JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4602–4603 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.01168-10


# Evaluation of a New Chromogenic Medium (StrepB *Select*) for Detection of Group B *Streptococcus* from Vaginal-Rectal Specimens[▽]

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Received 9 June 2010/Returned for modification 2 August 2010/Accepted 7 October 2010

**We compared StrepB *Select* medium (*Select*) after enrichment with conventional culture for the detection of Group B *Streptococcus* (GBS). Postenrichment sensitivities of *Select* and conventional culture were 98.8% and 92.2%, respectively ($P < 0.05$). *Select* was superior for detection of GBS from vaginal-rectal specimens. Growth of non-GBS colonies required additional work to exclude the presence of GBS, especially after 48 h of incubation. Incubation of *Select* beyond 24 h did not significantly increase the yield of GBS.**

As group B *Streptococcus* (GBS) remains a significant cause of neonatal morbidity and mortality, antenatal screening for GBS at 35 to 37 weeks of gestation is recommended to determine whether antimicrobial prophylaxis is warranted (4, 7). The use of newer, chromogenic media may improve the yield of GBS, while reducing labor and turnaround time (5). We evaluated a new chromogenic medium, StrepB *Select* (*Select*; Bio-Rad Laboratories, Marnes-la-Coquette, France), a selective medium for the detection and presumptive identification of GBS in vaginal and vaginal-rectal specimens (9). We compared the recovery of GBS from StrepB *Select* with that from conventional culture on colistin-nalidixic acid agar with 5% sheep blood (CNA; Oxoid, Nepean, Ontario, Canada) with and without broth enrichment using *Streptococcus* selective broth (SSB; Bio-Media Ltd., Woodbridge, Ontario, Canada).

From September to November 2008, a total of 1,025 specimens from 992 patients were submitted for GBS screening. These swabs were directly inoculated onto CNA plates and then placed into enrichment broth (SSB). After 24 h of incubation, the direct CNA plate was examined for the presence of colonies suggestive of GBS. If direct culture on CNA did not yield GBS, the SSB was subcultured onto CNA and incubated for 24 h. All broths were subcultured onto StrepB *Select* and incubated for up to 48 h at 37°C in ambient air, per the manufacturer's recommendations. Colonies suggestive of GBS, with a turquoise blue color on *Select* and gray colonies with or without hemolysis on CNA, were worked up by separate, experienced technologists blinded to each other's work. Identification of GBS was performed using conventional tests, including those with catalase, Gram stain, and Lancefield grouping antisera by using the PathoDx latex agglutination kit (Remel, Inc., Lenexa, KS). PCR testing for the *cfb* gene encoding the Christie-Atkins-Munch-Petersen (CAMP) factor (3), directly from the SSB enrichment broth, was used as the gold standard. A true positive was defined as growth of GBS on either medium.

Of the 1,025 specimens tested, a total of 243 (23.7%) yielded GBS, and the same 243 specimens were also positive by PCR for the *cfb* gene. Direct culture onto CNA yielded GBS from 201 samples (82.7%) (Table 1). SSB enrichment with CNA subculture at 24 h detected an additional 23 isolates (224/243 samples; 92.2%), while SSB enrichment with *Select* subculture detected GBS in 240 of 243 samples, for a sensitivity of 98.8% ($P < 0.0001$). There were no specimens that yielded GBS on direct culture that failed to grow in SSB. At 24 h postenrichment with *Select*, there were 50 specimens (6.4%) which grew turquoise blue colonies that were not GBS, the majority of which were *Enterococcus* spp. ($n = 35$) and *Streptococcus bovis* ($n = 15$). One specimen grew turquoise blue colonies on *Select* at 24 h postenrichment and tested positive for group B with PathoDx. Retrospective examination of the CNA plate for this specimen showed colonies with a large zone of beta-hemolysis, not characteristic of GBS. PCR for the *cfb* gene was negative, and 16S rRNA gene sequencing followed by BLAST analysis (National Center for Biotechnology Information [NCBI])

TABLE 1. Comparison of results for direct CNA blood agar culture, SSB enrichment with CNA subculture, and SSB enrichment with StrepB *Select* subculture for detection of GBS[e]

| Condition | No. (%) of samples positive for GBS |
|---|---|
| Preenrichment (CNA direct; 24 h)[a] | 201 (82.7) |
| Postenrichment | |
| CNA (24 h)[b] | 224 (92.2) |
| StrepB *Select* | |
| 24 h[c] | 240 (98.8) |
| 48 h[d] | 241 (99.2) |

[a] Swabs inoculated directly onto colistin-nalidixic acid blood agar (CNA).
[b] Postenrichment with *Streptococcus* selective broth followed by inoculation onto colistin-nalidixic acid blood agar and incubated for 24 h.
[c] Postenrichment with *Streptococcus* selective broth followed by inoculation onto StrepB *Select* agar and incubated for 24 h.
[d] Postenrichment StrepB *Select* agar incubated for 48 h.
[e] The total number of GBS-positive specimens was 243.

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▽ Published ahead of print on 20 October 2010.

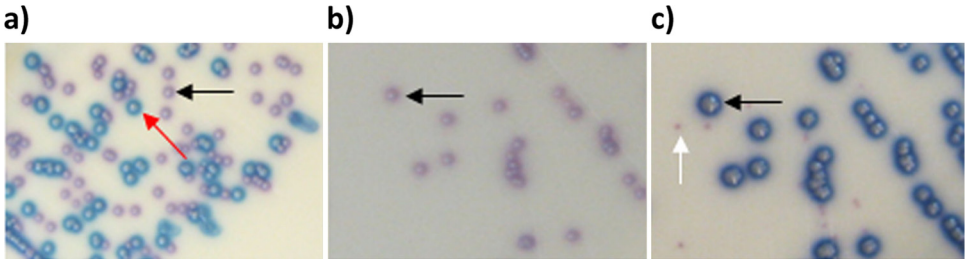


FIG. 1. Examples of the colony morphology of group B *Streptococcus* (GBS), *Enterococcus* spp., and *Lactobacillus* spp. after 24 and 48 h incubation at 37°C. (a) GBS and *Enterococcus* spp. at 24-h incubation (the red arrow indicates GBS [turquoise blue] and the black arrow indicates *Enterococcus* spp. [purple]). (b) *Enterococcus* spp. (purple colonies) at 24-h incubation (indicated by the black arrow). (c) *Enterococcus* spp. (same specimen as in panel b) with large blue colonies) at 48-h incubation (indicated by the black arrow) and *Lactobacillus* spp. (pinpoint purple colonies, indicated by the white arrow).

identified the isolate as *Streptococcus pseudoporcinus* (100% identity) (2). Incubation of *Select* plates for an additional 24 h yielded only one other specimen with GBS, for an overall sensitivity of 99.2%. There were 294 specimens (37.5%) which had blue colonies that were not confirmed as GBS (Fig. 1) at 48 h. *Select* recovered 17 more isolates following SSB enrichment than CNA. *Select* failed to detect GBS from two specimens that grew on CNA, as the colonies on *Select* agar were white in color. PCR performed from these white colonies confirmed the isolates as GBS.

Current guidelines for prenatal GBS screening recommend obtaining a vaginal-rectal swab and the use of selective broth enrichment, to maximize sensitivity (1, 7). Few studies have examined the use of chromogenic media for the detection of GBS from screening specimens (6, 8, 9). In this evaluation, although StrepB *Select* medium demonstrated excellent sensitivity for the detection of GBS, there are a few limitations that need to be mentioned. Because of the chromogenic substrates present, storage and incubation of the media must be in the dark, and minimal exposure to light is necessary for optimal performance. A number of non-GBS organisms, such as *Enterococcus* spp., group A *Streptococcus*, *Streptococcus bovis*, and *Streptococcus pseudoporcinus*, may grow as turquoise blue colonies. There are also rare strains of GBS that may produce no color or are very pale purple, not the typical turquoise blue, even after 48 h of incubation. In this study, only two (0.8%) out of all the GBS strains isolated failed to utilize the chromogenic substrates in the media. Overall, broth enrichment followed by *Select* subculture at 24 h was extremely sensitive (98.8%) compared with CNA subculture with a sensitivity of 92.2% ($P < 0.0001$). Based on our study results, *Select* appeared to be excellent for detection of GBS in low numbers (data not shown). Even though the use of *Select* facilitated the detection of GBS, the identification of suspicious colonies must be confirmed by conventional testing. A minimally increased yield of GBS was realized by prolonging the incubation of *Select* plates beyond 24 h following broth enrichment, but there was a significant increase in the work required to confirm the identity of non-GBS isolates that had a blue color. These were all *Enterococcus* spp. (bile esculin positive) that were purple at 24 h but turned blue at 48 h (Fig. 1c). One limitation of this study was that no comparison of postenrichment CNA subculture after 48 h incubation was made. However, in a study by Smith et al., no significant difference in sensitivity was noted between the 24 and 48 h results on CNA or sheep blood agar after enrichment (8). Studies with StrepB *Select* using direct inoculation would be of interest to determine whether the improved sensitivity and selective agents in the medium could decrease turnaround time (TAT) for the detection of GBS. Despite the introduction of rapid molecular methods, such as real-time PCR assays that are now commercially available, the cost and expertise required may limit use (1). Chromogenic media such as StrepB *Select* could be an excellent alternative.

We thank Bio-Rad Canada for providing the StrepB *Select* medium for this evaluation.

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