Evaluation of Direct and Rapid Identification of Group A Streptococci from Throat Swabs by Culturette 10-Minute Group A Strep ID Test Kit

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The Culturette Brand 10-Minute Group A Strep ID test kit (Marion Scientific, Division of Marion Laboratories, Inc., Kansas City, Mo.) was evaluated for its sensitivity and specificity in identifying the group A streptococci directly from 96 throat swabs, against the conventional culture method and serological grouping test. Our results indicated that the rapid test kit and conventional method are 93.8% accurate; percent sensitivity and specificity of the rapid test was 80.6% and 100%, respectively. None of the false-positive observed in rapid test kits occurred with the heterogeneous microorganisms. More than $8 \times 10^7$ colony forming unit per swab was required for the positive latex agglutination of the test kit. Since the Culturette method is simple to perform and correctly identifies group A streptococcal antigen, and also required no special instruments, it appears to be applicable in hospital laboratories and outpatients clinics.

GROUP A streptococcal pharyngitis is a self-limited infectious disease which may lead to the development of rheumatic fever, rheumatic heart disease and acute glomerular nephritis. It is important to identify streptococci in patient with suspected streptococcal infection so that appropriate chemotherapy can be instituted to eradicate the organisms from the upper respiratory tract. In most cases, such treatment prevents the onset of serious sequelae. Detection of group A streptococci by conventional culture requires at least 12 hours. For definitive identification, latex agglutination or coagglutination tests are generally used after primary isolation. Recently, a latex agglutination kit for the identification of group A streptococci directly from throat swabs has been made available to be examined for application in clinical use!–3 This latex reagent kit, the Culturette Brand 10-Minute Group A Strep ID (Marion Scientific, Kansas City, Mo.), contains the reagents required for the micro nitrous acid extraction of throat swabs and does not require special instruments in its procedure. The purpose of this study was to evaluate this kit in regard to sensitivity, specificity and suitability for the direct serogrouping of group A streptococci from throat swabs, compared with conventional throat swab culture methods.

MATERIALS AND METHODS

Swab specimens
Pharyngeal swab specimens were collected from 96 outpatients with pharyngitis using a single rayon-tipped swab (Culturette, Marion Scientific, Division of Marion Laboratories, Inc. Kansas City, Mo.)

Key Words:
- Streptococcal pharyngitis
- Group A streptococcal antigen
- Nitrous acid extraction
- Latex agglutination
- Rapid identification

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TABLE I  COMPARISON OF THE CULTURETTE 10-MINUTE GROUP A STREP ID TEST TO CULTURE CONFIRMED RESULTS

<table>
<thead>
<tr>
<th>Culture confirmed</th>
<th>Number of samples</th>
<th>Latex agglutination</th>
<th>%sensitivity</th>
<th>%specificity</th>
<th>%accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive 1+ &lt;</td>
<td>31</td>
<td>25</td>
<td>6</td>
<td>86.6 (25/31)</td>
<td></td>
</tr>
<tr>
<td>2+ &lt;</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>100 (25/25)</td>
<td>100 (90/90)</td>
</tr>
<tr>
<td>Negative</td>
<td>65</td>
<td>0</td>
<td>65</td>
<td>100 (65/65)</td>
<td>93.8 (90/96)</td>
</tr>
</tbody>
</table>

Percent sensitivity = (number of positive latex agglutination/number of confirmed positive) x 100
Percent specificity = (number of negative latex agglutination/number of confirmed negative) x 100

TABLE II  THE LIMITATION OF THE LATEX AGGLUTINATION BY THE KIT

<table>
<thead>
<tr>
<th>CFU per swab</th>
<th>8 x 10^5</th>
<th>8 x 10^4</th>
<th>8 x 10^3</th>
<th>Logaristic dilution of the culture of strain of group A streptococcus (ATCC 19615) was made, and swabs were charged with 100 μl of each dilution, and an antigen detection assay was carried out by the kit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex agglutination</td>
<td>4+</td>
<td>1+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Conventional culture method

The swab was rolled on a portion of a 5% sheep blood tryptcase soy agar plate (BBL). The inoculum was further distributed by streaking with a loop to obtain isolated colonies. The plates were then incubated at 37°C under aerobic conditions for 24 to 48 hours. These plates were then observed for the presence of the beta-hemolytic activity. The primary isolates of the beta-hemolytic streptococci were serogrouped with the Phadebact Strept Test reagent (Pharmacia Diagnostics, Uppsala, Sweden). Culture positive results were categorized semiquantitatively from 1+ to 4+ per plate: less than 10 colonies, 1+; 10–30 colonies, 2+; 30 or growth in 3rd quadrant, 3+; growth in 4th quadrant 4+.

Direct identification procedure

The kit procedure was performed according to the instruction. The swab was placed in a microtube and the acid extraction reagents 1 and 2 were added. The swab was then rolled and mixed with the reagents. After the swab was incubated for 5 minutes at room temperature, neutralizing reagents 3 were added and the swab was mixed with the reagent. The extraction mixture was then used for the identification. Fifty microliter of the extraction mixture was transferred to each of two circular areas on the black coated slide provided with the kit. The detection reagent 4 was added to one circle, and the negative control reagent was added to the other circle. The slide was then rocked for 2 to 3 min and examined for agglutination of the latex particles. The positive latex agglutination method was also scored from 1+ to 4+.

Sensitivity test

Sensitivity of the kit was quantified by the serial dilution of group A streptococcus (ATCC 19615).

Specificity test

Cross-reactivity with other microorganisms was tested. Group B, C, G streptococci, Streptococcus pneumoniae, Staphylococcus aureus, and Candida albicans were used for the test.

RESULTS

Reactivity of the kit in comparison with culture method

Of the 96 pharyngeal swabs tested by the kit, 31 were found to be positive and 65 were negative by the culture method. Of the 31 positive specimens, 25 were positive by the kit (80.6% sensitivity). Similarly, of the 65 negative samples, the kit showed negative result in these cases (100% specificity). The kit and culture methods are 93.8% accurate. The six negative direct tests were associated with the isolation of one to ten colonies of group A streptococci per primary culture plate. If the specimens that contained less than ten colonies per plate of group A streptococci were eliminated, all of the positive specimens in culture method were positive by the kit (Table I).

Sensitivity of the kit

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Sensitivity of the kit was quantified by the serial dilutions of group A streptococcus (ATCC 19615). More than $8 \times 10^4$ CFU per swab was required for the positive latex agglutination (Table II).

Specificity of the kit

Cross-reactivity with other microorganisms that may be found in the respiratory tract was tested. None of the bacteria tested demonstrated a positive latex agglutination (data not shown).

DISCUSSION

The most frequently used method of laboratory diagnosis for streptococcal pharyngitis is isolation and identification of group A streptococci from throat swabs cultured on blood agar plates. For definitive identification of group A streptococci, commercially available latex agglutination and coagglutination are generally used after primary isolation. Recently, these methods have been applied as direct use on clinical samples.

Slifkin and Gil\(^4\) and Gerber\(^5\) reported a sensitivity of 78% in the nitrous acid extraction-coagglutination assay when used with pharyngeal swabs. Edwards et al\(^6\) reported a sensitivity of 83.9% in a latex agglutination test, when used directly on trypsin-digested throat gargle. Otero and co-workers\(^7\) reported that when a latex system was used directly on enzyme-digested throat swabs, sensitivity was 89%. Calandra and Henson\(^8\) reported that latex agglutination assay system showed sensitivities in the range of $10^5$ CFU per test.

When we used the 10-Minute Group A Strep ID, a nitrous acid extraction-latex agglutination system, it was found that total accuracy, sensitivity and specificity were 93.8%, 80.6% and 100%, respectively. Reason for the variation of the sensitivity was not clear, but there were possibly some false-negative responses. The sensitivity of the kit is found to be limited, showing that $8 \times 10^4$ CFU per swab is the minimal requirement of the latex positive reaction.

It is clear that if the specimens that contain group A streptococci less than ten colonies per plate were eliminated, all the culture positive specimens would be found positive by the kit (100% sensitivity). Campos et al\(^3\) showed the decrease in sensitivity when the incubation time were prolonged more than 40 hours.

Our data did not show the false-positive latex agglutination. Possibly the false-positives, however, were not negligible when the growth of group A streptococci on the blood agar plate was inhibited by the normal throat flora of the host. Another possibility is that, non-hemolytic or non viable (state after chemotherapy) group A streptococci were present in the throat swab.

In conclusion, this rapid antigen detection system offers a distinct advantage over conventional tests in effecting timely patient management because the isolation of the etiologic agents, group A streptococci, in the throat swabs can be determined in only 10 minutes after the swab is obtained.

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REFERENCES