

## Letters to the Editor

### Detection of Group B Streptococcal Colonization in Pregnant Women Using Direct Latex Agglutination Testing of Selective Broth

Group B streptococcus (GBS) is the leading cause of neonatal infection in the United States. In May 1996, the Centers for Disease Control and Prevention (CDC) developed a strategy to reduce the morbidity and mortality of GBS-associated neonatal disease (2). They recommended culturing all pregnant women at 35 to 37 weeks gestation for GBS colonization using vaginal-rectal specimens. For maximum sensitivity of GBS detection, the CDC recommends the use of a selective broth containing antibiotics, such as Lim broth. Specimens are inoculated into selective broth, and after 18 to 24 h of incubation, the broth is subcultured onto a blood agar plate (BAP) and is incubated for 18 to 24 h. Colonies of suspected GBS are confirmed by either serological methods or by CAMP (3) testing. If colonies resembling GBS are absent after 18 to 24 h, reincubation for an additional 18 to 24 h and reexamination are recommended. In our laboratory, we found this traditional protocol to be costly and labor-intensive, which are important factors to consider since laboratories are forced to control costs and limit personnel. Investigators have previously attempted to modify the subculture protocol by using a selective solid medium or by DNA probe testing of Lim broth (1, 4). The purpose of the present study was to determine the efficacy of testing Lim broth after 18 to 24 h of incubation by using a direct latex agglutination procedure for detection of GBS antigen. Results were compared to those obtained by the traditional subculture method, and discrepancies were resolved by PCR testing and/or with neomycin discs placed on BAPs.

Vaginal-rectal specimens were obtained from 1,200 women receiving prenatal care at the Inova Fairfax Hospital. Samples were collected with a transport swab system containing modified Stuart's medium (Starplex Scientific, Etobicoke, Ontario, Canada). Specimens were delivered to the laboratory within 1 h of collection. One swab was used to inoculate 5 ml of Lim broth containing Todd-Hewitt broth with 10 µg of colistin per ml and 15 µg of nalidixic acid per ml (Becton-Dickinson Microbiology Systems). After 18 to 24 h of incubation, the broth was subcultured onto a BAP and incubated in 5% CO<sub>2</sub>. Both beta-hemolytic and nonhemolytic colonies morphologically resembling GBS were tested with the Phadebact Strep B kit, a particle coagglutination assay (Boule Diagnostics AB, Huddinge, Sweden). All nonhemolytic colonies with a positive coagglutination result were confirmed with the CAMP test. All negative subculture plates were reincubated for an additional 18 to 24 h and reexamined. In addition, latex testing for GBS was performed on all Lim broth cultures after 18 to 24 h of incubation according to the manufacturer's protocol. The testing kit included acid extraction and GBS latex reagents (Murex Diagnostics, Norcross, Ga.). The tubes of broth were vortexed briefly, and 40 to 50 µl (1 drop from a manufacturer's pipette) was dispensed into an extraction tube. After 1 min of incubation at room temperature, an alkaline reagent was added for

neutralization. One drop of the resulting solution was placed on a test card and mixed with 1 drop of GBS latex reagent. The card was placed on a rotator (Thermolyne Corp., Dubuque, Iowa) for 4 min at 110 rpm. Discrepant results were resolved in the following manner. The broth was subcultured onto a BAP and three neomycin discs (30 µg) were placed on top to suppress the growth of other gram-positive organisms. When GBS did not grow around the neomycin discs, broth tubes were submitted to a reference laboratory (Genetics & IVF Institute, Fairfax, Va.) for PCR testing to determine the presence of *cfb* genes (CAMP factor, group b gene) specific for GBS (6).

A total of 247 vaginal-rectal swab specimens were found to be GBS positive (20.6%) by both subculture and latex methods (Table 1). These results include 237 beta-hemolytic isolates and 10 nonhemolytic isolates of GBS. The direct latex method detected 244 GBS for a sensitivity of 98.8% and a specificity of 100%, while subculture detected 230 for a sensitivity of 93.1%. There were 17 specimens positive by the latex method but negative for GBS upon subculture. Since no visible colonies of GBS were seen, these results were considered false negatives. The broths were subcultured again onto a BAP, and three neomycin discs were placed on top. After overnight incubation, GBS colonies were detected in the area surrounding the neomycin discs in 10 of 17 specimens. The seven remaining GBS-negative specimens analyzed by PCR methodology were found to possess *cfb* genes. Therefore, the predictive values of negative and positive results using the latex and subculture methods were 99.5 and 100% (latex) and 97.3 and 100% (subculture), respectively.

We noticed that all seven of the GBS latex-positive samples with negative subcultures from Lim broth revealed heavy growth of *Enterococcus faecalis*, suggesting the occurrence of a possible antagonistic phenomenon. To support this observation, we combined suspensions of GBS (20 to 100 CFU/ml) and *E. faecalis* (> 2,000 CFU/ml) in Lim broth. Following 18 to 24 h of incubation and subculture, we observed that the number of GBS colonies was either significantly decreased or completely inhibited compared to the number from growth in a control broth containing only GBS. Isolates of *E. faecalis* (five strains) collected from different patients were analyzed as de-

TABLE 1. Recovery of GBS from Lim broth

Recovery results		No. of specimens
Subculture <sup>a</sup>	Latex	
+	+	227
-	-	953
+	-	3
-	+	17 <sup>b</sup>

<sup>a</sup> BAP (48 h).

<sup>b</sup> Inhibition of GBS by *E. faecalis*.

scribed above using five different strains of GBS, and testing demonstrated similar inhibitions. Heavy growth of GBS on a subcultured BAP usually lacked concomitant growth or had only very light growth of *E. faecalis*. Conversely, very light growth of GBS on a subcultured BAP revealed heavy growth of *E. faecalis*. This antagonism could not be demonstrated on strains of *Enterococcus faecium* or strains from viridans group streptococci. Inhibition activity could not be demonstrated on a BAP when both GBS and *E. faecalis* were growing side by side.

In our study, the three false-negative specimens by latex testing may be attributed to insufficient growth of GBS in Lim broth. The minimum cell density of GBS required to yield a positive latex test, directly from broth, is  $2 \times 10^8$  CFU/ml. If the minimum threshold for latex agglutination is not reached due to a competitive overgrowth, false-negative results may prevail. In all three cases of false-negative results, the BAP subcultured from Lim broth showed scant GBS growth in the presence of *E. faecalis* and other organisms. Bourbeau et al. (1) had a similar experience when five of nine GBS-positive specimens failed to multiply in Lim broth. A possible explanation was suggested by Dunne and Holland-Staley (4), who noticed that certain strains of GBS appear to be suppressed by a moderate to heavy growth of *E. faecalis*. One must be aware of the limitations of the traditional protocol of selective broth subculture, and alternative procedures should be examined.

Dunne and Holland-Staley (4) suggested that optimum recovery of GBS could be achieved by using both selective plating media and selective broth; however, we believe that their suggestion is too labor-intensive. In our hospital, where the average number of GBS cultures currently performed is 230 to 250 per month, elimination of subculture by using latex agglutination has shown the following advantages compared to the

traditional subculture method: a reduction in workload and cost, reduction in turnaround time for results by 24 h, and better sensitivity (98.8 versus 93.1%).

We thank Brian D. Mariani, Genetics & IVF Institute, Fairfax, Va., for the PCR analysis.

#### REFERENCES

1. Bourbeau, P. P., B. J. Heiter, and M. Figdore. 1997. Use of Gen-Probe AccuProbe Group B *Streptococcus* test to detect group B streptococci in broth cultures of vaginal-anorectal specimens from pregnant women: comparison with traditional culture method. *J. Clin. Microbiol.* **35**:145-147.
2. Centers for Disease Control and Prevention. 1996. Prevention of perinatal group B streptococcal disease: a public health perspective. *Morb. Mortal. Wkly. Rep.* **45**:1-24.
3. Christie, R., N. E. Atkins, and E. Munch-Peterson. 1944. A note on a lytic phenomenon shown by group B streptococci. *Aust. J. Exp. Biol.* **22**:197-200.
4. Dunne, W. M., Jr., and C. A. Holland-Staley. 1998. Comparison of NNA agar culture and selective broth culture for detection of group B streptococcal colonization in women. *J. Clin. Microbiol.* **36**:2298-2300.
5. Dunne, W. M., Jr. 1999. Comparison of selective broth medium plus neomycin-nalidixic acid agar and selective broth medium plus Columbia colistin-nalidixic agar for detection of group B streptococcal colonization in women. *J. Clin. Microbiol.* **37**:3705-3706.
6. Podbielski, A., O. Blankenstein, and R. Luitticken. 1994. Molecular characterization of the *cfb* gene encoding group B streptococcal CAMP-factor. *Med. Microbiol. Immunol.* **183**:239-256.

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