Evaluation of the Fluorescent Test for Office-Based Detection of Group A β-Hemolytic Streptococci Infection

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Primary care physicians are frequently called upon to diagnose and treat streptococcal illness. Group A β-hemolytic species have been associated with serious complications, making accurate diagnosis important to the clinician. This study was designed to compare a new test using a fluorescent tag with the traditional blood agar-bacitracin disc method and the capillary precipitin test.

The fluorescent test for group A β-hemolytic streptococci has been shown to be rapid and simple to perform. More important, the sensitivity and specificity appear to be equal to the capillary precipitin test and superior to the commonly used blood agar-bacitracin disc method.

Group A β-hemolytic streptococci are frequent causes of skin and upper respiratory tract infections. Group A streptococci are also responsible for two distinct clinical entities, scarlet fever and erysipelas, as well as two potentially serious nonsuppurative complications, rheumatic fever and acute glomerulonephritis. Accurate diagnosis and prompt treatment of streptococcal illness are essential to prevent spread and to reduce the risk of complication.[1,2]

Streptococci are gram-positive coccoid-shaped bacteria that are broadly classified on the basis of their ability to hemolize mammalian red blood cells. A zone of complete hemolysis surrounding colonies grown on blood agar distinguishes β-hemolytic streptococci from α-hemolytic (green or partial hemolysis) and nonhemolytic species.

β-Hemolytic streptococci can be categorized into several groups based on the presence of specific polysaccharide antigens located within the cell wall (Lancefield classification). Serological grouping by the Lancefield method is quite precise yet time consuming and, thus, not feasible as a rapid diagnostic test. Fortunately, group A organisms can be identified by differences in sensitivity to the antibiotic bacitracin. These two properties, β-hemolysis and inhibition of growth by bacitracin on blood agar, have been used for decades to identify organisms that are thus designated as group A β-hemolytic streptococci.

Pollock and Dahlgren[3] warned that if sensitivity to bacitracin was used as the major criterion for presumptive identification of group A streptococci, the potential for misidentification was significant, especially if the specimen was taken from the upper respiratory tract or from a wound.

Recently commercial tests have become available to detect group A streptococci using a fluorescent tag. The purpose of this study was to test this method against the standard culture technique.

METHODS

In 1969 Szewczuk and Mulczyk[4] reported the presence of the enzyme pyrrolidonyl peptidase in bacteria. Facklam et al[5] modified a test that utilizes this enzyme produced by group A streptococci to hydrolyze pyrrolidonyl-β-naphthalamide (PYR). More recently, a fluorescent tag has been added to PYR that can be cleaved and visualized under ultraviolet light. This reaction is summarized in Figure 1.

The cleavage of a fluorescent tag by group A streptococcus enzyme pyrrolidonyl peptidase is a rapid re-

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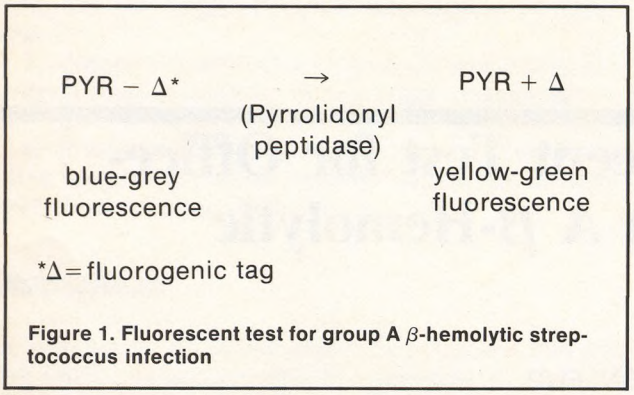

Figure 1. Fluorescent test for group A β-hemolytic streptococcus infection

TABLE 1. AGREEMENT BETWEEN FLUORESCENT TEST AND BLOOD AGAR CULTURE USING PATIENT THROAT SWABS

Test	Agreement			
Fluorescent test	+	+	−	−
Blood agar culture	+	−	+	−
Result	64	53	7	367

Sensitivity = (64/64 + 7) × 100 = 90.1%
Specificity = (376/376 + 53) × 100 = 87.6%

TABLE 2. AGREEMENT BETWEEN FLUORESCENT TESTS AND CAPILLARY PRECIPITIN WHEN USING KNOWN ISOLATED GROUP A β-HEMOLYTIC COLONIES

Test	Agreement			
Fluorescent test	+	+	−	−
Capillary precipitin	+	−	+	−
Result	271	0	0	134

Sensitivity = (271/271 + 0) × 100 = 100%
Specificity = (134/134 + 0) × 100 = 100%

TABLE 3. COST COMPARISON OF FLUORESCENT TEST WITH STANDARD BLOOD AGAR CULTURE

	Fluorescent Test ($)	Culture ($)
Cost per test	2.75	1.50-2.50
Additional equipment	50.00-70.00*	100.00-200.00**

**Ultraviolet light source*
***Incubator*

action that can be confirmed within ten minutes under an ultraviolet light source (254 nm, short wave length) by visualizing yellow-green fluorescence. Commercial kits (Strep A Fluor)* are now available for office use. Each kit contains sufficient materials for 50 determinations and includes test strips, buffer, positive standards, and Dacron swabs. Positive and negative control cultures and ultraviolet light source are not provided.

Between September 1984 and September 1985 a total of 500 patients presented to five primary care centers with the chief complaint of sore throat. Subjects' ages ranged between 2 and 64 years. No distinction was made based on the presence or absence of fever, history of exposure to persons with documented streptococcal infection, presence of strawberry tongue, or presence of tonsillar exudate. Patients were excluded from this study if antibiotics had been taken for any purpose within one month from the time of visit.

Pharyngeal swabs were then tested using the fluorescent group A detection kit (Strep A Fluor) and blood agar-bacitracin disc culture technique. Both tests were inoculated immediately from Dacron swabs. Cultures were incubated at 37°C and read from this primary inoculation at 24 and 48 hours. Cultures were considered positive if β-hemolysis was present and any zone of inhibition existed around the bacitracin disc. Fluorescent tests were interpreted as positive if any level of yellow-green fluorescence existed on the test strip that was clearly differentiated from the negative control. All cultures of fluorescent tests were read by physicians, nurse practitioners, or experienced laboratory personnel. The staff reading the throat cultures were unaware of the results obtained on the fluorescent test done at the time of the patient visit.

For the purpose of interpreting agreement between the fluorescent test and patient culture samples, a total of 405 tests were performed using the fluorescent test and the capillary precipitin test with isolated colonies. A total of 271 known group A β-hemolytic colonies were evaluated using the fluorescent test. One hundred thirty-four non-group A colonies were selected as negative controls. All colonies were obtained from stock laboratory controls. The fluorescent test results were interpreted by experienced laboratory personnel who were unaware of the source of the colonies being studied.

RESULTS

Of the 500 patients being tested, 64 had positive

*Available from Bio Spec, Inc., 179 Mason Circle, Suite A, Concord, CA 94520-1213; telephone (415) 689-0771.

fluorescent tests and positive cultures. Seven had negative fluorescent tests and positive cultures. Fifty-three had positive fluorescent tests and negative cultures. The remaining 367 had both negative fluorescent tests and cultures. These results are summarized in Table 1. All patients with either a positive fluorescent test or positive culture or both were subsequently treated with an appropriate antibiotic for ten days.

The results of the control study comparing the fluorescent test and the capillary precipitin test are shown in Table 2.

The approximate cost of the fluorescent test and culture method, including ancillary equipment, is summarized in Table 3.

DISCUSSION

Rosenstein et al[6] have reviewed the accuracy of identifying group A streptococci using blood agar-bacitracin disc culture in the office-based practice. Their overall results show that 18.9 percent of the positive isolates (false negatives) were missed by physicians. One source of error may be physicians' lack of skill in interpreting throat cultures. An additional source of error using the culture test is the susceptibility of the β-hemolytic non-group A, B, or D streptococci to bacitracin. Group C and G streptococci are occasionally (5 percent to 20 percent) susceptible to bacitracin and are, thus, erroneously presumed to be group A.[3,7,8]

Clinically, false-positive results are potentially less harmful than false-negative results. False-negative cultures may be obtained when the throat is swabbed poorly and a small inoculum obtained, or when the bacitracin disc contains too low a concentration of antibiotic.

The sensitivity of the fluorescent test, ie, the percentage of positive fluorescent tests that identifies infection based on patient-culture confirmation is 90 percent.

The specificity of the fluorescent test, ie, the percentage that a negative test identifies no infection when based on patient-culture confirmation is 87.6 percent.

The sensitivity and specificity of the fluorescent test based on the capillary precipitin test as defined by Facklam[9] using known group A β-hemolytic colonies were 100 percent, rspectively. The predictive value of a positive or negative test would be based on the incidence of disease in a community at any given time. Assuming the prevalence of β-hemolytic streptoccal infection in the community to be 10 percent at any given time, the predictive value of a positive test with 90 percent sensitivity and 87.6 percent specificity would be 55 percent.

The commonly used throat culture utilizing blood agar requires a 24-hour to 48-hour incubation period, which may delay diagnosis and treatment. The blood agar culture provides an overall accuracy of only about 70 percent to 80 percent in office-based studies.[8,10] Based on the results of this study, the fluorescent test for group A streptococcus may be considered superior to the traditional blood agar-bacitracin test when used in the office setting.

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