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Rapid, Cost-Effective Identification of Group A Streptococci and Enterococci by Pyrrolidonyl-β-Naphthylamide Hydrolysis

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Group A streptococci and enterococci were identified by two L-pyrrolidonyl-β-naphthylamide (PYR) hydrolysis tests. One uses Minitek PYR disks (BBL Microbiology Systems, Cockeysville, Md.); the other uses a swab impregnated with PYR reagent. Both tests and Strep-A-Chek (EY Laboratories, San Mateo, Calif.), a commercially available PYR test, were compared with conventional methods. The PYR methods correctly identified all strains of group A streptococci and enterococci tested.

Presumptive tests for identification of group A streptococci (GAS) and enterococci (ENT) include bacitracin susceptibility testing, bile-esculin hydrolysis, and salt tolerance (2, 3). Although inexpensive and easy to perform, traditional methods require overnight incubation and results are not always reliable. Latex and coagglutination tests provide rapid, accurate group identifications (2) but are expensive. ENT must be differentiated from other group D streptococci by a salt tolerance test that adds to the total cost and time to obtain results.

Godsey et al. (J. Godsey, R. Schulman, and L. Eriquez, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C84, p. 276) and others (3, 4) have demonstrated that L-pyrrolidonyl-β-naphthylamide (PYR) hydrolysis is a reliable test for the identification of GAS and ENT. Whereas earlier tests required incubation for 4 h to overnight to obtain results, recent modifications allow detection of PYR hydrolysis in 10 to 15 min (1, 4, 5). This study describes two PYR tests. One uses Minitek PYR disks (BBL Microbiology Systems, Cockeysville, Md.); the other uses swabs impregnated with PYR reagent. Both methods and Strep-A-Chek (EY Laboratories, San Mateo, Calif.), a commercially available PYR test, were compared with conventional biochemical and serological methods for accurate, cost-effective differentiation of GAS and ENT from other streptococci.

(This work was presented in part at the 87th Annual Meeting of the American Society for Microbiology, Atlanta, Ga., 1 to 6 March 1987 [S. Wellstood, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, C84, p. 337].)

A total of 198 isolates from fresh clinical specimens and 7 quality control strains (American Type Culture Collection, Rockville, Md.) were tested. Included were 60 GAS, 27 group B streptococci, 8 group C streptococci, 75 enterococci, 3 group D nonenterococci, 10 viridans group streptococci, 2 pneumococci, 4 group F streptococci, 15 group G streptococci, and 1 nongroupable strain. The isolates were initially screened with a Gram stain and catalase test. Beta-hemolytic streptococci from throat cultures were subcultured to a blood agar plate and tested for susceptibility to a 0.04-U bacitracin disk (BBL Microbiology Systems) or grouped directly from primary plates by the Streptex latex agglutination method (Burroughs Wellcome Co., Research Triangle Park, N.C.). Beta-hemolytic streptococci from other sources were grouped by the Streptex method. Alpha-hemolytic streptococci were classified as enterococci, group D nonenterococci, viridans group streptococci, or pneumococci based on bile-esculin hydrolysis, 6.5% NaCl tolerance, and susceptibility to a 6.0-mm optochin disk (BBL Microbiology Systems). Experimental tests were performed from primary plates when sufficient colonies were available. Otherwise, isolates were subcultured to a blood agar plate.

The Minitek method was performed by removing two to four colonies of each isolate from plates with a wooden applicator stick. The inoculum was rubbed onto a Minitek PYR disk contained in a petri disk lid; disks were rehydrated by adding 20 to 25 μl of phosphate buffer (pH 7.5). After incubation at room temperature for 10 min, a drop of cinnamaldehyde (Analytab Products, Plainview, N.Y.) was added to each disk. Final reactions were read at 2 min, and the color intensity was noted. Positive reactions were indicated by the formation of any color ranging from light pink to cherry red. Negative results were indicated by the absence of color on the disk. The disks were held for 30 to 45 min before being discarded to determine whether positive results faded or negative results turned falsely positive.

For the swab method, two to four colonies were removed from a blood agar plate with a cotton-tipped applicator stick. Swabs were moistened with two drops of PYR solution prepared by dissolving 25 mg of PYR (Sigma Chemical Co., St. Louis, Mo.) in 1 ml of methanol and adding distilled water to make 100 ml. After incubation in a petri disk lid for 10 min at room temperature, 1 drop of cinnamaldehyde reagent (Analytab Products) was added and reactions were read for color production as described above.

Strep-A-Chek tests were performed in accordance with the instructions of the manufacturer. Strips were incubated in a petri dish lid.

All three test methods correctly identified the 60 GAS and 75 ENT as PYR positive; all non-group A beta-hemolytic streptococci and nonenterococcal strains of alpha-hemolytic streptococci remained negative. Although the recommended incubation period for the Strep-A-Chek test is 10 min, strong positive reactions appeared immediately for most isolates. A few strains required up to 5 min of incubation to develop color. For the Minitek and swab methods, most positive reactions also occurred immediately after adding the color developer. Colors intensified slightly with time and did not fade for the observed period of 30 to 45 min; negative tests remained colorless. When the available inoculum was sparse (two colonies, each ≤1.0 mm), positive reactions sometimes

appeared as a small, pink or red dot on the tip of the swab or at the point of inoculation on the Minitek disk or Strep-A-Chek strip.

The PYR tests evaluated in this study were 100% sensitive and specific for the identification of GAS and ENT. The results obtained by the Minitek method were similar to those reported by Farmer et al. (P. Farmer, S. Wood-Helie, and R. Johnson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C265, p. 372). They added a Minitek PYR disk to a heavy suspension (McFarland standard of 2 to 3) of the organism and incubated the test mixtures at 37°C. The major advantages of the Minitek procedure reported here are direct inoculation of the disk, incubation at room temperature, and the use of a smaller inoculum. The cost per test is $0.18; the disks have a shelf life of 18 months and come ready to use.

The swab method was least expensive at a cost of $0.02 per test. The prepared reagent is stable for at least 5 months. However, colonies must be carefully picked from throat cultures or other specimens with a heavy growth of normal flora to avoid picking up nonstreptococci on the swab. Organisms other than streptococci may produce false-positive PYR results (5), and mixed cultures may result in false-negative tests.

The Strep-A-Chek results were comparable to those previously reported (5). Although this method offers the advantage of rapid positive results (<10 min), the color developer provided in the kit is unstable, must be reconstituted for each test or batch of five tests, and used within 30 min of reconstitution. Since there is insufficient developer provided to perform tests individually or in small numbers, low-volume laboratories would need additional tubes of developer, which would add to the cost of the test. Larger laboratories would have to batch tests to avoid purchasing additional reagents. A stable, prepared developer would add to the convenience and efficiency of the Strep-A-Chek test.

GAS and ENT are unique among the streptococci in their ability to hydrolyze PYR. Therefore, PYR tests provide a rapid, simple, cost-effective alternative to standard biochemical or latex agglutination tests for differentiating these organisms from other groups of streptococci. Tests may be performed conveniently at the bench, require little hands-on time or preparation, and save the time required for and expense of subcultures, antigen extractions, or remote incubation in a 35°C incubator. Positive results are available in 10 to 12 min with a small inoculum. The reagents are inexpensive, have a long shelf life, and may be purchased commercially or may be prepared in the laboratory for additional cost savings.

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