Evaluation of a New Chromogenic Medium (StrepB Select) for the Detection of Group B Streptococcus from Vaginal-Rectal Specimens

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Running title: StrepB Select and GBS
Abstract

We compared StrepB Select (Select) after enrichment with conventional culture for the detection of Group B Streptococcus (GBS). Post-enrichment 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 hours of incubation. Incubation of Select beyond 24 hours did not significantly increase the yield of GBS.
As group B *Streptococcus* (GBS) remains a significant cause of neonatal morbidity and mortality, antenatal screening at 35-37 weeks gestation for GBS is recommended to determine whether antimicrobial prophylaxis is warranted (4,7). The use of newer, chromogenic-based media may improve the yield of GBS, while reducing labor and turn-around-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 of 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 hours 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 hours. All broths were subcultured onto StrepB Select, incubated for up to 48 hours at 37°C in ambient air, as per the manufacturer’s recommendations. Colonies suggestive of GBS, turquoise-blue colour on Select, and grey colonies with or without haemolysis on CNA were worked up by separate, experienced technologists, blinded to each other’s work. Identification of GBS was performed using conventional tests, including catalase, Gram stain, and Lancefield grouping antisera 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 these same 243 specimens were also positive by PCR for the *cfb* gene. Direct culture onto CNA yielded GBS from 201 (82.7%) (Table 1). SSB enrichment with CNA subculture at 24 hours detected an additional 23 isolates (224/243 isolates, 92.2%) while SSB enrichment with *Select* subculture detected 240/243 GBS, 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 hours post-enrichment 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 hours post-enrichment 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-haemolysis, 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) identified the isolate as *Streptococcus pseudoporcinus* (100% identity) (2). Incubation of *Select* plates for an additional 24 hours 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 (Figure 1) at 48 hours. *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 mention. 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 may grow as turquoise-blue colonies, such as *Enterococcus* spp., Group A *Streptococcus*, *Streptococcus bovis*, and *Streptococcus pseudoporphirinus*. There are also rare strains of GBS that may produce no color or are very pale purple in color, not the typical turquoise-blue, even after 48 hours of incubation. In this study, only two (0.8%) of all GBS strains isolated failed to utilize the chromogenic substrates in the media. Overall, broth enrichment followed by *Select* subculture at 24 hours 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. Minimal increased yield of GBS was realized by prolonging the incubation of *Select* plates beyond 24 hours 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 hours but turned blue at 48 hours (Figure 1(c)). One limitation of this study was that no comparison of post-enrichment CNA subculture after 48 hours incubation was made. However, in a study by Smith et al, no significant difference in sensitivity was noted between the 24 and 48 hour results on CNA or sheep blood agar after enrichment (6). 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 turn-around time (TAT) for the detection of GBS. Despite the introduction of rapid molecular based 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.

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References


Table 1. Comparison of results for direct colistin-nalidixic acid (CNA) blood agar culture, *Streptococcus* Selective Broth (SSB) enrichment with CNA subculture, and SSB enrichment with StrepB *Select* subculture for the detection of group B *Streptococcus* (GBS).

<table>
<thead>
<tr>
<th>Pre-enrichment</th>
<th>Post-enrichment</th>
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<tr>
<td>CNA Direct (24 h)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CNA (24 h)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>No. GBS (%)</td>
<td>No. GBS (%)</td>
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<tr>
<td>201 (82.7%)</td>
<td>224 (92.2%)</td>
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<tr>
<td>StrepB Select (24 h)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>StrepB Select (48 h)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. GBS (%)</td>
<td>No. GBS (%)</td>
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<tr>
<td>240 (98.8%)</td>
<td>241 (99.2%)</td>
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</table>

<sup>a</sup>, swabs inoculated directly onto colistin-nalidixic acid blood agar (CNA)

<sup>b</sup>, post-enrichment with *Streptococcus* Selective Broth followed by inoculation onto colistin-nalidixic acid blood agar and incubated for 24 hours

<sup>c</sup>, post-enrichment with *Streptococcus* Selective Broth followed by inoculation onto StrepB *Select* agar and incubated for 24 hours

<sup>d</sup>, post-enrichment StrepB *Select* agar incubated for 48 hours
Figure 1. Examples of the colonial morphology of group B *Streptococcus* (GBS), *Enterococcus* spp., and *Lactobacillus* spp. after 24 and 48 hours incubation at 37°C. a) GBS and *Enterococcus* spp. at 24 hours incubation (red arrow indicates GBS (turquoise-blue) and black arrow indicates *Enterococcus* spp. (purple)); b) *Enterococcus* spp. (purple colonies) at 24 hours incubation (indicated by black arrow); c) *Enterococcus* spp. (same specimen as in panel b) with large blue colonies) at 48 hours incubation (indicated by black arrow); *Lactobacillus* spp. (pinpoint purple colonies, indicated by white arrow).