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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

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Detection of vaginal-anorectal colonization with group B streptococci (GBS) is critical to the prevention of neonatal GBS disease. The recommended method for the detection of GBS is culture of the distal vagina and anorectum in a selective broth medium followed by subculture to solid media and identification of GBS on the solid media. The purpose of this study was to compare this standard culture method with the detection of GBS directly from an enrichment broth by utilizing the Gen-Probe AccuProbe Group B Streptococcus Culture Identification Test (GPGB). A total of 502 specimens were tested in this study. Both culture and the GPGB detected 90 of 95 positive specimens (sensitivity, 94.7%). There were two false-positive GPGB results (specificity, 99.5%). An analysis of 100 consecutive specimens was performed to compare the costs associated with the use of a primary tryptic soy agar plate with 5% sheep blood (BAP) and a 3-ml tube of Todd-Hewitt broth supplemented with 10 μg of nalidixic acid per ml and 15 μg of colistin per ml (LIM broth) with subculture to another BAP and the costs associated with the GPGB. Our estimated costs were $3.68 for a negative culture including 7.0 min of labor, $5.41 for a positive culture including 8.9 min of labor, and $5.16 for the GPGB including 3.6 min of labor (based upon a test run of 10 specimens and two controls and a cost of $70.00 for a 20-test GPGB kit). Accessioning and reporting of results are not included in these costs. In conclusion, we found that the GPGB was equivalent in sensitivity to our standard culture method. While overall costs were somewhat higher for the GPGB, the GPGB has lower labor costs and offers the potential for incremental savings with higher test volumes and computer interface capability.

Group B streptococci (GBS) are the major infectious cause of illness and death in the newborn population in the United States. Approximately 80% of the GBS disease in newborns occurs during the first week of life and is referred to as early-onset disease to differentiate it from late-onset disease, which occurs after the first week of life (7). Early-onset GBS disease is caused by the transmission of GBS from the mother, who carries GBS in her genital tract or anorectum, to the newborn either during delivery or in utero just prior to delivery (1).

Intrapartum penicillin administration to mothers during labor and delivery is an effective way of lowering the risk of early-onset GBS infection. There are, however, contraindicatory factors to the administration of antibiotics to all women during childbirth; therefore, a variety of strategies have been developed or proposed to identify those mothers at highest risk for giving birth to a child with GBS disease (1).

Although there are several risk factors associated with early-onset GBS disease, genital tract or anorectal carriage of GBS at the time of delivery is the single most important risk factor. The rapid enzyme-linked immunosorbent assays available for assessing maternal colonization have insufficient sensitivity, compared with a broth-enriched culture method, to serve as an adequate replacement for culture (5). Culture, on the other hand, when performed at the time of delivery, does not generally provide a sufficiently rapid turnaround time to influence the decision of whether or not to initiate antimicrobial therapy. A recently published consensus statement on the prevention of GBS perinatal disease recommended that one of two strategies be adopted by institutions. One of these strategies includes the culturing of all pregnant women at 35 to 37 weeks of gestation (1).

The recommended culture method for detection of maternal colonization with GBS employs primary incubation in a selective broth for 18 to 24 h, subculture to plated media, and identification of suspected GBS from the subculture plated media. The need to identify both beta-hemolytic and nonhemolytic colony types of GBS, combined with the use of an enrichment broth, makes this a relatively labor-intensive procedure, particularly compared with culture for group A streptococci.

The purpose of this study was to compare the performance characteristics of a commercially produced GBS genetic probe, the AccuProbe Group B Streptococcus Culture Identification Test (GPGB; Gen-Probe, San Diego, Calif.), performed with an 18- to 24-h enrichment broth, with a rigorous culture method for GBS colonization in pregnant women.

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MATERIALS AND METHODS

Vaginal or vaginal-rectal specimens were obtained from women receiving prenatal care at Geisinger primary care sites. All specimens were collected with either Bacti-Swab II brand swabs (Remel, Lenexa, Kans.) or Culturette II brand swabs (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and transported to the laboratory at ambient temperature. Both of these products contain two swabs.

One swab was used for the routine culture, which was performed as follows. Upon receipt in the laboratory, the swab was used to inoculate one tryptic soy

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TABLE 1. Results of testing of specimens with discrepant results

Sample no.	Original result		Result of repeat subculture on:				Pledget result		Result interpretation
			Study broth		Routine broth				
	Routine culture	GPGB	Culture	GPGB	Culture	GPGB	Culture	GPGB	
10056	Broth only, GBS^+	0	0	0	+	+	0	0	False-negative GPGB
11164	Broth only, GBS^+	0	0	0	+	+	0	0	False-negative GPGB
1395	1 GBS colony	0	0	0	+	0	0	0	False-negative GPGB
1401	1 GBS colony	0	0	0	+	+	+	+	False-negative GPGB
11415	4 GBS colonies	0	+	0	+	0	+	0	False-negative GPGB
12013	0	+	+	+	0	0	+	+	False-negative culture
14565	0	+	+	0	+	0	ND[a]	ND	False-negative culture
14702	0	+	+	+	+	+	ND	ND	False-negative culture
29735	0	+	+	+	+	0	ND	ND	False-negative culture
31695	0	+	+	+	+	+	ND	ND	False-negative culture
22832	0	+	0	+	0	0	0	0	False-positive GPGB
14486	0	+	0	+	0	0	0	+	False-positive GPGB

[a] ND, not done.

agar plate with 5% sheep blood (BAP) and one Columbia agar plate with 5% sheep blood supplemented with nalidixic acid and colistin (CNA). The same swab was immersed for at least 1 min in a 3-ml tube of Todd-Hewitt broth supplemented with 10 μg of nalidixic acid per ml and 15 μg of colistin per ml (LIM broth [subsequently referred to as the routine LIM broth]). The tube was vortexed, and the swab was expressed on the inside of the tube and discarded. Plated and tubed media (all obtained from Remel) were incubated at 35 to 37°C in 5 to 7% enriched CO_2.

After overnight incubation, the primary plates were examined for beta-hemolytic and non-hemolytic colonies resembling GBS. Suspect colonies were Gram stained, and those with gram-positive cocci resembling streptococci were either directly tested with Strep B grouping latex reagent from a PathoDx Strep Grouping kit (Diagnostic Products Corporation, Los Angeles, Calif.) or subcultured for testing on the following day. All cultures negative for GBS on day 1 were reincubated for an additional 24 h and reexamined.

For those cultures with primary plates negative for GBS on day 1 of examination, subculture of the routine LIM broths was performed. Tubes were vortexed, and each was subcultured to one BAP and one CNA. Plates were incubated and examined for GBS in the same manner as the primary plates. Subcultures negative for GBS on first examination were reincubated and examined on the following day.

The amount of growth of GBS on primary cultures was recorded as follows: fewer than 30 colonies, exact number of colonies; 1+, growth in quadrants 1 and 2 (≥30 colonies); 2+, growth in quadrants 1 to 3; 3+, growth in all quadrants. Cultures with growth only from the LIM broth subculture were classified as positive from broth only.

The second swab from each specimen was saved at refrigerator temperature for batch testing with the GPGB. All study swabs were stored for less than 72 h in the laboratory before testing. Each swab was immersed for at least 1 min in LIM broth (subsequently referred to as the study LIM broth). The tube was vortexed, and the swab was expressed on the inside of the tube and discarded. After incubation at 35 to 37°C in 5 to 7% enriched CO_2 for 18 to 24 h, study LIM broth tubes were removed from the incubator, vortexed, and subcultured to BAP and CNA, which were then incubated and worked up in the same way as the subculture of the routine LIM broth from the other swab.

The GPGB was performed with the study LIM broth at essentially the same time as the subculture was done. The GPGB was performed as outlined in the product insert. The tubes were read in a Gen-Probe Leader 450 Luminometer. As indicated in the package insert, the following relative light unit (RLU) values were used to interpret test results: <40,000 RLUs, negative; 40,000 to 49,999 RLUs, indeterminant, repeat; ≥50,000 RLUs, positive. Positive and negative controls were included in each test run.

Some of the pledgets (rayon plugs) in the swab holders were cultured in broth to resolve discrepant results. This was done by carefully cutting the swab holder with a pair of scissors just above the pledget. The pledget was removed with sterile forceps and placed in a 3-ml tube of LIM broth. Following overnight incubation at 35°C, the broth was subcultured to BAP and CNA and examined in the same manner as the routine culture.

To determine how prolonged incubation in LIM broth affects the detection of GBS by the GPGB, a study using seeded specimens was performed. Six Bacti-Swab II samples which had previously been used to inoculate routine cultures of vaginal specimens were placed in 12 tubes of LIM broth (one swab in each broth tube). Two control strains of GBS were grown in broth and diluted to a concentration of about 10^5 CFU/ml. Six tubes were inoculated with 100 μl of one strain of GBS, and the other 6 tubes were inoculated with 100 μl of the second strain. The LIM broths were incubated at 35 to 37°C and assayed with the GPGB after 24, 48, 72, and 96 h of incubation.

RESULTS

A total of 502 specimens were tested in this study. For those specimens with discrepant test results (i.e., positive routine culture or positive GPGB result with the other test result negative), a two-step resolution process was performed. Initially, to exclude technical error, culture plates from specimens with discrepant results were reexamined and the GPGB was repeated on saved study LIM broth. Following this, these results were noted: 85 test results were positive by both methods, 405 test results were negative by both methods, 7 test results were GPGB positive and routine culture negative, and 5 results were GPGB negative and routine culture positive.

For the five specimens that were routine culture positive and GPGB negative, further testing was performed, including: (i) repeat subculture of the study LIM broths to BAP and CNA; (ii) placement of the pledgets in LIM broth with subsequent overnight incubation, subculture to BAP and CNA, and GPGB testing of the LIM broth in which the pledgets were incubated; and (iii) testing of the five GBS isolates for reactivity with the GPGB probe. The results of these tests are summarized in Table 1. On subculture GBS grew from one of the five study broths and two of the five pledgets. All five of the colony isolates were positive for GBS by probe test. As also noted in Table 1, the routine cultures from these five specimens all contained small numbers of organisms, with two specimens growing GBS only from the routine LIM broth and the other three growing from 1 to 4 colonies on the routine, primary plated media. These five results were classified as false-negative probe and true-positive culture results.

For the seven specimens that were routine culture negative and GPGB positive, further testing was performed, including: (i) repeat subculture of the study broths to BAP and CNA; (ii) repeat subculture of the routine broth to BAP and CNA (for specimens with repeat GBS-negative subcultures of the routine LIM broth, the pledgets were subcultured and broths were tested with the GPGB as described above); and (iii) testing of each isolate of GBS which grew for reactivity with the GPGB. The results of these tests are also reported in Table 1. For five of the these seven specimens, GBS grew from either the resubculture of the routine LIM broth or the pledget broth. All five of these GBS isolates were GPGB positive. Five of these seven results were classified as true-positive GPGB and false-negative culture results. For the other two specimens, no GBS were recovered by any culture method although two of the study broths and one of two pledget broths were repeatedly

TABLE 2. Comparison of culture with GPGB for detection of GBS

Test	No. of results				Sensitivity (%)	Specificity (%)	Predictive value of:	
	True positive	False negative	True negative	False positive			Positive result	Negative result
Culture	90	5	407	0	94.7	100	100	98.8
GPGB	90	5	405	2	94.7	99.5	97.8	98.8

GPGB positive. These two were classified as false-positive GPGB results.

Based upon the 490 results in agreement and the discrepant test analysis for the other 12 specimens, 95 specimens (18.9%) were positive for GBS and 407 were negative for GBS. Both the GPGB and culture detected 90 of 95 positive results (sensitivity, 94.7%). There were two false-positive GPGB results (specificity, 99.8%). These results are summarized in Table 2.

The routine culture method used in this study included primary culture to BAP and CNA, as well as overnight enrichment in LIM broth with subculture of the LIM broth to BAP and CNA. Of the 90 culture-positive specimens, 24 (26.6%) showed growth of GBS only upon subculture in LIM broth. An additional 11 specimens grew one to four colonies of GBS on the primary media. Thus, 35 (38.8%) of the 90 specimens grew four or fewer colonies in culture. The remaining 55 specimens included 24 with 1+ growth, 26 with 2+ growth, and 5 with 3+ growth of GBS on the primary plates.

A comparison of the costs associated with culture compared with the GPGB was made. For comparison, only the costs associated with plating and workup of the LIM broth and BAP (primary BAP as well as BAP for subculture of LIM broth) were calculated. We saw little, if any, benefit for the CNA and believe that a more realistic cost comparison could be made by excluding costs associated with the CNA. The cost comparisons are summarized in Table 3. The total estimated costs and minutes of labor per test were as follows: negative culture, \$3.68 and 7 min; positive culture, \$5.41 and 8.9 min; GPGB, \$5.16 and 3.6 min. The costs of the GPGB were calculated based upon a test run of 10 patient specimens and two controls, with an acquisition cost of \$70.00 for the Gen Probe reagents required to perform 20 tests. No costs associated with special equipment necessary to perform the GPGB are included. Labor costs associated with accessioning and reporting test results were not included in these calculations because of laboratory-to-laboratory variation associated with manual versus computer accessioning and reporting, as well as differences between laboratory information systems.

This study was performed with specimens incubated for 18 to 24 h in LIM broth prior to testing with the GPGB. To determine if incubation for longer than 24 h would have any detrimental effects on test results, seeded specimens were evaluated for time periods of 24 to 96 h. Table 4 is a summary of the RLU values for 12 specimen-GBS strain combinations. Results for all 12 tubes remained above the cutoff value (50,000 RLUs) which separates positive results from negative and indeterminate results. However, RLU values declined overall from 24 to 96 h. At 24 h, 12 of 12 results exceeded 800,000 RLUs, while only 10 of 12, 4 of 12, and 5 of 12 results exceeded 800,000 RLUs at 48, 72, and 96 h, respectively.

DISCUSSION

A recently published consensus paper has proposed two strategies to lessening GBS newborn disease. It has been estimated that the first strategy, which includes prenatal culture for GBS at 35 to 37 weeks of gestation, would prevent 86% of early-onset GBS disease. The other approach, which proposes no prenatal cultures, relying entirely on the presence of intrapartum risk factors to determine which women receive intrapartum antibiotics, has been estimated to prevent 69% of early-onset GBS disease (1).

Depending upon which strategy is implemented, laboratories face the potential for significant increases in requests for GBS screening of pregnant women. In our laboratory, we have already noted a significant increase in these requests. As volumes of any tests in the laboratory increase, potential cost saving for alternative testing methods must be explored.

Two factors contribute to the increased cost for the culture of GBS compared with, e.g., culture for group A streptococci. First, because even a low concentration of GBS in a vaginal-rectal specimen increases the risk of perinatal GBS disease, an enrichment broth is required to increase the culture sensitivity (3). A second factor contributing to the cost is the need to screen not only beta-hemolytic colonies but also non-hemolytic colonies for GBS. In this study, 1 of the 95 isolates of GBS was non-hemolytic.

The GPGB kit was designed to determine if bacteria growing in pure culture on solid media or in broth media are GBS. In this study, we evaluated the ability of the GPGB to detect GBS in a LIM broth culture of a patient specimen after 18 to 24 h of incubation. It is reasonable to assume that all specimens containing GBS also contained a mixture of vaginal and/or rectal flora and thus were not pure cultures of GBS.

We chose the 18- to 24-h incubation period because that is the recommended incubation period for broth cultures for GBS before a subculture is performed. Ideally, we would have performed this study with a single swab, performing the routine culture and GPGB from the same LIM broth. However, we needed to perform the routine culture by utilizing our normal laboratory protocol to satisfy patient turnaround time requirements. On the other hand, the GPGB was performed in a batch mode by one of us (B.J.H.) several times a week. Accepting the potential inherent variability in organism con-

TABLE 3. Estimated average cost for performance of GBS culture and GPGB

Source of expenses	Cost[a] in dollars (min of labor)		
	Negative culture	Positive culture	GPGB[c]
Labor			
Plating at \$0.16/min	0.32 (2.0)	0.32 (2.0)	0.19 (1.2)
Workup[d] at \$0.32/min	1.60 (5.0)	2.21 (6.9)	0.77 (2.4)
Supplies-reagents	1.76	2.88	4.20
Total	3.68 (7.0)	5.41 (8.9)	5.16 (3.6)

[a] Does not include labor costs associated with accessioning and reporting test results.

[b] Culture costs are average costs associated with 100 consecutive cultures, including 28 positive and 72 negative results.

[c] GPGB results are based upon a cost of \$70.00 for a 20-test kit and a test run of 10 patient specimens and two controls. Workup labor of 2.0 min/specimen becomes 2.4 min with inclusion of controls.

[d] Culture workup includes reading the primary plate for up to 2 days, smears, LIM broth subculture and reading, and GBS identification.

TABLE 4. RLU values for vaginal specimens seeded with GBS as a function of time[a]

GBS strain no.	Vaginal specimen no.	RLUs after incubation for:			
		24 h	48 h	72 h	96 h
1	1	858,000	734,000	283,000	552,000
	2	906,000	917,000	777,000	331,000
	3	980,000	1,014,000	836,000	667,000
	4	989,000	934,000	968,000	918,000
	5	965,000	918,000	787,000	122,000
	6	913,000	843,000	128,000	141,000
2	1	866,000	655,000	724,000	824,000
	2	956,000	896,000	867,000	830,000
	3	935,000	933,000	712,000	831,000
	4	959,000	933,000	712,000	950,000
	5	967,000	986,000	781,000	311,000
	6	965,000	849,000	126,000	128,000
Positive control		970,000	1,023,000	859,000	1,020,000

[a] RLU values were rounded to the nearest thousand.

centration between two swabs, we adopted a rigorous protocol to reconcile discrepant test results.

In this study, the GPGB was equivalent in sensitivity to culture for detection of GBS. Both culture and the GPGB detected 90 of 95 positive samples. The results of the discrepant analysis testing suggest that the failure of each method to detect the five positive results was due to sampling error, i.e., GBS present on one swab and not on the other or present in very low numbers on one swab compared with the other. Similar sampling differences have been noted in studies evaluating testing methods for group A streptococci. The sensitivity of the GPGB for low concentrations of GBS is validated by the fact that 66% of all cultures had 1+ growth on the primary plates or grew only from the LIM broth subculture. The specificity of the GPGB (99.5%) was also excellent.

Other investigators have also evaluated the GPGB on broth cultures of primary specimens. Yancey et al. demonstrated sensitivities of 44% and 71% after 2.5 and 3.5 h, respectively, for the GPGB in broth culture of vaginal specimens compared with their routine culture method (6). Kircher et al. demonstrated a sensitivity of 95% for the GPGB following 8 h of incubation in broth and concentration of the specimen by centrifugation (4). In a preliminary report, Fuller et al. reported a sensitivity of 100% for the GPGB when the test was performed on a centrifuged aliquot of a broth culture after 4 to 6 h of incubation (2).

While it may be possible to shorten the incubation period to less than the minimum of 18 h used in this study, we believe that this would offer little incremental clinical utility. In our opinion, the GPGB, as used in this study, is best adapted to culture screening performed by batch testing of specimens collected at 35 to 37 weeks of gestation. We do not believe that it is practical to perform this test on specimens collected at the onset of labor and expect that the test results can be obtained in sufficient time to influence the clinical decision of whether or not to give intrapartum antibiotics. Thus, shortening the incubation period by 6 or 12 h would have little benefit in our institution. We do not offer this test as a stat assay in our institution.

With the GPGB best suited to batch testing, the time when the LIM broth is inoculated may have an effect on the test result, particularly if there is a period, such as over a weekend, when the test is performed less frequently. In our evaluation of a limited number of specimens, we demonstrated that the GPGB RLU values dropped between 48 and 72 h of incubation for most specimen-organism combinations. Although all of the RLU values would have been interpreted as positive, we believe that it is prudent to limit incubation in the LIM broth to 48 h. More extensive studies may be able to demonstrate that longer incubation does not affect the test result interpretation. On the other hand, we (data not shown) and others have demonstrated that GBS maintain viability in transport swabs for up to 96 h. Depending on the frequency with which the test is performed in a lab, better test sensitivity may be achieved by keeping the swab in the lab before inoculating the LIM broth rather than incubating the LIM broth for more than 48 h.

Having demonstrated that the performance characteristics of the GPGB are essentially equivalent to those of a rigorous culture method, cost and adaptability to workflow patterns should determine whether the GPGB is routinely utilized in a laboratory. In our analysis of comparative costs of the GPGB and culture, we made several assumptions which significantly impact estimated costs. The number of specimens per test run, the cost of the GPGB reagents, and labor rates all influence the cost per test. For laboratories performing large volumes of GBS testing, the lower labor costs for the GPGB than for culture offer the potential for substantial labor savings. We included no incremental equipment costs in our cost comparison, assuming that the laboratories most likely to consider performing the GPGB are already performing other Gen-Probe assays and would utilize existing equipment.

In summary, the GPGB performed on a broth culture demonstrated performance characteristics equivalent to those of the standard culture method for GBS utilized in our laboratory. Depending upon the frequency with which the GPGB is performed in a laboratory, the time to test completion for the GPGB could be up to 48 h shorter than that of culture. While overall costs are slightly higher for the GPGB than for culture, the GPGB has lower labor costs and offers the potential for incremental savings with higher test volumes, as well as the ability to interface with laboratory information systems.

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