

## Evaluation of a Chromogenic Agar for Detection of Group B Streptococcus in Pregnant Women<sup>∇</sup>

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**We compared ChromID Strepto B agar (STRB; bioMérieux, Inc.), a selective and differential medium for group B streptococcus, with culture using neomycin-nalidixic acid agar (NNA) and LIM broth. STRB alone was more sensitive (87.7%) than NNA alone (79.0%), while each had a sensitivity of 100% when used in conjunction with LIM broth.**

Meningitis, pneumonia, and septicemia due to group B *Streptococcus* (GBS) infection are leading causes of infant mortality and morbidity (7). In 1996 the American College of Obstetricians and Gynecologists (ACOG) and the CDC issued recommendations for intrapartum antibiotic prophylaxis according to either a risk-based or a screening-based approach to prevent GBS transmission to neonates. The risk-based guidelines called for intrapartum prophylaxis in the event of delivery at <37 weeks of gestation, a temperature of >100.4°F, or the rupture of membranes for ≥18 h, while the culture-based approach called for the screening of all pregnant women between 35 and 37 weeks of gestation for vaginal or rectal colonization with GBS and subsequent intrapartum prophylaxis of those who were culture positive. In 2002 these guidelines were revised to recommend that culture-based prenatal GBS screening at 35 to 37 weeks of gestation be universally adopted (8). This time point was chosen as an appropriate predictor of intrapartum colonization (11). U.S. national estimates of early-onset (<7 days of life) neonatal invasive GBS disease using data collected by the CDC Active Bacterial Core Surveillance system (ABCs) indicate a decrease in incidence since the institution of GBS screening and prophylaxis guidelines, from 0.7 case/1,000 live births in 1997 to 0.34 case/1,000 live births in 2007 (3).

The CDC and ACOG recommend that vaginal/rectal swabs be inoculated into a selective broth medium to aid in the recovery of GBS. The broth is then to be subcultured on sheep blood agar plates after 18 to 24 h of incubation (1, 8). The addition of a solid agar medium inoculated upon receipt of the swab in the laboratory can allow for the identification of GBS after 24 h of incubation, instead of the 48 h required when swabs are inoculated only into selective broth. Neomycin-nalidixic acid agar (NNA) medium is a GBS-selective solid agar that can be used for primary plating, since it is significantly more sensitive than colistin-nalidixic acid agar (CNA) for this

purpose (4). However, NNA should never be used as a substitute for a selective broth medium, because the combination of a selective broth medium and NNA improves the detection of GBS by 15% over that with either medium used alone (5).

ChromID Strepto B agar (STRB; bioMérieux, Inc.) is a newly developed selective and differential medium for the presumptive identification of GBS. STRB contains a chromogenic substrate that, in the presence of actively growing GBS, generates pink to red colonies that are easily identified during routine inspection. The growth of bacteria of other species either is inhibited or appears in other colors (violet, blue, or colorless). We evaluated the performance of STRB in our laboratory compared to that of NNA, with or without the addition of a selective broth medium, as a suitable screening method for the detection of GBS colonization among pregnant women.

Our established protocol calls for the inoculation of vaginal/rectal swabs onto NNA, which is incubated at 35°C under 5% CO<sub>2</sub>, and into LIM broth, which is incubated at 35°C in room air. The NNA plate is examined at 24 h for the presence of characteristic beta-hemolytic colonies, which are further evaluated by Gram staining and by catalase, PYR (pyrrolidonyl-β-naphthylamide), and rapid latex agglutination tests to identify GBS. If beta-hemolytic colonies are not present after 24 h of incubation, then (i) the NNA plate is held for an additional 24 h and is examined the following day, and (ii) the LIM broth is subcultured onto 5% sheep blood agar, which is incubated for 48 h and is examined daily for the presence of beta-hemolytic colonies. To evaluate the performance of the STRB agar, primary swabs were processed by our routine protocol and were also inoculated onto the STRB plate, which was incubated at 35°C for 48 h in the dark. A single swab was collected from each patient, and the order of inoculation onto STRB or NNA plates was randomized. Plates were examined daily for the presence of pink colonies, which were then subjected to latex agglutination testing to confirm the identification of GBS. Gram staining and catalase and latex agglutination testing can be performed directly from STRB agar, while PYR testing cannot. However, according to the manufacturer, the appearance of pink to red colonies that type as group B by latex agglutination is sufficient for the identification of GBS.

A total of 250 prenatal screening swabs were included in the

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TABLE 1. Performance characteristics of STRB, NNA, and LIM broth

Medium	No. of GBS-positive specimens	Sensitivity (%)	Specificity (%)
STRB	71	87.7	97.6
NNA	64	79.0	97.6
LIM	74	91.4	100
LIM or STRB	81	100	100
LIM or NNA	81	100	100

study. Eighty-one of these samples (32.4%) were positive for GBS by our reference method (i.e., they were recovered from either NNA or LIM broth). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of either combination (NNA plus LIM broth subcultured onto 5% sheep blood agar or STRB plus LIM broth subcultured onto 5% sheep blood agar) were all 100%. A direct comparison of NNA to STRB without broth amplification as a screening medium revealed sensitivities of 79.0% (95% confidence interval [95% CI], 68.3% to 86.9%) for NNA and 87.7% (95% CI, 78.0% to 93.6%) for STRB (Table 1). For both NNA and STRB, the specificity was 97.6% (95% CI, 93.8% to 99.3%), with four isolates being incorrectly identified as group B *Streptococcus*. The four isolates from NNA were identified as *Streptococcus porcinus*, while three of the four *S. porcinus* isolates from NNA were also incorrectly identified on STRB, along with a fourth isolate that was identified as *Streptococcus thoraltensis*. There is some potential for misidentification of other streptococcal species as GBS by using STRB. *S. porcinus* isolated from NNA would likely be evaluated further if a wide zone of beta-hemolysis, which is not characteristic of GBS, were recognized. This would not be the case, however, if isolates were identified from STRB alone, since there would be no potential to observe the hemolysis pattern. Conversely, GBS can exhibit a nonhemolytic phenotype that may be overlooked on NNA, but these isolates would be identified using chromogenic agar (10).

Several recent publications have assessed the use of molecular methods for GBS screening. Real-time PCR for GBS has not proven to be sufficiently sensitive to replace culture-based screening (2, 9). An evaluation of a nonamplified nucleic acid hybridization assay indicated that in order to achieve a sensitivity similar to that of culture, the sample must be incubated in LIM broth for 24 h before molecular testing (9), thereby negating the potential advantage of a rapid testing methodology. El Helali et al. report good performance (sensitivity, 98.5%; specificity, 99.6%) for a rapid real-time PCR test for GBS in comparison to culture on Columbia ANC sheep blood agar and broth medium (6). However, the authors' suggestion that current guidelines for antenatal testing may not be relevant due to the low PPV of antenatal screening (58.3%) was based on a comparison of PCR, not to culture of intrapartum samples in broth medium and Columbia ANC agar, but to

culture of antenatal samples on Columbia ANC agar alone, not to culture of intrapartum samples in broth medium and Columbia ANC agar (6). The use of Columbia ANC agar alone without the addition of a selective broth medium to screen the antenatal samples would lead to decreased sensitivity and would likely cause a falsely low PPV.

In conclusion, we report that STRB used in combination with LIM broth performs as well as NNA with LIM broth and that chromogenic agar has a higher sensitivity than NNA. The 95% confidence intervals of the sensitivities of STRB or NNA culture alone overlap, indicating that there is not a significant difference between these two culture methods. However, chromogenic agar has advantages: the ease of detecting a color change on this medium, as opposed to looking for weakly beta-hemolytic colonies on NNA, and the need to perform only one additional test from STRB medium. The rare chance of a false-positive result is balanced by the increased sensitivity of STRB and the potential for detecting nonhemolytic colonies of GBS. real-time PCR (RT-PCR) may provide an alternative to culture in situations where a result is needed before culture can be completed (i.e., birth at <37 weeks of gestation, or lack of appropriate prenatal care and screening), though this would likely not be a cost-effective alternative to routine antenatal testing.

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