

Comparative Evaluation of the AccuProbe Group B Streptococcus Culture Test, the BD GeneOhm Strep B Assay, and Culture for Detection of Group B Streptococci in Pregnant Women[∇]

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We compared a rigorous culture method with the Gen-Probe AccuProbe Group B Streptococcus Culture Test (APGB) and the BD GeneOhm StrepB assay (GOSB) for the detection of group B streptococci (GBS) from an 18- to 24-h LIM broth. Culture (95.3%) and GOSB (95.3%) were more sensitive than APGB (86.5%) for the detection of GBS.

Prenatal screening for group B streptococci (GBS) is routinely performed on women at 35 to 37 weeks of gestation (10, 11, 12). We and others have previously presented data supporting the use of the AccuProbe Group B Streptococcus Culture Test (APGB; Gen-Probe, San Diego, CA) for the detection of GBS from LIM broths (3, 13).

The advent of PCR-based technology for the detection of GBS offers the potential for increased sensitivity and a shorter turnaround time than culture (2, 4, 11). APGB is a nonamplified nucleic acid hybridization assay, while the BD GeneOhm StrepB assay (GOSB; BD Diagnostics, Ste-Foy, Quebec, Canada) is a real-time PCR nucleic acid amplification assay. Moreover, since the time when we performed our initial evaluation of APGB, improved culture media for the detection of GBS have been developed (5, 6). The purpose of this study was to compare the performance of APGB with a commercially available PCR assay, GOSB, and a rigorous culture method.

Vaginal and vaginal/rectal specimens submitted to the Geisinger Medical Center microbiology laboratory for prenatal testing for GBS colonization were included in this study. All of the specimens were collected with Copan Venturi Transystem double swabs and liquid Stuart's medium (Copan Diagnostics, Marietta, CA).

Swabs were first used to inoculate a neomycin-nalidixic acid blood agar plate (NNA; Becton-Dickinson Microbiology, Cockeysville, MD) and then placed into a 3-ml tube of LIM broth (Remel, Lenexa, KS). Following 18 to 24 h of incubation, LIM broths were vortexed and subcultured to an NNA plate. Primary plates and LIM broth subculture plates were incubated at 35°C in a 5 to 10% CO₂ incubator and examined after overnight incubation and a second time 24 h later if the first reading of the plates yielded no GBS. Organisms were identified by the routine bacteriology procedures used by the Geisinger Medical Center microbiology laboratory, including

Lancefield identification of GBS with the PathoDX kit (Remel).

After 18 to 24 h of incubation, a 50- μ l aliquot of each LIM broth was tested with APGB. The directions in the product insert were followed, with one exception. The product insert specifies that when an enrichment broth such as LIM broth is used, the swab should be dipped into the LIM broth and then discarded. We followed the standard procedure that is used in our laboratory for APGB and did not remove the swab from the LIM broth after inoculation.

The product insert for GOSB provides instructions for direct testing of swab specimens for GBS. We utilized a protocol that tested a sample from a LIM broth rather than a swab (9). Briefly, 0.2 ml (200 μ l) of the LIM broth growth was transferred to sample buffer tubes (blue cap; contains 1 ml of sample buffer) with extended, filtered pipettes. The sample buffer tube stood for 5 min (in a cold block) and was then vortexed at high speed for 15 s. Fifty microliters of each cell suspension was transferred to a corresponding lysis tube (yellow cap), and the cap was closed tightly. The standard GOSB protocol was followed to complete the performance of the assay.

For this study, a true positive result was defined as one of the following: GBS from the primary plate culture, GBS from the LIM broth subculture, or GBS from both the primary culture plate and the LIM broth subculture; GBS culture negative but GOSB and APGB positive; GOSB positive, primary culture and APGB negative, but culture positive for GBS following additional culture testing; or APGB positive, primary culture and GOSB negative, but culture positive for GBS following additional culture testing. For any specimen that did not yield the same result with APGB, GOSB, and culture, the result was classified as discrepant. For any specimen with discrepant results, the following protocol was followed: APGB was repeated from the LIM broth; the GOSB was repeated from the reserved lysis tube, as well as from the LIM broth; the LIM broth was subcultured to NNA, and plates were examined for GBS as with the primary culture; and primary plates and initial LIM broth subcultures were reexamined for GBS. Importantly, none of the results were changed based upon the use of the discrepant protocol. The discrepant analysis protocol was used only to correctly classify the initial result. For example, two

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TABLE 1. Performance characteristics of culture, APGB, and GOSB

Method	No. of true positive results (sensitivity [%]) (<i>n</i> = 148)	True negative results (specificity [%]) (<i>n</i> = 350)
Primary culture	128 (86.5)	350 (100)
LIM broth subculture	136 (91.9)	350 (100)
Primary culture or LIM broth subculture	141 (95.3)	350 (100)
APGB	128 (86.5)	350 (100)
GOSB	141 (95.3)	347 (99.1)

isolates were initially GOSB positive and culture and APGB negative. Repeat LIM broth subcultures yielded GBS, so these two specimens were classified as GOSB true positive and culture false negative. Statistical analysis was performed as described by Ilstrup by using McNemar's asymptomatic chi-square test (7).

A total of 498 specimens were tested in this study. One hundred twenty-three specimens yielded positive results by all of the methods, APGB, GOSB, primary culture, and/or LIM broth subculture. Three hundred forty-seven specimens were negative by all of the methods. Of the 28 specimens that yielded discrepant results, 25 were determined to be true positives by the above-described criteria. Among these 25 specimens, 3 were culture negative but positive by APGB and GOSB and thus were classified as true positive results. Three specimens were GOSB positive only and were considered false positives. The overall performance characteristics are summarized in Table 1. The comparative performance characteristics of the three methods are summarized in Table 2. Culture was more sensitive than APGB ($P = 0.025$). GOSB was more sensitive than APGB ($P = 0.002$). Culture and GOSB were equivalent in sensitivity.

Residual GOSB lysates from 10 specimens were sent blinded to the laboratory of Jeanne Jordan (Magee-Women's Research Institute, University of Pittsburgh, Pittsburgh, PA) for confirmatory PCR testing with alternative GBS primers. These included four from specimens that were negative by all of the tests in our laboratory, three from specimens that were APGB and GOSB positive and culture negative in our laboratory, and three from specimens that were only GOSB positive in our laboratory. Jordan's laboratory confirmed that the first four were negative for GBS, the second three were positive for GBS, and one of the final three was positive for GBS. However, for this evaluation and using the definitions of positive and negative results established for this study, we reported this as a false-positive result.

In an earlier published evaluation from our laboratory, APGB performed from a LIM broth incubated for 18 to 24 h demonstrated sensitivity equivalent to that of the culture method then utilized in our laboratory (3). In our present study, the culture method was significantly more sensitive than APGB. However, the reference culture methods were different in the two evaluations. The earlier evaluation used LIM broth and two plated media—Columbia agar with 5% sheep blood supplemented with nalidixic acid and colistin (CNA) and a standard blood agar plate. The present evaluation utilized LIM broth and NNA plated medium. NNA has been shown to be

TABLE 2. Paired performance characteristics of three methods for detection of GBS

Comparison and parameter	No. of test results				Total
	Positive GOSB	Positive culture	Negative GOSB	Negative culture	
GOSB vs culture ^a					
Positive culture	136		5		141
Negative culture	8 ^b		349		357
Total	144		354		498
GOSB vs APGB ^c					
Positive APGB	126		2		128
Negative APGB	18 ^d		352		370
Total	144		354		498
Culture vs APGB ^e					
Positive APGB		123		5	128
Negative APGB		18		352	370
Total		141		357	498

^a Total number of true positives = 146; total number of true negatives = 352. GOSB sensitivity/specificity, 96.6%/99.1%. Culture sensitivity/specificity, 96.6%/100%.

^b Includes three false-positive GOSB results.

^c Total number of true positives = 143; total number of true negatives = 355. GOSB sensitivity/specificity, 98.6%/99.1%. APGB sensitivity/specificity, 89.5%/100%.

^d Includes three false-positive GOSB results.

^e Total number of true positives = 146; total number of true negatives = 352. Culture sensitivity/specificity, 96.6%/100%. APGB sensitivity/specificity, 87.7%/100%.

superior to CNA for the detection of GBS from prenatal screens, and our results indirectly support those results (5, 6). Our data clearly suggest that NNA is a superior agar for the growth of GBS and should be used as the predicate device in studies of molecular assays for the detection of GBS.

The advent of GOSB and other similar molecular tests offers the opportunity for a rapid result that may help in the management of a woman who goes into labor with no prenatal testing for GBS. However, the use of a direct PCR test is predicated on adequate test sensitivity, as well as successful integration into the work flow of the laboratory where it is performed.

Performance of GOSB from a LIM broth enrichment culture offers two potential advantages over direct use of GOSB with patient specimens. First, an organism is available from the LIM broth on test-positive patients for susceptibility testing in patients allergic to penicillin. Second, although there are limited data to support this point, we believe that sensitivity for GBS is greater when the GOSB is performed from LIM broth, as opposed to direct testing from a swab, because the GBS present in very low numbers have an opportunity to multiply prior to testing (1, 8). Atkins et al. reported that the use of a direct GOSB provided insufficient sensitivity (86.8%) to serve as a standard screen for GBS (1). With the same reference culture method that Atkins et al. used, our sensitivity for GOSB performed from a LIM broth was 95.9% and would have been higher had we characterized one false-positive GOSB result as a true positive result. Maloney et al. also

reported that the sensitivity of GOSB was greater for specimens preincubated in LIM broth than for the same specimens with direct testing (8).

Clearly, performance of GOSB from an enrichment broth delays a test result if a woman has no prenatal testing for GBS and the specimen is collected at the onset of labor. An unanswered question is whether a rigorous culture, such as we have utilized for this study or a GOSB performed from a LIM broth enrichment at 35 to 37 weeks of gestation, is equivalent in sensitivity to a direct PCR performed at delivery. If this were the case, the results for a PCR test performed at 35 to 37 weeks of gestation would be available at delivery for women who did not have premature labor prior to completion of the testing. Laboratories would also have the advantage of batch testing such as we currently utilize. Direct PCR testing could be reserved for those women for whom prenatal testing was not performed.

In conclusion, we report the successful use of GOSB performed from a LIM broth enrichment. The sensitivity of GOSB is equivalent to that of a rigorous culture method and provides us with labor savings compared to a rigorous culture method.

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