

Comparison of Two Culture Media and Three Sampling Techniques for Sensitive and Rapid Screening of Vaginal Colonization by Group B Streptococcus in Pregnant Women

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The Centers for Disease Control and Prevention (CDC) recommend universal screening of all pregnant women between 35 and 37 weeks of gestation for group B streptococci (GBS) by use of a selective broth medium. Recent reports suggest that Granada medium can be used for rapid and direct visual identification of GBS colonies. However, studies comparing the Granada medium method to the selective broth method are few, and while some report comparable sensitivities, others have found significant differences in detection rates between the two methods. This prospective study compared a method using Granada agar to a Todd-Hewitt broth method with subculture to blood agar in order to determine which GBS detection method is more sensitive and less labor-intensive and has a more rapid turnaround time. Detection rates for three sampling techniques (rectovaginal, vaginal only, and cervical only) were also compared. Consecutive specimens for GBS screening received over a 6-month period from 1,635 pregnant women were included. Overall, GBS was detected in 390 (23.8%) women. The Granada medium gave positive results for 348 of these women, and the selective broth gave positive results for 385, indicating sensitivities of 89.2% for the Granada medium and 98.7% for the selective broth. These findings show that the Granada medium method is less sensitive than the selective broth method and should not replace it as the only method for screening pregnant women for GBS. However, the Granada medium method reduced detection time to 1 day and also reduced the use of ancillary tests in approximately 90% of positive cases. Additionally, no significant differences were noted in the detection rates with rectovaginal, vaginal, and cervical specimens.

Infection by group B streptococcus (GBS; *Streptococcus agalactiae*) continues to be a leading cause of neonatal morbidity and mortality in spite of a reduction in its incidence due to routine prenatal screening (2, 13). Colonization of the vagina by GBS can be transient, chronic, or intermittent and may affect 10 to 30% of pregnant women (2, 13). The Centers for Disease Control (CDC) recommend universal screening of pregnant women between 35 and 37 weeks of gestation for GBS, since a risk-based approach is unreliable and may miss up to 50% of infants born with GBS disease when used as the sole approach (2, 3, 13). Screening should be performed at 35 to 37 weeks of gestation (less than 5 weeks prior to the anticipated date of delivery) because although GBS colonization may be transient, women who test positive for GBS at this gestational age are likely to remain colonized at the time of delivery (2). Rectovaginal swabs have been reported to provide high bacterial yields, as the gastrointestinal tract is a natural reservoir for GBS and a potential source of vaginal colonization (1, 2, 6, 10, 11, 13). Either a health care provider or the woman herself may collect the specimen, as GBS yields are reported to be similar in either case (2). The swab should be transported in a nonnutritive medium, inoculated in selective broth medium for 18 to 24 h, and subcultured onto sheep blood agar for GBS screening. Colony characteristics (including a narrow zone of beta-hemolysis), Gram staining, catalase reac-

tions, and biochemical tests are used to identify GBS. This process typically takes between 48 and 72 h.

Granada medium (Hardy Diagnostics, Santa Maria, Calif.), a commercially available selective agar, allows for direct visual identification of GBS colonies since it produces an orange-red carotenoid pigment that is unique to GBS from human sources (Hardy Diagnostics catalog) (4, 8). This eliminates the need for ancillary tests to confirm the presence of GBS colonies. Since swabs can be inoculated directly onto this medium, its use reduces the detection time for GBS to 1 day. A review of world literature revealed only a few studies that have compared a selective broth medium to Granada medium in clinical settings. These studies reported contradictory findings; a few reported comparable sensitivities, while others found significant differences in detection rates between the two screening methods (4, 7–9, 12, 13). The sensitivities of Granada medium and selective broth for detection of GBS in pregnant women ranged from 40 to 97% and 82 to 98%, respectively, in these studies. Some authors have also reported that colistin-nalidixic acid (CNA) agar is a low-cost alternative for GBS screening, albeit with a lower sensitivity than that of selective broth (5).

Thus, while the CDC's recommended broth method has been reported to be sensitive, it has a longer turnaround time and requires the technologist to perform additional tests. The Granada medium allows rapid and direct visual detection of GBS but has a wide range of reported sensitivities. With these considerations in mind, we designed this study to compare a Todd-Hewitt broth method with subculture to blood agar to the Granada medium method in an effort to determine which of these methods is best for screening pregnant women for

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TABLE 1. GBS colonization rates determined by three sampling techniques using different culture media

Specimen type (<i>n</i>)	No. (%) of specimens positive		
	On broth	On Granada medium	Total
Rectovaginal (1,315)	303 (23.0)	278 (21.1)	306 (23.3)
Vaginal (168)	40 (23.8)	36 (21.4)	40 (23.8)
Cervical (152)	42 (27.6)	34 (22.4)	44 (28.9)
Total (1,635)	385 (23.5)	348 (21.3)	390 (23.8)

GBS colonization. This study also compared three sampling techniques, namely, rectovaginal, vaginal only, and cervical only. This study included over 1,600 GBS screening specimens from a diverse population group served by our tertiary-care hospital, which has a large community outreach program.

MATERIALS AND METHODS

Specimens from pregnant women that were submitted to St. John Hospital and Medical Center Laboratory, Detroit, Mich., for screening for GBS colonization were included in this prospective study. Specimens were received over 6 months, from July 2003 through December 2003. Each screening specimen consisted of multiple swabs that were collected in a sterile fashion and transported to our laboratory. These swabs were inoculated onto Granada medium and placed in Todd-Hewitt broth with gentamicin, nalidixic acid, and blood (Trans-Vag Broth w/Blood; Remel Inc., Lenexa, Kans.). The culture plates and the broth were incubated for 18 to 24 h at 36°C. Samples from the Todd-Hewitt broth were then subcultured onto blood agar plates. The Granada medium was incubated anaerobically and examined at 18 to 24 h for orange-red colonies of GBS and reexamined at 48 h if results were negative at 24 h. The blood agar was incubated in 5% CO₂, and colonies of GBS were identified based on colony morphology, Gram staining, catalase reaction, and latex agglutination tests (Streptex B; Murex Biotech Ltd., Darford, England). Statistical analysis (chi-square analysis) was performed using SPSS version 10.0 (SPSS Inc., Chicago, Ill.), with a *P* value of <0.05 considered significant.

RESULTS

Over the duration of the study, 390 (23.8%) of 1,635 consecutive specimens were found to be positive for GBS by either the Granada medium method or the selective broth method. GBS colonization was found in 306 (23.3%) of 1,315 rectovaginal specimens, 40 (23.8%) of 168 vaginal specimens, and 44 (28.9%) of 152 cervical specimens (Table 1). GBS detection rates for the rectovaginal samples were not significantly different from detection rates for either the vaginal samples (*P* = 0.90) or the cervical samples (*P* = 0.23). The broth method was more sensitive than the Granada medium method overall, as well as for each of the sampling techniques (Table 2).

GBS was detected in 385 of 390 positive specimens by the

TABLE 2. Sensitivity of GBS detection by the two culture methods

Sampling site (no. of samples)	No. (%) of samples detected			<i>P</i> value
	Broth method	Granada medium method		
Rectovaginal (306)	303 (99.0)	278 (90.8)		<0.001
Vaginal only (40)	40 (100.0)	36 (90.0)		0.04
Cervical only (44)	42 (95.4)	34 (77.3)		0.01
Total (390)	385 (98.7)	348 (89.2)		<0.001

Todd-Hewitt broth method, with a sensitivity of 98.7%. All subcultures demonstrated good growth by 48 h postincubation. The five false-negative specimens detected only by the Granada medium method had colony counts of less than 10. The Granada medium method detected GBS colonization in 348 of the 390 GBS-positive specimens, a sensitivity of 89.2%. Most GBS colonies on Granada medium developed a dark-orange to red color. All plates that were positive grew GBS within 18 to 24 h. Other organisms that grew on the Granada plates, including *Enterococcus* species, were either colorless or a light-yellow color. Colonies that were only light salmon or pink were found to be positive for GBS when further tested by Gram staining, determination of colony characteristics on blood agar (including a narrow zone of beta-hemolysis), catalase reactions, and biochemical tests. Orange-red colonies on Granada medium tested positive for GBS without exception. Five Granada plates (1.3%) had colorless GBS colonies that were detected only by ancillary tests, including Gram staining, determination of colony characteristics on blood agar (including a narrow zone of beta-hemolysis), catalase reactions, and biochemical tests, which were performed to resolve discrepancies between the two culture methods. These five specimens were not included in the 348 positive specimens on Granada medium in the final analysis.

DISCUSSION

In our study, GBS was detected with a sensitivity of 98.7% (385 specimens) by the selective Todd-Hewitt broth method, which is consistent with the results described in most published studies (4, 8, 12, 13). The Granada medium method (348 specimens; 89.2% sensitivity) was less sensitive than the selective broth method. The broth method failed to detect GBS in only 5 of the 390 positive specimens, and these 5 specimens yielded rare GBS colonies on the Granada medium. This result may be due to the fact that the Granada agar was inoculated before the swabs were placed into the broth. Thus, there may have been too few GBS colonies to grow in the broth and to be detected subsequently on blood agar. The sensitivity of Granada medium (89.2%) in this study is similar to that described in some published studies and to that reported by the manufacturer (Hardy Diagnostics catalog) (7, 8).

The vast majority (1,315, or 80.4%) of the specimens received by the laboratory were rectovaginal, which is the specimen recommended by the CDC. Only 168 (10.3%) were vaginal specimens, and only 152 (9.3%) were cervical specimens. Although rectovaginal swabs are reported to increase the yield of GBS, this finding was not supported by this study (1, 2, 6, 10, 11, 13). Detection rates of GBS by use of rectovaginal, vaginal, and cervical swabs were 23.3, 23.8, and 28.9%, respectively. The differences between these values are not statistically significant. Thus, it appears that specimens collected by swabbing the lower vagina and/or the rectum, a simple procedure that can be performed by a health care worker or by the woman herself with appropriate instructions, will yield adequate samples for screening. A speculum examination for visual inspection and sampling of the cervix may be unnecessary.

Although the broth method proved to be the more sensitive method in this study, it has potential drawbacks, including a longer turnaround time (approximately 48 h), the requirement

for additional subculturing to blood agar, a need for ancillary tests for identification of GBS colonies, and additional technologist time. The Granada medium method reduces the time required to detect GBS to 1 day, and the characteristic pigment produced by GBS colonies on this medium makes ancillary tests unnecessary. All of the 348 positive specimens on this medium showed growth of pigmented colonies within this time period. Although this medium was not entirely selective, colonies of other organisms that grew on this medium were either colorless or light yellow. Five (1.3%) GBS colonies on Granada plates were colorless and detected only when ancillary tests were performed to evaluate discrepancies between the results shown by the Granada medium and by the selective broth. This low percentage of colorless GBS colonies is consistent with previously reported results, and it may be found that those colorless GBS colonies are non-beta-hemolytic (13). These 5 specimens with colorless GBS colonies were analyzed as false negatives and were not included in the 348 specimens that were positive on Granada medium. Colonies of GBS will produce pigment when incubated anaerobically. However, published studies report the achievement of good growth of pigment-producing GBS colonies by placement of a cover slide over the inoculated areas of Granada medium and incubation in 5% CO₂ (12).

The Granada medium method was found in this study to be a sensitive method for detection of GBS in pregnant women. However, it cannot be recommended as the sole method for screening for GBS colonization in such women, as it missed up to 11% of the GBS-infected women in our study. In this era when cost containment and rapid result reporting are paramount, the broth method may not be the best alternative either. The CDC guidelines on GBS testing allow inoculation of an additional culture medium (CNA medium or sheep blood agar) in parallel with incubation of the screening sample in a selective broth. According to these guidelines, the broth may be discarded if GBS colonies are identified in the additional culture medium at 24 h. Based on the findings of our study, we feel that Granada medium is a much better alternative than CNA medium or sheep blood agar for simultaneous testing for GBS. Thus, swabs received for GBS screening should be inoculated directly onto Granada plates and then incubated in a selective broth for 18 to 24 h. After that, the Granada plates should be examined for orange-red colonies of GBS. If no GBS

colonies are identified, the broth can be subcultured onto a blood agar. This process will detect GBS by direct visual inspection of the Granada medium within 24 h in about 90% of positive cases, making the Granada medium a component of a sensitive and rapid detection method.

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