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Micronitrous Acid Extraction-Coagglutination Test for Rapid Diagnosis of Streptococcal Pharyngitis

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A micronitrous acid extraction-coagglutination test for the rapid diagnosis of streptococcal pharyngitis was examined in a busy pediatric clinic and found to be a simple, rapid, and inexpensive procedure with a sensitivity of 78% and a specificity of 98% when compared with blood agar culturing.

Although rapid diagnostic tests have recently been developed for many infectious diseases (6), the diagnosis of streptococcal pharyngitis has for almost 3 decades (2) been made primarily by throat cultures on blood agar plates. Recently, Slifkin and Gil (11) described a micronitrous acid extraction-coagglutination test (MCT) for the rapid diagnosis of streptococcal pharyngitis that can be performed with a single throat swab in approximately 20 min. We evaluated the MCT in a busy pediatric ambulatory clinic to determine its practicality and its reliability in the diagnosis of streptococcal pharyngitis when compared with throat culturing.

Three specially trained nurses obtained throat swabs from 414 consecutive patients with pharyngitis from our pediatric ambulatory clinic by simultaneously rubbing two sterile cotton swabs over the posterior pharynx and both tonsils (or tonsillar fossae). One of the swabs was sent immediately to the diagnostic microbiology laboratory at St. Mary's Hospital, Waterbury, Conn., where it was streaked onto a blood agar plate (tryptic soy agar with 5% sheep blood; Scott Laboratories, Inc., Fiskeville, R.I.) and an undercut was made in the agar. After overnight incubation in 5% CO_2 at 37°C, the plate was examined for the presence of beta-hemolytic streptococci and quantified as follows: 1+ culture, <10 colonies of streptococci per plate; 2+ culture, >10 but <50 colonies; 3+ culture, >50 colonies but not a pure culture; 4+ culture, >50 colonies in pure culture. Beta-hemolytic streptococci from the primary culture plate were then identified as either group A or non-group A by fluorescent-antibody staining (FA Streptococcus Group A reagent, Difco Laboratories, Detroit, Mich.) (9) or by a bacitracin disk sensitivity test (Taxo A Disc; BBL, Cockeysville, Md.) (7). All positive readings were confirmed.

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The second of the paired swabs was processed by a slight modification of the method of Slifkin and Gil (11). The swab was placed in a 1.0-ml Micro Product Vial (Wheaton Scientific, Millville, N.J.) containing 0.3 ml of sterile water and then vigorously rolled and pressed against the inner wall of the vial. After the vial was capped and centrifuged for 10 min at 3000 × g at 25°C, the supernatant was decanted. Excess fluid was drained from the vial through a piece of filter paper. Sodium nitrite (4 N, 20 μl) was then added to the pelletted material in the vial. Then, 2.5 μl of glacial acetic acid diluted 1:1 with sterile water was added to the vial, and the pelletted material was mixed into the solution. The uncapped vial was then incubated for 5 min at room temperature. Approximately 18 to 24 mg of sodium bicarbonate was placed on a microspatula and added to the vial. A 10-μl portion of the supernatant from the uncentrifuged extract was then placed on a glass microscope slide, mixed with 10 μl of Phadebact Strep A Test reagent (Pharmacia Diagnostics, Piscataway, N.J.), and examined under indirect lighting for agglutination.

Of the 414 blood agar cultures performed, 100 (24%) were positive for group A beta-hemolytic streptococci (GABHS). The MCT was positive for 78 (78%) of the 100 patients with positive throat cultures (Table 1). The MCT was negative for 307 (98%) of the 314 patients with negative throat cultures (Table 1). The MCT therefore had a sensitivity of 78%, a specificity of 98%, a positive predictive value of 92%, and a negative predictive value of 93% when compared with blood agar cultures. When throat cultures with greater than 50 colonies of streptococci per plate (3+ and 4+ cultures) were considered positive, the sensitivity of the MCT was 94%, and the negative predictive value was 99%.

Over the years, physicians disturbed by the expense and delay (24 to 48 h) inherent in blood agar culturing that is used to diagnose strepto-

TABLE 1. Comparison of MCT and blood agar culturing

MCT	No. of cultures that were					
	Positive				Negative	
	4+	3+	2+	1+	No beta-hemolytic streptococci	Non-group A beta-hemolytic streptococci
Positive	55	13	8	2	5	2
Negative	4	0	8	10	269	38

coccal pharyngitis have sought alternative methods. Numerous attempts have been made to develop clinical algorithms for the diagnosis of streptococcal pharyngitis; however, clinical diagnoses have proven to be relatively inaccurate when compared with diagnoses by throat culturing (2). Fluorescent-antibody staining of throat swabs has been suggested as a possible alternative to throat culturing (10). Although fluorescent-antibody staining has been a reliable method of grouping streptococci after isolation on blood agar, it has been unreliable when used as a primary method of identification directly from throat swabs (1). Recently, a Gram-stained smear of pharyngeal secretions has been proposed as a possible adjunct to clinical evaluation and throat culturing in the diagnosis of streptococcal pharyngitis (3). The limited data that are currently available suggest that gram-staining requires considerable technical expertise and is relatively insensitive when compared with blood agar culturing.

In 1978, El Kholy et al. (4) described a rapid, modified nitrous acid extraction technique that correctly identified positive throat cultures in 28 of 34 (82%) patients and produced a false-positive result in only 1 patient of 166 (1%) patients with negative throat cultures. Recently, Slifkin and Gil (11) have simplified this nitrous acid extraction technique and combined it with a coagglutination method to produce the MCT for the identification of GABHS on throat swabs. In their diagnostic microbiology laboratory, the MCT correctly identified positive throat cultures in 48 of 62 (77%) patients and produced no false-positive results in 311 patients with negative throat cultures.

Interestingly, all of the false-negative results in the reports by El Kholy et al. (4) and Slifkin and Gil (11) were from patients with <30 colonies of GABHS per blood agar plate. It has been suggested that patients with few streptococcal colonies after primary isolation may be streptococcal carriers (positive throat culture but no immunological response to GABHS) and not actually have streptococcal infections (positive throat culture and an immunological response to GABHS) (8). Since streptococcal carriers are not at risk of developing suppurative or nonsuppurative sequelae and rarely transmit GABHS to others, there is little need to identify and treat these patients (5). In this study, 82% of the false-negative MCT results were from patients with 1+ and 2+ blood agar cultures which suggests, along with the data from earlier studies, that the true sensitivity of the MCT (the ability to identify bona fide streptococcal infections) may be 94% or higher. Further studies are needed to determine whether patients with 1+ and 2+ blood agar cultures are truly infected with GABHS or are streptococcal carriers and to determine the ability of the MCT to identify those patients with true infections.

In conclusion, the MCT is a simple, rapid, inexpensive (~$0.25 per test) procedure for the diagnosis of streptococcal pharyngitis that can be performed simultaneously with 18 throat swabs in approximately 1 h in a busy pediatric ambulatory clinic. The reliability of this method needs to be confirmed by further investigation; however, the preliminary results are encouraging, particularly with respect to patients with 3+ and 4+ blood agar cultures.

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