

Ten-Minute Detection of Group A Streptococci in Pediatric Throat Swabs

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A 10-min latex agglutination test kit, Culturette Brand Ten-Minute Group A Strep ID (Marion Scientific, Div. of Marion Laboratories, Inc., Kansas City, Mo.), was assessed for the rapid detection of group A beta-hemolytic streptococcus directly from a throat swab. Four hundred and thirty-five throat swab specimens from children with suspected group A streptococcal infection were tested by the Ten-Minute Group A Strep ID and by concurrent conventional culture. The strep test was effective in detecting group A streptococcal infection; 90% (63/70) of culture-positive specimens gave positive latex tests. The specificity of the test was 99.2% (362/365). The predictive values were 95.5% for positives and 98.1% for negatives. Overall agreement with culture was 98% (425/435). This test offers a sensitive and specific method for the early detection of group A beta-hemolytic streptococci in throat swabs of children and would be most useful in a hospital laboratory or pediatrician's office.

Group A beta-hemolytic streptococcal (GABS) infection of the pharynx is often difficult to differentiate clinically from viral pharyngitis. It is estimated that in children clinical diagnosis without culture is only 43 to 75% accurate (2). Conventional culture and identification of the organism require 24 to 48 h. This time requirement has mandated one of two options: treating all patients in whom the diagnosis is suspected or delaying appropriate therapy in all patients for 24 to 48 h.

This study is an evaluation of the Culturette Brand Ten-Minute Group A Strep ID (strep test; Marion Scientific, Div. of Marion Laboratories, Inc., Kansas City, Mo.) for the detection of GABS antigen directly from a throat swab. This kit uses a micronitrous acid extraction coupled with latex agglutination.

Gerber has demonstrated reasonable success with a micronitrous acid extraction-coagglutination method for group A streptococci on swab specimens (3), and more recently, Slifkin and Gil evaluated the strep test for swab specimens from adults (8).

The purpose of this study was to determine the sensitivity and specificity of this kit compared with conventional culture for the direct detection of GABS infection in throat swabs from children.

Specimen collection. Four hundred and thirty-five throat swab specimens were collected from children with suspected GABS pharyngitis and submitted to Clinical Microbiology between September and December 1983. The throats were swabbed with a sterile rayon-tipped swab; the swab was transported to the laboratory in modified Stuart bacterial transport medium (Marion Culturette I) at 25°C.

Culture and identification. The swab from each patient was inoculated on Trypticase soya agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) and streaked for isolation. Cultures were incubated in ambient atmosphere at 35 to 37°C for 18 to 24 h and then examined for the presence of beta-hemolytic colonies (6).

Group A beta-hemolytic colonies were presumptively identified as white-gray colonies surrounded by a clear zone of hemolysis. Colony counts of streptococci on the plate

were recorded and approximated as 1+ to 4+. The gradations were as follows: 1+, <10 colonies per plate; 2+, 10 to 30 colonies per plate; 3+, >30 colonies per plate or growth in the third quadrant, and 4+, growth in the fourth quadrant.

All presumptive beta-hemolytic streptococci were subcultured from the primary plate and grouped by the Meritec-Strep beta-hemolytic streptococcus grouping set (Meridian Diagnostics Inc., Cincinnati, Ohio). This procedure has shown 100% correlation with the Lancefield precipitin method for organisms whose colony morphology allows identification by this method (Meridian Diagnostics Inc.).

Strep test. After inoculation of the blood agar plate, the same swab was subjected to the strep test. The swab was incubated for 5 min in 1 drop each of extraction reagents 1 and 2. At the end of 5 min, 2 drops of extraction reagent 3 was added to the microtube and allowed to rest briefly. The swab then was firmly rolled over appropriately labeled circles on an agglutination tile, so that 40 to 50 µl of liquid was transferred. One drop of negative latex reagent was added to one set of specimen circles, and one drop of detection latex reagent was added to a duplicate set of specimen circles. The slides were rocked for 3 min on a slide rotator at 110 to 180 rpm and observed under a high-intensity lamp. The agglutination or clumping reactions were graded 0 to 4+, depending upon the size of the clumps and the clarity of the background. All swabs were subjected to the strep test within 24 to 48 h of arrival in the laboratory.

Four hundred and thirty-five throat cultures were evaluated by conventional culture and by the strep test during a 4-month period. Seventy cultures (16%) were positive for GABS infection by culture; 63 (14%) were positive by the strep test. The strep test had a sensitivity of 90% (63 of 70). There were three false-positive reactions; the specificity of the strep test was 99% (362 of 365). The predictive values of a positive and negative strep test were 95.5% (63 of 66) and 98.1% (363 of 369), respectively. The intensity of agglutination in 84% of specimens was 3+ to 4+. A trend toward more intense agglutination in specimens with higher colony counts was demonstrated. Of the throat specimens with colony counts of >30 per plate, 80% had an agglutination of 3+ to 4+, 70% with 10 to 30 colonies per plate had a reaction

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of 3 to 4+, and only 50% of the throat specimens with <10 colonies had a reaction of 3 to 4+. A total of 362 specimens were negative by both conventional culture and Strep test, and there were 7 false-negative Strep tests. These showed no correlation with numbers of CFU. The overall agreement with culture was 98% (425 of 435).

Three percent (15/435) of the cultures were positive for beta-hemolytic streptococci other than group A: six were group B, six were group C, one was group F, one was group G, and one was nontypable. None of the corresponding swabs was positive on strep test.

During the study of the last 137 specimens, a retrospective examination of the sheep blood specimen plates was conducted when a discrepancy between strep test and culture results existed. In three instances group A beta-hemolytic streptococci were recovered after a second careful reading or upon subculture. In one of these instances, a 3+ beta-hemolytic streptococcus failed to produce a zone of inhibition around a Taxo A disk but typed as a group A beta-hemolytic streptococcus and gave a latex agglutination reaction of 4+.

When these 137 specimens were analyzed separately, the sensitivity and specificity of the strep test were 100% (29/29) and 99% (107/108), respectively.

Clinically, the diagnosis of streptococcal pharyngitis will be missed in 20 to 30% of children with culture-proved GABS infection (7). Throat cultures have been the accepted means for accurate diagnosis of GABS pharyngitis before initiating antimicrobial therapy (4). Unfortunately, throat cultures require 24 to 48 h, for results and variability in the performance of the test may result in a high percentage of false-negatives (1, 10).

The strep test offers reliability and reproducibility and surpasses conventional culture in speed and simplicity. In this evaluation the strep test performed well, with a sensitivity of 90% and a specificity of 99%. The predictive values for positives and negatives were 95.5 and 98.1%, respectively, in our pediatric population in which the prevalence of GABS infection was 16%.

Agglutination reactions from this kit were easy to read and interpret. Of throat specimens positive by the strep test, 84% had an agglutination reaction of 3+ to 4+. The three false-positives in our study exhibited an agglutination reaction of 1+. These throat specimens may have had low colony counts which were overgrown by normal throat flora or were inhibited by bacteriocins of other gram-positive bacteria (9). A small percentage of mutant nonhemolytic streptococcal strains that are difficult to detect by culture also may account for these false-positives (5). The false-neg-

atives are difficult to explain. Of the specimens in our study, 2% were negative by the strep test but positive by culture. The colony counts on the blood agar plate ranged from 10 to 30 colonies. Only one swab was used to perform both tests; since the plates were seeded first, perhaps the swab was left with too few organisms to be detected by the strep test. Data from Slifkin and Gil suggest that use of the same swab for culture and rapid identification should yield excellent results (8).

In this study it was assumed that throat culture is a perfect method for diagnosing streptococcal pharyngitis. This clearly is not the case. No clinical correlation with latex agglutination results was made, nor was the issue of this method and the streptococcal carrier state addressed.

In summary, the strep test had an overall correlation of 98% with culture. The test is simple, accurate, rapid, and attractive, both for a hospital laboratory and a pediatrician's office.

LITERATURE CITED

1. **Battle, C. U., and L. A. Glasgow.** 1971. Reliability of bacteriologic identification of beta-hemolytic streptococci in private offices. *Am. J. Dis. Child.* **122**:134-136.
2. **Breese, B. B., and F. A. Disney.** 1954. The accuracy of diagnosis of beta streptococcal infection on clinical grounds. *J. Pediatr.* **44**:670-673.
3. **Gerber, M. A.** 1983. Micronitrous acid extraction-coagglutination test for rapid diagnosis of streptococcal pharyngitis. *J. Clin. Microbiol.* **17**:170-171.
4. **Honikman, L. H., and B. F. Massell.** 1971. Guidelines for the selective use of throat cultures in the diagnosis of streptococcal respiratory infection. *Pediatrics* **48**:573-582.
5. **James, L., and R. B. McFarland.** 1971. An epidemic of pharyngitis due to a non-hemolytic streptococcus at Lowry Air Force Base. *N. Engl. J. Med.* **284**:750-752.
6. **Libertin, C. R., A. D. Wold, and J. A. Washington II.** 1983. Effects of trimethoprim-sulfamethoxazole and incubation atmosphere on isolation of group A streptococci. *J. Clin. Microbiol.* **18**:680-682.
7. **McCracken, G. H., Jr.** 1982. Diagnosis and management of group A streptococcal pharyngitis. *Pediatr. Infect. Dis.* **1**(Suppl.):30-32.
8. **Slifkin, M., and G. M. Gil.** 1984. Evaluation of the Culturette Brand Ten-Minute Group A Strep ID technique. *J. Clin. Microbiol.* **20**:12-14.
9. **Tagg, J. R., A. S. Dajani, and L. W. Wanamaker.** 1976. Bacteriocins of gram-positive bacteria. *Bacteriol. Rev.* **40**:722-756.
10. **Taranta, A., and M. D. Moody.** 1971. Diagnosis of streptococcal pharyngitis and rheumatic fever. *Pediatr. Clin. N. Am.* **18**:125-143.