KS5300/KS530020 FLUO-CARD MILLERI

PRINCIPLE / DISCUSSION:

The "*Streptococcus milleri* group," long recognized as more virulent than other viridans streptococci and often associated with abscess formation, has gone through numerous name changes and taxonomic challenges. Recently, however, they have been recognized as a legitimate taxonomic group of three species that appear to be associated with distinctive clinical syndromes (2). They share several phenotypic characteristics that differentiate them from other streptococci; all Lancefield group F streptococci are "S. Milleri group," but the converse is not true (4). Members of the group may possess Lancefield group antigens other than F. "*S. milleri*" can exhibit any type of hemolysis on blood agar. A distinctive caramel-like odor may be observed in some isolates, which serves as a preliminary clue to their identity. All viridans streptococcal isolates from sterile body sites and those associated with abscesses, pneumonia, and other serious disease should be identified. Fluo-Milleri substrates are designed to differentiate the three *Streptococcus milleri* group species based on the enzymes produced by each (1,3,5). The last three substrates are all 4-methylumbelliferyl (4-MU) fluorogenic compounds. Enzymes produced by the organisms attack and degrade the target substrate bound to 4-MU, which releases blue fluorescent 4-methylumbelliferone.The first circle is 7-amido-4-methylcoumarin which also fluoresces blue when released. This blue fluorescence can be observed under a hand-held long-wave-length UV lamp (i.e. Wood's Lamp). Colonies must be identified as belonging to the "*S. milleri* group" before they can be tested. The following minimal characteristics are necessary to assign an isolate to this group: Gram positive cocci (may be elongated) in pairs and chains; Catalase negative; Voges-Proskauer positive; Arginine positive; Sorbitol negative and PYR negative.

MATERIALS REQUIRED:

FLUO-CARDMilleri test is provided in sets of 10 or 20 trays. FLUO-CARD Milleri requires fresh 24 hour growth of the organism on appropriate agar media, usually a nutrient agar base with blood or other comparable enrichment additive. A loop or stick for harvesting the organisms and distilled water, pH 6.5 to 7.5 are needed but not provided.

LIMITATIONS / COMMENTS:

Only viridans streptococci of the "*milleri* group" should be tested using this system. Other organisms may yield positive results in any of the test wells. Occasionally other colors of fluorescence occur. These are not reactions due to the respective enzyme activity and should be disregarded, except in the case of circle one. If in doubt, hold the product label under the Wood's lamp as a color guide.

STORAGE:

Store in freezer in original packaging. It is not necessary to thaw before use.

PROCEDURE:

(1) Put 2 drops of water in each circle.

(2) Smear 5-10 isolated colonies (a visible paste) of the test organism on each circle using a fresh loop or stick for each circle.

(3) Incubate covered or uncovered for 15-20 minutes at 35-37°C.

(4) After incubation, observe for fluorescence by holding the tray under a Wood's Lamp.

INTERPRETATION:

The appearance of an intense blue fluorescence where the organism was smeared is a positive result (note: test #2 is not as bright as 3 and 4). Negatives in well 1 will fluoresce purple. Interpret well 1 first. If well 1 fluoresces blue, the organism is not Milleri group *Streptococci* since they are negative for PYR. This will invalidate the test. If well 1 is negative, proceed with identification, following the flow chart or the reaction chart at the end of this insert. When following the flow chart, ***stop at the first positive circle. The identification is printed directly below the (+). Disregard any subsequent positive circles or other color fluorescence.***

QUALITY CONTROL:

Dispose of all used material in a manner appropriate for bacterial contamination. We recommend testing each lot. Some suggested strains are listed on the chart.

REFERENCES:

(1) Whiley, R.A., K. Fraser, J. M. Hardie, and K. Beighton. 1990. Phenotypic differentiation of Streptococcus intermedius, Streptococcus constellatus and Streptococcus anginosus strains within the "Streptococcus milleri" group. J. Clin. Microbiol. 28:1497-1501.

(2) Whiley, R.A., D. Beighton, T.G. Winstanley, et al. 1992. Streptococcus intermedius, Streptococcus constellatus and Streptococcus anginosus (the "Streptococcus milleri" group): association with different body sites and clinical infections. J. Clin. Microbiol. 30:243--244.

(3) Beighton, D., and R. Whiley. 1990. Sialidase activity of the "Streptococcus milleri group" and other viridans group streptococci. J. Clin. Microbiol. 28: 1431-1433.

(4) Ruoff, K. and M. J. Ferraro. 1986. Presumptive identification of "Streptococcus milleri" in 5 h. J. Clin. Microbiol. 24:495-497.

(5) Flynn, Cynthia E. and Ruoff, Kathryn L.. 1995. Identification of "Streptococcus milleri" Group isolates to the Species Level

FLOW CHART

1. (-) ☞ 2. (-) ☞ 3. (-) ☞ 4. (-) STOP
(+) (+) (+) (+)
STOP Not Milleri | Strep. Intermedius | Strep. anginosus | Strep. constellatus

EXPECTED REACTIONS: % POSITIVE				
STRAIN	1.Pyr	2. ß-fuco.	3. ß-gluco.	4. a-gluco.
S. intermedius QC=ATCC 27335	0	90	47	100
S. anginosus QC=ATCC 33397	0	0	96	19
S. constellatus QC=ATCC 27823	0	0	4	90

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