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Presumptive Identification of "*Streptococcus milleri*" in 5 h

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Rapid miniaturized tests for acetoin production, arginine hydrolysis, and sorbitol fermentation were used for presumptive identification of non-beta-hemolytic "*Streptococcus milleri*" isolates in 5 h. All 77 "*S. milleri*" strains tested were Voges-Proskauer positive, arginine hydrolysis positive, and sorbitol fermentation negative. On the basis of these reactions, "*S. milleri*" was differentiated from isolates of other viridans group streptococcal species and from *Streptococcus bovis*.

Streptococci referred to as "*Streptococcus milleri*" have been implicated as agents of purulent infection in numerous body sites (6, 13–18, 22, 23). These organisms may be beta-hemolytic, non-beta-hemolytic, or alpha-reacting on blood agar, and Facklam (8) has proposed that, based on hemolytic as well as physiological characteristics, these streptococci be separated into the species *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus*. Non-beta-hemolytic "*S. milleri*" isolates are likely to be identified only as viridans group streptococci and possibly regarded as clinically insignificant unless they are fully identified to the species level. Identification can be accomplished with tubed and plated media (10) or commercially available kits (1, 2, 9, 11, 20), but these methods may be considered either too time-consuming or expensive for routine use in many laboratories.

We propose the use of three rapid miniaturized physiological tests for presumptive identification of non-beta-hemolytic "*S. milleri*" isolates. These tests could be used as a screening method to identify organisms for further study or for referral if confirmatory identification is desired.

Presumptive identification of beta-hemolytic "*S. milleri*" isolates from throat cultures was recommended by Bucher and von Graevenitz (4) to differentiate beta-hemolytic large colony group C and G streptococci from beta-hemolytic "*S. milleri*" strains with group C or G antigen. A rapid Voges-Proskauer (VP) test was found to distinguish "*S. milleri*" (VP positive) from other group C and G isolates (VP negative). The utility of this test was substantiated by other authors (12, 21).

The VP test alone is insufficient for identification of non-beta-hemolytic "*S. milleri*" because other viridans group species, as well as *Streptococcus bovis*, are usually VP positive (5, 17). We propose use of a test for arginine hydrolysis to separate "*S. milleri*" (VP and arginine hydrolysis positive) from *S. bovis*, *Streptococcus salivarius*, and most strains of *Streptococcus mutans*, which are usually VP positive and arginine hydrolysis negative. Finally a test for sorbitol fermentation can be used to differentiate arginine hydrolysis-positive *S. mutans* (usually sorbitol fermenting) from "*S. milleri*" (sorbitol fermentation negative).

The VP test was performed as described by Bucher and von Graevenitz (4) by inoculating 0.2 ml of MR-VP broth (Northeast Laboratories Services, Waterville, Maine) in a tube (10 by 75 mm) with a loopful of growth from an overnight blood agar plate culture. The resulting turbid suspension was incubated for 5 h at 35°C, and then 1 drop each of 0.5% creatine, alpha-naphthol (Analytab Products, Plainview, N.Y.), and 40% potassium hydroxide was added to the tube. After thorough shaking, the suspensions were examined for the development of a pink to red color within 15 min, indicating a positive test. For the arginine hydrolysis test, turbid suspensions of each organism were made in tubes (10 by 75 mm) containing 0.2 ml of Moeller decarboxylase broth base and Moeller base supplemented with arginine (GIBCO Diagnostics, Madison, Wis.). The suspensions were overlaid with a few drops of mineral oil and incubated for 5 h at 35°C. Acid reactions (yellow) in both tubes denoted no action on arginine, whereas hydrolysis was indicated by an acid reaction in the basal medium coupled with an alkaline (purple) reaction in the arginine-containing medium. Phenol red broth containing sorbitol (Remel, Lenexa, Kans.) was dispensed, inoculated, and incubated with a mineral oil overlay as described above. A definite yellow or light orange color indicated fermentation of sorbitol, whereas any shades of red or dark orange were considered negative.

The results obtained when these three tests were performed on a number of streptococcal species are shown in Table 1. The streptococci consisted of clinical isolates and stock strains; some of the *S. mutans* strains were isolated from the mouths of healthy volunteers. The organisms were identified by previously described conventional methods (10, 19) or with the Rapid Strep system (1, 11, 20) or in some cases by both conventional methods and the Rapid Strep kit. The "*S. milleri*" isolates examined are subdivided in Table 1 by the nomenclature of Facklam (8). All "*S. milleri*" isolates tested could be differentiated from all other streptococci tested by virtue of positive reactions in the VP and arginine hydrolysis tests and negative reactions in the sorbitol fermentation test. Positive VP reactions were noted with all isolates of *S. mutans* and *S. bovis* and all but two isolates of *S. salivarius* and one isolate of *S. bovis* variant. The rapid VP-negative *S. salivarius* and *S. bovis* variant isolates gave strongly positive VP reactions in the Rapid Strep system with pyruvate as the substrate for acetoin production. When grown in MR-VP broth containing glucose as the substrate, the *S. salivarius* strains gave positive VP reactions after 24 h of incubation and after 48 h of incubation the *S. bovis* variant strain reacted positively. In the isolates examined, sorbitol fermentation differentiated the VP-positive, arginine hydro-

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TABLE 1. Reactions of various streptococci in rapid miniaturized tests for acetoin production, arginine hydrolysis, and sorbitol fermentation

Species	No. tested	No. of isolates positive in the following test:		
		VP	Arginine hydrolysis	Sorbitol fermentation
S. mutans	12	12	1[a]	11[b]
S. salivarius	30	28	0	0
S. sanguis I	31	0	24	0
S. mitis	31	0	4	0
S. sanguis II	31	0	2	0
S. bovis	29	29	0	0
S. bovis variant "*S. milleri*"	15	14	0	0
S. intermedius	31	31	31	0
S. intermedius (mannitol positive)	20	20	20	0
S. constellatus	26	26	26	0

[a] This isolate was also sorbitol fermentation positive.
[b] The sorbitol fermentation-negative isolate was also arginine hydrolysis negative.

lysis-positive *S. mutans* strain from "*S. milleri*." The sorbitol fermentation-negative *S. mutans* isolate was also arginine hydrolysis negative. All isolates of *Streptococcus sanguis* I, *Streptococcus mitis*, and *Streptococcus sanguis* II were VP negative and could be differentiated from "*S. milleri*" on that basis.

The data presented suggest that non-beta-hemolytic and alpha-reacting "*S. milleri*" isolates can be presumptively identified by their ability to produce acetoin and hydrolyze arginine coupled with their inability to produce acid from sorbitol. Although all 77 "*S. milleri*" strains tested gave typical reactions, isolates with aberrant reactions would not be detected with the use of only three tests. In addition, strains of other species which display atypical reactions (e.g. *S. mutans* which is arginine hydrolysis positive and sorbitol fermentation negative) could be misidentified as "*S. milleri*." However, it seems that in the great majority of cases the three-test system described here will correctly differentiate "*S. milleri*" from other non-beta-hemolytic streptococci.

Methods used for determining acetoin production and arginine hydrolysis seem to affect the results obtained. In studies using conventional media, not all organisms identified as "*S. milleri*" were scored as VP positive (3, 5, 17) and arginine hydrolysis positive (3, 5, 7, 17). In our experience, these growth-dependent methods tend to produce false-negative results because of the poor growth of some "*S. milleri*" strains in the media used (unpublished observations). Conversely, a false-negative VP result in the rapid test was observed with one *S. bovis* variant and two *S. salivarius* isolates, but none of the 77 "*S. milleri*" strains tested behaved similarly.

The estimated cost of materials for performing the three tests described here is about $0.25 per isolate with commercially prepared media which is redistributed in 0.2-ml amounts. Thus, these methods afford a simple and economical means of presumptive identification of "*S. milleri*." This organism stands out among viridans group streptococci as an agent of purulent disease, and its identification can have important clinical implications. For example, identification of an agent of bacteremia as "*S. milleri*" can provide a clue to the presence of an otherwise inapparent pyogenic focus of infection. Even presumptive identification of non-beta-hemolytic streptococci as "*S. milleri*" by the methods reported here could provide clinically useful information.

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