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Sensitivities of Antigen Detection and PCR Assays Greatly Increased Compared to That of the Standard Culture Method for Screening for Group B Streptococcus Carriage in Pregnant Women

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Group B streptococcus (GBS) is a major cause of serious infections in neonates. The 2002 revised guidelines of the Centers for Disease Control and Prevention (CDC) for the prevention of perinatal GBS disease recommend that all pregnant women be screened for GBS carriage at between 35 and 37 weeks of gestation and that intrapartum antibiotic prophylaxis be given to carriers. We studied the performances of four different GBS detection assays in the context of antenatal screening. Between May and August 2004, the 605 vaginorectal swab specimens received at our bacteriology laboratory for GBS antenatal detection were tested by the four assays. The standard culture method was done according to the CDC recommendations. The three experimental assays performed with the growth from the selective enrichment (LIM) broth (Todd-Hewitt broth with 15 μg/ml nalidixic acid and 10 μg/ml colistin) after overnight incubation were a GBS antigen detection assay (PathoDx) and two PCR assays (for *cfb* and *scpB*). The most accurate assay was the *scpB* PCR (sensitivity, 99.6%; specificity, 100%), followed by the *cfb* PCR (sensitivity, 75.3%; specificity, 100%), GBS antigen detection (sensitivity, 57.3%; specificity, 99.5%), and standard culture (sensitivity, 42.3%; specificity, 100%). The GBS antigen detection assay was found to be more sensitive than the standard culture method, and moreover, the assay has a low cost and is easy to perform in all obstetrical centers which have access to the most basic of diagnostic microbiology services. We believe that antigen detection on incubated LIM broth should replace the standard culture method for screening for GBS carriage at 35 to 37 weeks of gestation. The impact of the greater sensitivities of PCR assays on the diminution of neonatal GBS infections remains to be demonstrated.

Group B streptococcus (GBS) has been the leading cause of early-onset neonatal sepsis in industrialized countries for more than 30 years. Infection occurs through vertical transmission from a GBS-colonized mother to the newborn during labor and birth. Intrapartum antibiotic prophylaxis (IAP) has been shown not only to interrupt the transmission of GBS from mother to infant (28) but also to reduce the incidence of early-onset GBS disease (23). Guidelines from professional organizations issued in 1996 recommended two different strategies for the selection of candidates for IAP: either screening for GBS vaginorectal carriers or identification of maternal clinical risk factors for early-onset neonatal GBS disease (5).

Results from a large prospective study showed the superiority of the screening approach in preventing early-onset GBS neonatal disease (22). This finding was confirmed in a retrospective review of GBS disease in infants (26). In 2002, the Centers for Disease Control and Prevention (CDC) recommended that all pregnant women be screened for carriage of GBS at between 35 and 37 weeks of gestation and that IAP be offered to the carriers (20). The culture method has a slow turnaround time requiring 36 to 72 h before results can be issued. Besides being time-consuming, this method requires an experienced technician to identify the suspected colonies, which are not always beta-hemolytic (14). Moreover, the suppression of GBS growth by enterococci present in the vaginal and rectal flora could lead to false-negative results (11).

Molecular biology-based assays are the only methods that have been shown to be reliable and more rapid than the standard culture-based method. A real-time PCR assay has been approved by the Food and Drug Administration (FDA) for the detection of GBS DNA directly from combined vaginal and rectal swab specimens (17). However, implementation research will have to be conducted to confirm that this rapid test can determine colonization status in time for the IAP to be administered more than 4 h antenatally (12, 20, 21).

The purpose of our study was to evaluate a rapid, reliable, easy-to-perform, and inexpensive test to detect antenatal GBS colonization in pregnant women. We compared the performance characteristics of an antigen detection assay for GBS to those of the standard culture method and two nucleic acid detection assays, all of which were performed on LIM broth that had been incubated overnight.

MATERIALS AND METHODS

Study design. Sainte-Justine Hospital is a pediatric tertiary care center where 4,000 infants are delivered yearly. Obstetricians at Sainte-Justine Hospital monitor pregnant women antenatally, at 35 to 37 weeks of gestation, by culturing vaginorectal specimens for the detection of GBS, according to the recommendations of CDC (20). The swabs are placed in Amies transport medium and sent to the microbiology laboratory. The 605 vaginorectal swab specimens processed in our laboratory between May and August 2004 were tested by four assays: the standard culture method, a GBS antigen detection method, and two PCR assays.

Culture identification of GBS. Swab specimens were plated on 5% sheep blood agar and were then placed into the LIM selective enrichment broth (Todd-Hewitt broth with 15 μg/ml nalidixic acid and 10 μg/ml colistin). After

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TABLE 1. Results of culture and GBS antigen detection methods

GBS antigen detection result	No. of specimens with the following culture results:		Total
	Positive	Negative	
Positive	96	36	132
Negative	0	473	473
Total	96	509	605

overnight incubation (mean, 20 h 33 min; standard deviation, 3 h 36 min), the broths were subcultured onto 5% sheep blood agar. Solid and liquid media were incubated at 35°C with 5% CO_2, and solid media were read daily for 2 days.

GBS was identified as follows: the presence of catalase-negative, beta-hemolytic colonies and a positive reaction for the GBS antigen by a slide agglutination test (PathoDx; Diagnostic Products Corporation, Los Angeles, CA), as recommended by the CDC guidelines (20). Nonhemolytic colonies were tested for the GBS antigen reaction, based on colony appearance and catalase-negative and bile esculin-sensitive reactions.

GBS antigen detection on incubated LIM broth. The PathoDx kit used for the identification of GBS among isolated colonies was adapted and used for GBS antigen detection. The test was performed daily with the incubated LIM broth just before subculture. Nitrous acid extraction of the antigen was done prior to the slide agglutination test by using the reagents included in the PathoDx kit: 2 drops (approximately 75 μl) of reagent one, 2 drops of reagent two, 2 drops of incubated LIM broth, and 4 drops of reagent three were mixed in that order in a plastic tube. One drop of the extracted broth was mixed with 1 drop of Strep B grouping latex reagent on a PathoDx slide, which was rocked for 60 s.

A result was considered positive if there was at least a 2+ agglutination reaction within the 60-s period, as defined in the product documentation. This assay was demonstrated to be reproducible (data not shown). The PCR results were unknown to the technician performing the culture and the antigen detection assays.

GBS nucleic acid detection on incubated LIM broth. GBS nucleic acid detection was based on two different PCR assays, one targeting the *cfb* gene, which encodes the CAMP factor, and the other targeting the *scpB* gene, which encodes C5a peptidase. Aliquots of the remaining incubated LIM broth were frozen at −70°C immediately after the antigen detection and PCR assays were performed with batched samples. Two microliters of incubated LIM broth was mixed with 20 μl of Gene Releaser (Bioventures, Murfreesboro, TN), and the DNA was extracted by using a nine-temperature one-cycle program (65°C for 15 s, 8°C for 15 s, 65°C for 45 s, 97°C for 90 s, 8°C for 30 s, 65°C for 90 s, 97°C for 30 s, 65°C for 30 s, and hold at 80°C). Fifty microliters of the PCR mixture (20 mM Tris-HCl [pH 8.4], 50 mM KCl, 2.5 mM $MgCl_2$, 0.4 mM each deoxynucleotide, 0.4 μM each primer, 1 U Platinum *Taq* polymerase [Invitrogen] and internal control DNA, when needed) was added to the 22-μl DNA preparation. An internal control was added to each sample submitted for *cfb* PCR (13). For the *cfb* PCR, we used primers Sag59 and Sag190, defined by Ke et al. (13), to amplify both the genomic DNA and the internal control template. For the *scpB* PCR, we used the primers described by Dmitriev et al. (10). Amplifications were performed after 5 min at 94°C by 45 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. The amplified reaction products were analyzed by electrophoresis on 2% agarose gels. In the case of PCR inhibition (no amplification of *cfb* or the internal control), DNA was reextracted with the DNeasy tissue kit from QIAGEN (Mississauga, Ontario, Canada). A result was considered positive if the amplicon was 153 bp for the *cfb* PCR and 255 bp for the *scpB* PCR. Positive and negative controls were included in each PCR batch; and three different areas were used for specimen preparation, PCR mix preparation, and PCR amplification and analysis. The culture and the antigen detection results were unknown to the technician performing the PCR assays.

Both strands of the amplicons from the *spcB* PCR were sequenced in-house by using the same primers used for the initial PCR amplification and an ABI PRISM 3100-*Avant* genetic analyzer (Applieds Biosystems), following the manufacturer's instructions. The 56 contigs were compared to the sequences present in the GenBank database and were aligned to the GBS *scpB* sequence (GenBank accession number AAF04282) by using the MultAlin alignment program (7).

Statistical analysis. A specimen was considered positive for GBS if it was positive by culture or by one of the two PCR assays. Sensitivity and specificity were then calculated for each of the assays. Statistical work was performed by using STATA8 (StataCorp LP, College Station, TX) on a Windows XP (Microsoft, Redmond, WA) operating system. Confidence intervals for proportions were calculated by using the exact binomial confidence intervals (1), as implemented by Seed (24).

TABLE 2. Results of GBS antigen detection and PCR assays with culture-positive specimens

GBS antigen detection result	No. of specimens with the indicated results:				Total
	cfb PCR positive		*cfb* PCR negative		
	scpB PCR positive	*scpB* PCR negative	*scpB* PCR positive	*scpB* PCR negative	
Positive	95	1	0	0	96
Negative	0	0	0	0	0
Total	95	1	0	0	96

RESULTS

Six hundred five specimens were tested by the four assays. Culture identified 96 positive specimens among the 605 specimens tested (16%), GBS antigen detection identified 132 positive specimens (22%), *cfb* PCR identified 171 positive specimens (28%), and *scpB* PCR identified 226 positive specimens (37%). A total of 227 specimens (37.5%) were considered true positive.

All specimens found to be positive by culture ($n = 96$) were also positive by GBS antigen detection (Table 1), and the result was confirmed by at least one PCR assay. Of these 96 specimens, 95 were positive by the two PCR assays and 1 was positive by the *cfb* PCR only. The *scpB* PCR amplicon obtained for that one specimen was not of the expected size; two bands were seen, suggesting a modification of the gene (data not shown), and thus, the result by *scpB* PCR was considered false negative (Table 2).

Among the 509 culture-negative specimens, 36 were positive by GBS antigen detection (Table 1). When these samples were analyzed by the PCR assays (Table 3), 34 of 36 were confirmed to be positive by the use of both sets of primers. The remaining two samples, which were negative by the use of both sets of primers, were considered false positive by GBS antigen detection. Among the 473 culture-negative and GBS antigen detection method-negative specimens, 41 were positive by both PCR assays, and the results were considered true positive; 56 were positive only by the *scpB* PCR, and the results were considered true positive because the sequences of all amplicons were identical (>99% homology) to the *scpB* GBS sequence (data not shown); and 376 were negative by both PCR assays, and the

TABLE 3. Results of GBS antigen detection and PCR assays with culture-negative specimens

GBS antigen detection result	No. of specimens with the indicated results:				Total
	cfb PCR positive		*cfb* PCR negative		
	scpB PCR positive	*scpB* PCR negative	*scpB* PCR positive	*scpB* PCR negative	
Positive	34	0	0	2	36
Negative	41	0	56	376	473
Total	75	0	56	378	605

TABLE 4. Performance characteristics of the four assays for GBS detection with antenatal vaginorectal specimens[a]

Method	% Sensitivity (95% CI)	% Specificity (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Culture	42.3 (36–49)	100 (99–100)	100 (96–100)	74.3 (70–78)
GBS antigen	57.3 (51–64)	99.5 (98–100)	98.5 (95–100)	79.5 (76–83)
cfb PCR	75.3 (99–100)	100 (99–100)	100 (98–100)	87.1 (84–90)
scpB PCR	99.6 (98–100)	100 (99–100)	100 (99–100)	99.7 (98–100)

[a] Abbreviations: PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

results were considered true negative. Of interest, there was a very low level of PCR inhibition; 18 of the 605 samples (3%) showed PCR inhibition, and the results for 10 of those 18 samples were positive by both PCR assays on retesting.

The performance statistics for the four assays are summarized in Table 4. The most accurate method was the *scpB* PCR (sensitivity, 99.6%; specificity, 100%), followed by the *cfb* PCR (sensitivity, 75.3%; specificity, 100%), GBS antigen detection (sensitivity, 57.3%; specificity, 99.5%), and standard culture (sensitivity, 42.3%; specificity, 100%).

The distributions of the turnaround times for positive cultures were 24 h for 46 of 96 (48%) specimens, 48 h for 42 of 96 (44%) specimens, and 72 h for 8 of 96 (8%) specimens. The negative culture results for 509 of 605 (84%) specimens were issued at 72 h, according to the CDC recommendations. The GBS antigen detection assay and the PCR assays were performed directly on incubated LIM broth and therefore could generate final results within 24 h.

DISCUSSION

The intrapartum use of prophylactic antibiotics in pregnant women colonized with GBS has been shown to be very successful at reducing the incidence of GBS early-onset neonatal disease, which declined from 1.5/1,000 live births in 1990 to 0.5/1,000 live births in 1999, according to surveillance in the United States (20). The selection of the appropriate candidates for prophylaxis has been a major concern. Although a clinical risk-based approach appeared to be easier for obstetricians to implement, "vaginal colonization with GBS at the time of delivery is by far the most powerful indicator of risk" for early-onset neonatal disease (3). A large multistate retrospective cohort study conducted in 1998 and 1999 showed that the culture-based screening approach not only was 50% more effective at preventing perinatal GBS disease but also resulted in improved compliance (22). Those results led to the 2002 CDC revised guidelines for the prevention of perinatal GBS disease: universal screening of all pregnant women at 35 to 37 weeks of gestation and intrapartum chemoprophylaxis for the carriers as a first-line approach (20).

We have demonstrated that our three alternative assays for screening for GBS on incubated LIM broth have greatly increased sensitivities over that of the culture method recommended by CDC. In our population the prevalences of GBS carriage were found to be 16% by the standard culture method, 21% by antigen detection, and 37% by *scpB* PCR. Although we cannot exclude the possibility of detection of nonviable GBS by the antigen and PCR assays, several conditions could explain the false-negative culture results: antibiotics and feminine hygiene products have been shown to inhibit the detection of GBS by culture but have no detrimental effect on antigenic detection (16). Suppression of the growth of GBS by the enteroccoci present in the vaginal and rectal flora has also been documented (11). These criteria were not evaluated in our study. Inadequate specimen collection and transport from obstetrical clinics to the laboratory could also result in false-negative results, especially in the presence of light colonization (19, 25). The culture method is suspected to lack sensitivity, given that studies of confirmed early neonatal GBS infection have demonstrated disease in neonates born to mothers negative for GBS by culture (15, 18), and our results confirm this hypothesis.

The detection of GBS by the antigen detection method is superior to that by the standard culture method and is clearly the simplest of the four methods to implement. Because it requires only LIM broths, an incubator, an antigen detection kit, and minimal technician training, this method is easily adaptable to small satellite laboratories located in community clinics; and subculture for susceptibility testing could be done, if needed. In comparison to the standard culture method, the PCR method is the most accurate method at present, but it is not available in all obstetrical centers.

Several screening methods for the detection of GBS have been evaluated directly with vaginorectal specimens immediately prepartum because the ability of antenatal cultures at 35 to 37 weeks of gestation to predict intrapartum culture results has been reported to vary between 54 and 87% (8, 27). In 1997, the FDA issued a safety alert with regard to direct antigenic test kits for GBS producing false-positive and false-negative results (2). The consequence was the withdrawal of these methods from the diagnostic armamentarium. A real-time PCR targeting the *cfb* gene is the only assay with a 1- to 2-h turnaround time and has been approved by the FDA for the detection of GBS DNA directly from vaginorectal specimens (17). The assay initially demonstrated a sensitivity of 97% (95% confidence interval, 83 to 99%) when it was evaluated against the standard culture method (4). This reduced sensitivity was confirmed in a recent multicenter study, in which it was found to have an overall sensitivity of 94%, with a sensitivity range from 85% to 99%, depending on the site (8). This approach presents two other challenges: first, the test result needs to be available to clinicians more than 4 h prior to delivery for the prophylaxis to be administered as recommended (20), and second, the cost of the equipment and the number of technical personnel needed to process the specimen on-site and on a "stat" basis are considerable (12, 21). A limited number of obstetrical centers can afford these re-

sources, and standard cultures would still need to be done for antimicrobial susceptibility testing, especially for penicillin-allergic patients.

The increased sensitivities of our three assays may allow us to better understand the dynamics of vaginorectal GBS carriage in pregnant women. Colonization thought to be intermittent or transient (9) might rather be continuous, with variations in the bacterial load. Consequently, the predictive value of an antenatal GBS detection assay could increase significantly and be a better indicator of colonization at the time of delivery. This would be an important hypothesis to address in a future study. The use of an assay with increased sensitivity would result in an increase in the number of women who received IAP and, above all, could further reduce the incidence of early-onset neonatal GBS disease beyond the reduction seen by use of the standard culture-based method. Such an increase in antibiotic use may raise the concern of selecting for non-GBS or ampicillin-resistant organisms. Most studies have not found any significant changes in the rate of early-onset neonatal sepsis caused by pathogens other than GBS, including those that are antibiotic resistant (6, 20). However, ongoing surveillance is needed.

In conclusion, GBS-specific PCR and antigen detection assays with the growth from incubated LIM broth are more sensitive and more rapid methods for the detection of GBS carriers during pregnancy than the standard culture method. GBS antigen detection is much more sensitive than the standard culture method, is the easiest method to perform, is inexpensive, and could be made available in any obstetrical center. We believe that antigen detection in the growth on incubated LIM broth should replace the standard culture method for screening for GBS carriage at 35 to 37 weeks of gestation. Both PCR assays with the growth from incubated LIM broth have greatly increased sensitivities compared to those of the standard culture method and GBS antigen detection. We do not believe that PCR assays with the growth from incubated LIM broth should be adopted as standard practice before quantitative studies of GBS carriage in vaginorectal specimens can demonstrate whether a threshold predictive of neonatal disease exists. Without such studies, the increase in sensitivity would result in a much larger number of women receiving IAP, and it remains to be demonstrated whether it would significantly diminish the number of infants with neonatal GBS disease compared to the numbers that would result by the use of culture and the detection of antigen in the growth on incubated LIM broth.

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