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New Granada Medium for Detection and Identification of Group B Streptococci

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A methotrexate-containing medium for the detection of beta-hemolytic group B streptococci from clinical specimens on the basis of detection of pigment is described. The medium contained peptone, starch, serum, $MgSO_4$, glucose, pyruvate, methotrexate (as pigment enhancer), phosphate-morpholinepropanesulfonic acid buffer, and selective agents. The recovery of beta-hemolytic group B streptococci was comparable to that obtained with selective broth.

Streptococcus agalactiae (Lancefield group B streptococcus [GBS]) is an important cause of perinatal and infant morbidity worldwide and can also cause serious infections in adults (3).

The production of an orange carotenoid pigment is a unique characteristic of beta-hemolytic GBS isolated from humans, which varies according to the culture medium used (12) and serves as the basis for several media for the detection and identification of GBS from clinical specimens. The sensitivities of these media, however, are low (2, 5–8, 10, 13–15). In this report, we describe a new, selective, and differential medium for the rapid detection of beta-hemolytic GBS (New Granada Medium [NGM]); methotrexate (amethopterin) is used in the medium as a pigment-enhancing folic acid antagonist (2, 10).

(This work was presented in part at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 29 September to 2 October 1991 [11].)

Preparation of NGM. For the preparation of NGM, 10 g of agar (3 g if the medium is used in tubes), 25 g of proteose peptone no. 3 (Difco), 20 g of soluble starch (1252; Merck), 11 g of morpholinepropanesulfonic acid (MOPS) hemisodium salt (Sigma), and 8.5 g of anhydrous Na_2HPO_4 were dissolved in up to 1,000 ml of water by heating gently (and stirring several times). After dissolution was complete, the solution was autoclaved at 0.5 atm for 20 min (or at 1 atm for 7 min). The solution was allowed to cool (50 to 55°C); and then 10 ml of a filter-sterilized solution containing 250 g of glucose per liter, 100 g of sodium pyruvate per liter, 20 g of anhydrous $MgSO_4$ per liter, 0.6 g of methotrexate sodium salt (Lederle) per liter, 0.02 g of crystal violet (15940; Merck) per liter; 0.5 g of colistine sulfate per liter, and 1 g of metronidazole (this solution is stable for up to 6 months when it is kept frozen at −20°C) were added. Fifty milliliters of sterile horse serum was added, and the solution was poured into plates or dispensed into tubes. The final pH was 7.45 ± 0.1 (no pH adjustment was necessary). To avoid the formation of a precipitate, it is essential to add $MgSO_4$ in solution after the phosphate, MOPS, proteose peptone no. 3, starch, and agar are dissolved. The addition of glucose and pyruvate after autoclaving avoids caramelization and the formation of inhibitors. Plates were kept in plastic bags, and tubes were tightly closed and kept at 4 to 8°C. Although NGM gradually turns a translucent white during storage, its performance is not affected for up to 1 month.

The specificity of NGM was assessed by identifying, by accepted serological and biochemical procedures (4), several colonies from each of 100 clinical specimens that yielded orange colonies in NGM. These colonies were identified as beta-hemolytic *S. agalactiae* in all cases. To determine the sensitivity of NGM, 100 fresh strains of beta-hemolytic GBS isolated from different unselected clinical specimens plated in blood agar were tested. All strains tested produced an orange pigment in plates and tubes of NGM incubated aerobically and anaerobically.

A field evaluation of NGM was carried out with 700 clinical samples (Table 1). All swabs were brought to the laboratory in Stuart medium and were processed within 10 h. Plates and tubes of NGM were compared with (i) plates of selective Islam medium (1 μg of gentamicin per ml plus 10 μg of metronidazole per ml; Oxoid) incubated anaerobically, (ii) tubes of Rapid GBS medium (Oxoid) (15); and (iii) selective Todd-Hewitt broth (THB; 0.8 μg of gentamicin per ml plus 15 μg of nalidixic acid per ml) (9). Each swab was placed in a tube with 0.5 ml of brain heart infusion broth and swirled vigorously. Two additional swabs were immersed in this broth; one of them was stabbed in a tube of NGM, and the other was stabbed in a tube of rapid GBS medium. The original swab was used to inoculate one Islam and two NGM plates and was then put back into the tube, and 2 ml of selective THB was added. One plate of NGM and the Islam plate were incubated anaerobically, whereas the other NGM plate was incubated aerobically (5% CO_2). All plates were read after 18 h. Tubes of NGM and rapid GBS medium were incubated (with the swabs) for 18 h at 37°C in a water bath and were read after 12 and 18 h. Tubes of selective THB were incubated aerobically overnight at 37°C and were then subcultured onto plates of blood agar, Islam, and NGM.

Beta-hemolytic GBS were detected in 115 specimens (Table 1). Use of NGM in tubes incubated aerobically (12 h) and in plates incubated anaerobically (18 h) was the most sensitive means of detecting beta-hemolytic GBS by pigment production (98 and 95%, respectively). Islam plates (18 h) and Rapid GBS tubes (12 h) were the least sensitive methods (66 and 69%, respectively). Two specimens were positive in NGM tubes only, 2 were positive in selective THB only, 1 was positive in NGM anaerobic plates and NGM tubes only,

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TABLE 1. Number of GBS in 700 clinical specimens detected by six methods

Specimen	No. of specimens cultured	No. (%) of positive samples detected by[a]:						
		NGM plates (anaerobic)[b]	NGM plates (aerobic)[b]	Islam plates (anaerobic)[b]	NGM tubes[c]	Rapid GBS tubes[c]	Selective THB[b]	Any method
Perianal	100	20	17	16	21	16	21	22
Urethral	100	18	15	12	18	12	18	18
Vaginal	300	48	41	33	50	33	49	51
Cervical	200	23	19	15	24	15	24	24
Total	700	109 (95)	92 (80)	76 (66)	113 (98)	76 (69)	112 (97)	115

[a] NGM, New Granada Medium; Islam, selective Islam medium; selective THB, selective Todd-Hewitt broth. Percentages indicate percentage of positive samples of total positive samples detected by any method.
[b] After 18 h of incubation at 37°C.
[c] After 12 h of aerobic incubation at 37°C.

and 76 (66%) were detected by all six methods. Pigment intensity and density of growth were weakest in Islam medium and rapid GBS medium. Positive specimens could often be detected after 7 h in NGM tubes, but these early positives were more difficult to distinguish than were those read after 12 or 18 h. NGM also supported growth of some other streptococci, enterococci, staphylococci, yeasts, and *Proteus* spp.; but growth of most of the accompanying flora was inhibited, and brightly pigmented colonies were readily seen (Fig. 1). In bloody or colored specimens (e.g., meconium), the initial reading of NGM tubes may be misleading, and these tubes should be assessed after 18 h. Most beta-hemolytic GBS strains, when incubated aerobically (5% CO_2) in NGM plates, also grew as pigmented colonies, and when they were assessed in tubes, no differences between tubes incubated aerobically or anaerobically were found. There were no differences in pigment production throughout the length of the agar tubes.

Among the methods used to identify beta-hemolytic GBS, the CAMP test is not a rapid test; antigen detection (1) is expensive, its sensitivity is low, and it is better for batch work than it is for work that must be done immediately. The most straightforward method for detecting and identifying beta-hemolytic GBS from clinical specimens is pigment detection (5, 10, 13–15). This method has been reported to be 100% specific (8), and its sensitivity with pure cultures of beta-hemolytic GBS is 97 to 100% (2, 5, 13). Our work confirms earlier observations (6) of the striking difference in the atmosphere requirement for pigment production between cultures in agar plates and in broth or inside agar-containing media. In general, we found great differences in pigment yield between plates incubated aerobically and anaerobically, although there were no differences between tubes incubated in the different atmospheres.

Recently, cloning of the gene(s) that encodes pigment production and hemolysis in GBS (16) has been reported, providing evidence of the genetic linkage between pigment and hemolysin production in these bacteria. Although they are found infrequently and are probably less virulent (10, 13, 16), nonhemolytic (and nonpigmented) GBS have been implicated in cases of neonatal sepsis; because these strains are not identified by use of NGM, other detection methods must be used if these strains are prevalent.

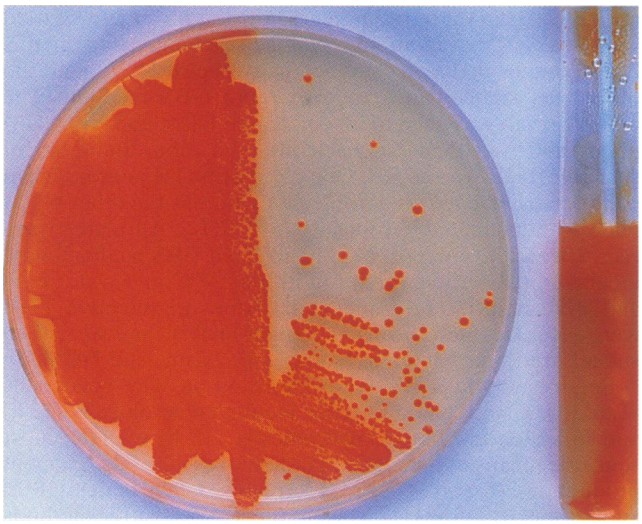

FIG. 1. Appearance of beta-hemolytic *S. agalactiae* from a heavily colonized vaginal specimen after 18 h of anaerobic incubation in NGM (plate) and aerobic incubation (tube).

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