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Evaluation of the Strep-A-Fluor Identification Method for Group A Streptococci

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Strep-A-Fluor (Bio Spec, Inc., Dublin, Calif.) is a new test designed for the rapid identification of group A streptococci. A filter paper strip impregnated with a synthetic substrate is used to detect a specific aminopeptidase present in group A streptococci by UV fluorescence. In a blind study, 305 beta-hemolytic streptococcal isolates were correctly categorized as group A or non-group A.

Group A beta-hemolytic streptococci have traditionally been identified by susceptibility to bacitracin (4) or by the capillary precipitin test after appropriate extraction (8). More recently, latex and coagglutination methods have been used for the rapid identification of streptococcal groups (1, 5).

In 1981, Godsey et al. (Abstr. Annu Meet. Am. Soc. Microbiol. 1981, C84, p. 276) reported a rapid technique to identify groups A and D streptococci based on the hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR) by a specific aminopeptidase found only in these organisms. Facklam et al. (3) modified this technique by incorporating the substrate into agar. Hydrolysis of the substrate was observed after 16 to 20 h of incubation by the addition of *N,N*-dimethylaminocinnamaldehyde, which formed a red color with free β-naphthylamine.

Strep-A-Fluor (Bio Spec, Inc., Dublin, Calif.) is a new, rapid group A streptococcal identification system which incorporates a synthetic substrate, similar to PYR, on a filter paper strip. By using short-wave UV light, hydrolysis of the substrate by streptococcal enzymes can be observed in less than 15 min with the unaided eye. We evaluated the Strep-A-Flour system for the routine identification of group A streptococcal isolates in a laboratory setting.

The test organisms consisted of 305 beta-hemolytic streptococcal isolates which were obtained from throat cultures seen in a local private pediatric practice and from a variety of patient specimens received in the Bacteriology Laboratory at North Carolina Baptist Hospital, Winston-Salem. The organisms consisted of 240 group A, 30 group B, 14 group C, 4 group F, and 17 group G isolates. No group D isolates were included in this study because beta-hemolytic isolates are rarely encountered in routine clinical practice and are usually morphologically distinct from other beta-hemolytic groups. All isolates were identified by Gram reaction, catalase test, and capillary precipitin reaction after autoclave extraction (7). All streptococcal isolates were numerically coded and kept frozen at −70°C before testing.

A sheep blood agar plate was inoculated with each coded isolate and incubated for 18 to 24 h at 35°C. With the aid of an applicator stick, a small portion of the growth was removed and placed on a Strep-A-Fluor filter paper strip. One drop of 0.1 M Tris buffer (pH 6.5) was added to the inoculated portion of the strip. The strip was placed in a clear plastic envelope and incubated unsealed at 35°C for 15 min. After incubation, each strip was examined under 254-nm UV light (UVP, Inc., San Gabriel, Calif.). Any yellow-green fluorescence, indicative of enzymatic hydrolysis of the synthetic substrate, was considered positive; negative reactions showed no fluorescence. Known groups A and B streptococcal isolates were tested concurrently as quality control procedures. The technologist performing the tests had no prior knowledge of the group of the coded isolates until the completion of the study.

Of the 305 streptococcal isolates tested with Strep-A-Fluor, all 240 group A streptococci gave positive reactions. The remaining 65 non-group A isolates gave negative reactions.

Although bacitracin has been used for years for the identification of group A streptococci, the results are only presumptive, since false-negative and false-positive reactions do occur (2, 6). Definitive identification of these organisms requires extraction and testing by the capillary precipitin method. Extraction methods usually require overnight incubation and are labor-intensive. Coagglutination and latex procedures have reduced the labor involved in streptococcal identification, but most isolates still require enzymatic or acid extraction before testing.

Godsey et al. (ASM Annu. Meet. 1981, p. 276) originally reported the use of PYR for the rapid identification of *Streptococcus pyogenes* and group D streptococci. In his study, 95% of the *S. pyogenes* isolates hydrolyzed PYR. Facklam et al. (3) used a modified version of Godsey's procedure for the presumptive identification of streptococci by incorporating PYR into agar. The modification was 98% effective for the differentiation of groups A and B streptococci after overnight incubation. Both of these tests required significant incubation time and the addition of a reagent to observe the hydrolytic reaction. Strep-A-Fluor requires only 15 min of incubation and needs no additional reagents. Group A streptococcal isolates can be rapidly identified without extraction or overnight incubation.

Strep-A-Fluor provides the laboratory with an innovative method that is easy to use and interpret and requires minimal technical time for accurate determination of group A streptococci.

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