NOTES

Improved Medium for Detecting Pigment Production by Group B Streptococci

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The production of pigment which can be used for presumptive identification of group B streptococci is sometimes weak and difficult to observe in Columbia agar stab cultures. A medium which is whiter and affords better visibility of this pigment is described.

Pigment production for presumptive identification of group B streptococci has been reported using Columbia agar or Columbia blood agar (3, 6). Fallon recently reported that 85% of his clinical isolates formed pigment on the surface of Columbia agar plates incubated anaerobically (3). We subsequently reported that 96% of our clinical isolates showed pigment in stabs of Columbia agar deeps and suggested the use of the more convenient stabs as a screening test for group B streptococci in busy clinical laboratories not performing serological studies (6).

The Columbia agar stab cultures that we have used have the disadvantage that the yellow tinge of the medium obscures the pigment formed by those strains producing a small amount of yellow-orange pigment. Interpreting the stab cultures of these weaker pigment producers required some experience. We decided to prepare an agar medium that was not yellow but would support the growth of and pigment production by the group B streptococci. In this communication we describe components required by the organisms for the production of pigment.

Media requirements. Several media were prepared, and it was noted that GC basal medium (Difco) was white and contained components similar to those in Columbia agar. The GC basal medium alone did not support the production of pigment by the group B streptococci. However, the addition of proteose peptone to the medium resulted in a white medium that allowed easy observation of the yellow to orange pigment even when using weakly pigmented strains. We recommend the use of this medium (which we will call DMS medium), which can be prepared by adding 1% additional proteose peptone no. 3 (Difco) to the GC

basal medium made full strength without hemoglobin. The resulting medium contains 25 g of proteose peptone, 1 g of starch, 4 g of K_2HPO_4 , 1 g of KH_2PO_4 , 5 g of NaCl, and 10 g of agar per liter. The medium is dispensed, 7 ml into 16- by 125-mm or 3 ml into 13- by 100-mm screw-capped tubes, autoclaved, and then stored for use for stab cultures. The stabs are incubated at 37°C for 24 h. Pigment production is evident at 24 h.

We have not defined the component of proteose peptone required for the pigment production. Technical-grade Casamino Acids, but not vitamin-free Casamino Acids, can be used in place of the 1% proteose peptone added to the basal medium. Fallon reported (3) that starch was a requirement for pigment production in his technique. However, in our study, when the DMS medium was prepared without starch, pigment production was very good. The pigment tended to be quite red without the starch; however, the growth of the organism was poorer and the pigment production was not uniform throughout the stab. Therefore, although the starch is not required for pigment production, we recommend the inclusion of starch for slightly more uniform results. The discrepancy between our results and Fallon's results cannot be explained; however, Fallon used agar plates under specific anaerobic conditions and we used stab cultures in agar deeps.

Since our previous report (6) we have obtained 90 additional cultures documented by counterimmunoelectrophoresis (4, 6) and hippurate hydrolysis (2) to be group B streptococci. Of these, 84 were positive for pigment formation on Columbia agar and the other 6 were called negative on Columbia agar by one or more observers. However, all 90 of these pro-

duced readily visible pigment on the DMS medium.

Anaerobic requirements. Fallon (3) described specific anaerobic conditions required for the production of pigment. We found the stab culture incubated under atmospheric conditions to be easier and more reliable in our hands. We have noticed that with some cultures, pigment production does not occur within 2 mm of the surface, indicating the need for anaerobic conditions.

To further document Fallon's observations on anaerobic conditions, we prepared petri dishes with DMS medium. Twenty-four organisms were taken at random from our collection and each plated on two plates. One set of plates was incubated at 37°C in a candle jar and the other was incubated at 37°C in an anaerobic Gas-Pak jar (BBL). Our results are in agreement with Fallon's since no organisms produced pigment in the candle jar and in the Gas-Pak jar only 17 out of 19 of the group B streptococci produced pigment. The non-group B streptococci did not produce pigment. All 19 group B organisms produced pigment in stab cultures in DMS medium. Thus, the growth conditions in stab cultures are superior to those obtained on the surface of Gas-Pak anaerobic plates for producing pigment. This may explain why Fallon, using plates under his anaerobic conditions, reported that only 85% of his clinical isolates formed pigment, whereas we, using stabs, found that a much higher percentage of our clinical isolates formed pigment.

Over the past year, our laboratory has collected beta-hemolytic streptococci that are not group A or D. Of these, 158 were proven to be group B by serological tests and hippurate hydrolysis; 156 of these produced pigment. However, further studies with more clinical isolates will be needed before the true percentage of false negatives in this test (group B streptococci not forming pigment in stabs of DMS medium) is known. Our previous study found 2 of 68 isolates (3%) that failed to produce pigment in Columbia agar. The present investigation shows that DMS medium is superior to Columbia medium. However, until further studies are completed, it will continue to be very important to test all non-group A or D beta-hemolytic streptococci which are not pigmented in DMS medium by the more conventional serological

tests (4, 5), hippurate hydrolysis (2), or the CAMP test (1) before the organisms can be concluded not to be group B streptococci. Although hippurate hydrolysis is also a useful test for identification of group B streptococci, some streptococci, other than group B, hydrolyze hippurate (2) and care must be taken to recognize these organisms.

The production of pigment in DMS medium and Columbia agar has been studied using isolated colonies of beta-hemolytic streptococci from clinical cultures. We have not studied the production of pigment by other genera and care must therefore be taken when using this test to be sure a well-isolated colony of beta-hemolytic streptococci is used for the stab culture. We have studied over 170 clinical isolates of beta-hemolytic streptococci that are serologically not group A and not group B, and none of these have produced pigment. Similarly, none of the 50 cultures found to be group A by fluorescent antibody which we have studied have produced pigment. We have had no false positives (streptococci which are not group B producing pigment) in our studies to date.

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