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Evaluation of Three Commercial Broth Media for Pigment Detection and Identification of a Group B *Streptococcus* (*Streptococcus agalactiae*)[▽]

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Detection of group B *Streptococcus* (GBS) strains at various bacterial concentrations was evaluated using three pigment-producing broth media. At 10^3 CFU/ml, StrepB carrot broth (SBCB), Granada instant liquid biphasic (IGLB), and Northeast Laboratory GBS screening medium (NEL-GBS) showed 100% detection, but at the lower bacterial counts, SBCB and IGLB were more sensitive than NEL-GBS after 24 h.

Streptococcus agalactiae remains a significant cause of morbidity and mortality among newborns despite major efforts to detect colonization in pregnant women. The current Centers for Disease Control and Prevention guidelines for the prevention of perinatal GBS infections recommend that pregnant mothers be tested at 35 to 37 weeks of pregnancy for carriage of group B *Streptococcus* (GBS) by a selective enrichment method using anorectal/vaginal swabs in LIM (Todd-Hewitt broth supplemented with selective antibiotics) or TransVag broth, followed by subculture on blood agar plates (BAP) (2). Although microbiology-based screening techniques have been shown to be an effective means of preventing the onset of perinatal GBS disease, this process can be labor intensive and typically requires 2 days to provide results. In recent years, alternative methods have become available that have improved sensitivity, decreased test turnaround time, and increased test efficiency and that are easier to perform in the laboratory (1). Such methods include the use of modified media, such as Granada medium (GM) (5, 6), which utilizes the unique ability of beta-hemolytic strains of *S. agalactiae* to produce a red-orange pigment, thereby allowing detection and identification of GBS in a single step. Most reports describing this medium have included direct plating of swabs on the agar medium, and variable results for the sensitivity of this medium have been reported, with some investigators considering it to be unacceptably low (4, 7, 10, 11). Enrichment broth modified from the original Granada medium has been developed and investigated more recently (1, 3, 8, 9). These broth media have shown a good diagnostic performance compared to LIM broth with respect to sensitivity and specificity and have the added advantage of decreasing turnaround time for positive cultures by 24 h. The formulations of both these solid and liquid media that enhance pigment production have changed considerably over the past few years, and comparisons between these new formulations are few. This study evaluated each of three commercial broth media for the identification of GBS and their ability to produce pigment at various bacterial concentrations.

Reference strain cultures ($n = 10$) for each of the GBS serotypes and clinical isolates ($n = 36$) representing various types were selected from the CDC's Active Bacterial Core Surveillance collection of invasive GBS. *Enterococcus faecalis* ATCC 29212 was used as a negative control, and a single nonhemolytic GBS strain was included. Organisms were supplied in a blinded manner, and the laboratory tests were performed by a single laboratory technician. Each GBS culture was inoculated on a BAP and grown overnight. Culture suspensions were prepared in saline to achieve a 0.5 McFarland density, followed by serial dilutions to obtain 10^3, 10^2, and 10^1 CFU per ml. Tubes of LIM, StrepB carrot broth (SBCB), Granada instant liquid biphasic (IGLB), and Northeast Laboratory GBS screening medium (NEL-GBS) broth were each inoculated with 1.0 ml of each of the three concentrations, and tests were repeated three to four times over a 4-month period. LIM broths were examined for growth as judged by the increase in turbidity of the broth after 24 and 48 h. If the broth showed no visible growth after 24 h of incubation, the broth was subcultured to BAP and these plates were examined for growth after overnight incubation at 35°C. The SBCB, IGLB, and NEL-GBS broths were examined for pigment production at 24 and 48 h. If a peach, orange, reddish-orange, or red color was evident, positive pigment production was recorded. If no color change was detected after 24 h of incubation, the tubes were subcultured to BAP and incubated overnight. If growth of GBS on BAP was evident, the culture was recorded as positive for growth.

All three pigment-producing broths were positive for all hemolytic GBS isolates ($n = 46$) tested at the higher concentration of 10^3 CFU/ml. *E. faecalis* ATCC 29212 used as a negative control, as well as the single nonhemolytic GBS strain that was tested, did not produce pigment in any of the media but did grow on BAP upon subculture. Any pigment production in the broths, detected by the presence of a peach, orange, red-orange, or red color regardless of the intensity of color, was recorded as positive. This method assumes that growth of GBS is necessary for pigment production. The SBCB was evaluated on three separate days,

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TABLE 1. Color detection for beta-hemolytic GBS strains by the use of three pigment-detecting broth media[a]

Medium and test date[b]	No. (%) of strains with pigment at indicated concn (CFU/ml)				No. of isolates negative for pigment production and positive on BAP subculture at indicated concn (CFU/ml)/total no. of subcultured isolates	
	After incubation at 24 h		Only after incubation at 48 h			
	10^2	10^1	10^2	10^1	10^2	10^1
Carrot (SBCB)						
TD-1	45 (97.8)	45 (97.8)	1 (2.1)	1 (2.1)		
TD-2	46 (100)	46 (100)				
TD-3	46 (100)	45 (97.8)		0		0/1
Granada (IGLB)						
TD-1	46 (100)	46 (100)				
TD-2	46 (100)	45 (97.8)		0		0/1
TD-3	46 (100)	44 (95.7)		1 (2.1)		0/1[c]
TD-4	46 (100)	43 (93.5)		0		0/3
NEL-GBS						
TD-1	37 (80.4)	38 (82.6)	2 (4.3)	4 (8.6)	6/7[c]	4/4
TD-2	43 (93.4)	43 (93.4)	2 (4.3)	2 (4.3)	1/1	1/1
TD-3	37 (80.4)	37 (80.4)	3 (6.5)	4 (8.6)	6/6	3/5
TD-4	40 (86.9)	39 (84.7)	4 (8.6)	3 (6.5)	2/2	2/4

[a] Forty-six strains were used.
[b] TD-1, test date 1; TD-2, test date 2; TD-3, test date 3; TD-4, test date 4.
[c] Subculture to BAP for one isolate that was negative after 24 h in broth was not determined.

and pigment production was detected in 97.8% to 100% of beta-hemolytic GBS inoculated into SBCB at 10^2- and 10^1-CFU concentrations after a 24-h incubation (Table 1). IGLB medium was tested at four separate times, and pigment production was observed for all reference and clinical isolates at 10^2 CFU and for 93.5% to 100% of the 10^1-CFU concentrations after 24 h. NEL-GBS broth were tested on four separate testing dates, and pigment production was observed to be poor for both 10^2 CFU/ml and 10^1 CFU/ml (80.4% to 93.4%) in NEL-GBS broth, with around 30% of the reactions varying from very weak to no pigment production. At the lower bacterial counts (primarily 10^1 CFU/ml), all three broths had isolates that were not detected by pigment production and were also negative on subculture (Table 1). Pigment production that was either not detected or poorly observed after 24 and 48 h at the lower bacterial counts was associated primarily with serotypes V and Ib. All three media performed optimally within their commercially assigned shelf life periods, and there was no loss in sensitivity of the media over the 4-month test period.

Although laboratory methods for the detection of GBS have evolved over the past decade, there remains a clinical need for further speed and accuracy. A number of previous studies using pigmented broth formulations for the identification of GBS from clinical specimens have shown this method to be sensitive (1, 8, 9) and cost-effective, and it can allow the improvement of diagnostic efficiency by dramatically reducing the number of subcultures that need to be performed (3). Results from this study and others suggest that these media may be appropriate alternatives to those recommended by the current CDC guidelines, and further evaluations using clinical specimens to compare the current formulations of the three broth media are required. All three media evaluated in this report are reliable for GBS detection when bacterial loads are above 10^2 CFU/ml, but in simulations of low GBS count, SBCB and IGLB broths were the most sensitive, with weak or delayed pigment production common in the NEL-GBS broth. A positive reaction for pigment in these media is very specific for GBS and therefore obviates the need for subsequent subculture or further identification steps in most cases. The requirement for subculturing onto conventional BAP, all broths that do not produce a pigment, allows a necessary second level of detection for increased sensitivity. Also, in cases where patients report allergies to penicillin, broths will need to be subcultured to obtain GBS cultures to determine antimicrobial susceptibilities.

Use of trade names and commercial sources is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services.

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