strains of streptococci, which included clinical isolates and reference strains representing groups A to H and K to P and clinical isolates of Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, S. mutans, S. sanguis, Neisseria lactamica, N. sicca, N. subflava, and Branhamella catarrhalis, were obtained from the Wellcome Bacterial Culture Collection. These were incubated overnight at 37°C on plates of nutrient agar (Wellcome Research Laboratories, Beckenham, England) supplemented with 5% defibrinated sheep blood in an atmosphere of 5% CO2 in air. For preparation of an extract for testing, an individual colony was picked off the agar by using the blunt end of a wooden toothpick and processed immediately through the extraction system (see below). The cultures were coded and tested as unknowns.

Sample processing. The extraction procedure, based on the simplified nitrous acid method (5), was similar to that used for currently available commercial kits (4) with the following modifications. (i) The extraction tubes were specially de-
signed with a narrow, parallel-sided base to fit the swabs, so that when a swab was moved in and out of a tube, a plunger effect was created to force the extraction mixture through the material of the swab, thus improving the extraction process. (ii) Extraction reagent 1 (sodium nitrite) was air dried in extraction tubes prior to use. Tubes could be stored in a dry cupboard at room temperature for several months without loss of activity, saving one dispensing step during the extraction process. (iii) Extraction reagent 2 (acetic acid) was supplemented with 30 μg of bromocresol purple indicator per ml to provide a visual indication, by a color change from yellow to pale purple, and a degree of control over neutralization. (iv) The period of nitrous acid extraction was 2 min for both swabs and toothpick samples.

**Latex reagent.** Preparation of the latex reagent has been described previously (Hadfield et al., in press). Blue polystyrene latex particles (0.2-μm diameter, Estapor; Rhône-Poulenc Specialités Chimiques, Courbevoie, France) were passively sensitized with affinity-purified antibodies to group A streptococci, and red polystyrene latex particles were sensitized in a matching process with immunoglobulin G obtained from the sera of immunized rabbits. The sensitized red and blue suspensions were mixed in equal proportions.

**Test method.** Reactions were performed on white disposable cards in circles with 2.6-cm diameters. A sample of the extract (40 μl) was transferred to the card and mixed with a single drop (40 μl) of the latex reagent by using a wooden toothpick. The card was either rocked slowly by hand for up to 2 min or placed on a flat-bed rotator (16-mm orbital radius) at a speed of 150 rpm for the same length of time. The latter method was used for culture tests, and the former was used for throat swabs. Examples of reactions are shown in Fig. 1. Agglutinated clumps of particles spread to the edge of the reaction area with manual rotation (Fig. 1a and c) but concentrate at the center of the reaction area on the flat-bed rotator (Fig. 1b and d). In a positive reaction, signifying the presence of group A streptococcal antigen, the aggregated blue particles are seen against a pink background (Fig. 1a and b); in a nonspecific reaction, demonstrated with a drop of dilute goat anti-rabbit serum (Miles Laboratories, Inc., Elkhart, Ind.), all the latex particles aggregate into purple clumps seen against a clear background (Fig. 1c and d). With the development of a concentrated spot of color on a rotator, this method is easier to read. However, although the agglutination patterns differ, the sensitivity of the two methods is similar. In the absence of agglutination, the mixture remains uniformly purple (Fig. 1e).

**RESULTS**

**Throat swabs.** Beta-hemolytic group A streptococci were isolated from 101 of the 646 swabs examined; of these, 91 were detected by latex agglutination. The 10 culture-positive swabs from which nonreactive extract was obtained included 7 which yielded less than 10 colonies on the culture plate, but 3 other swabs with fewer than 10 colonies were detected. The relationship between the number of colonies isolated from a swab and the latex agglutination result is shown in Table 1. Extracts from five specimens which gave a positive latex agglutination result were culture negative for group A streptococci. Two of these patients had commenced antibiotic therapy before their swabs were taken, and two patients had self-medicated with proprietary throat lozenges before their swabs were taken. The remaining 540 swabs were culture negative for group A streptococci and gave

**TABLE 1.** Relationship between the number of colonies grown from throat swabs and positive latex agglutination reactions

<table>
<thead>
<tr>
<th>No. of colonies</th>
<th>No. of swabs that were latex agglutination</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>&gt;50</td>
<td>68</td>
</tr>
<tr>
<td>20–50</td>
<td>17</td>
</tr>
<tr>
<td>10–19</td>
<td>3</td>
</tr>
<tr>
<td>&lt;10</td>
<td>3</td>
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</tbody>
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**FIG. 1.** Examples of reactions obtained with the group A streptococcal colored latex reagent. (a and b) Positive reactions; (c and d) nonspecific reactions (not interpretable); (a and c) reaction patterns obtained with manual rotation; (b and d) reaction patterns obtained on a flat-bed rotator; (e) negative reaction.
negative results in the latex agglutination test. Other beta-hemolytic streptococci were recovered from 56 of these specimens: 1 group B, 15 group C plus 5 S. milleri group C, 2 group D, 16 group G, and 17 strains not belonging to group A, B, C, D, F, or G. No nonspecific reactions were observed with any of the specimens.

If all swabs from which any group A streptococci were isolated were included in the analysis, the sensitivity of the latex test was 90.1%, and the specificity was 99.1%. If swabs yielding less than 10 colonies on the culture plate were excluded, the sensitivity increased to 96.8%.

Cultures. Tests of single colonies of stock cultures produced the following results. All 30 group A streptococci were positive. None of the following cultures were positive: 30 each of group B and C streptococci, 28 group D streptococci, 2 group E streptococci, 17 group F streptococci, 21 group G streptococci, 2 group H streptococci, 6 group K streptococci, 2 each of group L, M, N, O, and P streptococci, 1 S. sanguis, 4 S. pneumoniae, 2 S. mutans, 12 Staphylococcus aureus, 8 Staphylococcus epidermidis, 2 each of N. lactamica and N. sicca, and 1 each of N. subflava and B. catarrhalis.

DISCUSSION

The major aim of screening tests for group A streptococci is to provide a rapid indication of the etiology of a throat infection to enable a scientific approach to therapy to be pursued. Results must be available in a time frame within which appropriate action can be taken, and as a consequence, many of the tests can be performed by relatively inexperienced personnel in the vicinity of the patient. Ideally, therefore, a suitable test system should be easy to perform and read. Several commercial kits based either on latex agglutination technology or on an enzyme immunoassay technique have recently been introduced for this purpose.

In general, latex agglutination tests are technically simple and rapid to perform, but reading of the results, particularly with weak antigens, is subjective, and difficulty can be encountered even by experienced operators. Color changes are usually associated with greater clarity of results, but the enzyme immunoassays which provide these are technically more complex and intricate than latex agglutination tests. The color test described in this report incorporates the desirable features of both latex agglutination tests and enzyme immunoassays, and although this study was performed by experienced personnel in clinical and research laboratory surroundings, it is suggested that equally good results should be obtainable by anyone. In particular, the subjectivity of reading has been eliminated.

The test system provides a result rapidly enough to be of value, that is, within 5 min of swabbing the throat. Extraction of antigen from the swab requires only two reagent additions, color coded to eliminate mistakes. The color test incorporates both the test latex and the control latex in one bottle, reducing the number of reagents to be dispensed and the volume of sample required for testing.

Color discrimination plays an important role in the overall process. It has been found that people with a range of color vision difficulties have no problems with the test, only a few individuals with extreme deficiencies failing to identify the color changes which occur.

Many scientific papers reporting the evaluation of some of the direct test kits commercially available for group A streptococci have been published, and a survey of these reveals considerable conflict. For example, the sensitivity of one product was as high as 100% in one study (R. J. Lesher and F. Dreher, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C257, p. 370) but as low as 48% in another (M. Tyburski, R. Almon, C. Cavanagh, C. Mannone, and E. Baron, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C210, p. 335), with a series of figures in between. There are very real problems in designing valid trials and interpreting the results in this area. Fundamental is a decision either to culture and process a single swab or to obtain duplicate swabs (6); both approaches have drawbacks. Other variables, some of which can be controlled more effectively than others, include the efficiency of swabbing the throat, particularly with children (3), the use of transport medium with the swab, and time delays between sampling and testing (8). Probably of greatest significance is the efficiency of the reference culture procedure in recovering organisms from the samples (1, 4). Therefore, results of the different studies should be interpreted with appropriate caution, and the results of this study may not be comparable in absolute terms with those in other publications. However, they do suggest that our method is capable of improving upon clinical judgement in direct tests and can, in addition, provide useful results in clinical laboratories, particularly for rapid identification of beta-hemolytic cultures.

The sensitivity of the color test was not identical to that of the culture method used. Relatively small numbers of samples which yielded less than 10 colonies were detected; this is the usual borderline of sensitivity of antigen detection methods. The significance of these cases as to infection or carrier status remains controversial (2, 7). Indeed, it may be dangerous to suggest such a correlation in view of the sampling variables already mentioned. It is unlikely that direct tests are sensitive enough to detect 10 CFU, because 10 colonies in a culture probably result from 10^3 to 10^6 CFU within the swab itself. The lack of complete correspondence between culture sensitivity and antigen detection tests may also reflect bacterial strain variation but is more likely due in part to an uneven distribution of organisms in the swab.

A small number of specimens were antigen positive but culture negative. All but one of these patients had received prior therapy which could have affected the viability of the organisms. No attempt was made to rule out the alternative possibility, the presence of nonhemolytic group A organisms in these patients.

Beta-hemolytic group A streptococci were isolated from 15.6% of the patients in this study. Other beta-hemolytic streptococci were cultured from a further 8.7% of samples. Whether any of these might have been responsible for the symptoms of pharyngitis cannot be determined, but none gave reactions in the color test.

Although the test system was designed primarily with direct tests in mind, it is flexible enough for use in individual or batch situations in clinical laboratories and may be of particular value in the identification of single colonies growing on blood agar isolation media. All the group A streptococci reacted correctly. None of the streptococci tested from other serological groups, none of the staphylococci, and none of the representative species of normal oropharyngeal flora gave any reaction, and all colony identifications were correct. The test should prove useful to physicians and laboratory personnel alike.

LITERATURE CITED


