Diagnostic Accuracy of a Rapid Real-Time Polymerase Chain Reaction Assay for Universal Intrapartum Group B Streptococcus Screening

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Background. Intrapartum antibiotic prophylaxis is currently given to mothers who test positive for group B streptococcus (GBS) by antenatal culture-based screening, with a risk-based approach for cases with an unknown GBS status. A rapid real-time polymerase chain reaction (PCR) assay for the detection of GBS became available recently, making intrapartum screening possible. We aimed to assess its diagnostic accuracy and to compare it with antenatal screening.

Methods. We conducted a prospective study in a French hospital. All pregnant women giving birth at the maternity ward were considered for inclusion, except those with planned cesarean delivery, with delivery at <35 weeks gestation, and who received antibiotic therapy before admission. We performed GBS culture (the reference standard) and a molecular GBS test (Xpert GBS; Cepheid) on intrapartum specimens. Decisions about intrapartum antibiotic prophylaxis were based on the current GBS screening by culture at 35–37 weeks gestation.

Results. We prospectively enrolled 968 pregnant women from April 2007 through March 2008. The overall molecular GBS test yield was 89.2%. Among the 863 women with available results, the molecular GBS test had a sensitivity of 98.5%, specificity of 99.6%, positive predictive value of 97.8%, and negative predictive value of 99.7%. The positive predictive value of antenatal culture for identifying colonization status at delivery was low (58.3%), whereas the negative predictive value was imperfect (92.1%).

Conclusions. This real-time PCR assay is a highly accurate test to identify intrapartum GBS carriers at point of care. This new tool could enhance the exact identification of candidates for intrapartum antibiotic prophylaxis, including women with preterm rupture of membranes or preterm labor.

Group B streptococcus (GBS) is the leading cause of neonatal morbidity and mortality in developed countries. Maternal GBS colonization is one of the most important risk factors for early-onset GBS disease, because of the vertical transmission from GBS-colonized mothers to their babies. The prevalence of GBS vaginal colonization in pregnant women ranges from 6.5% to 36% in European countries [1]. Other risk factors for transmission include GBS bacteriuria during pregnancy; a previous infant with GBS infection, intrapartum fever, preterm labor at <37 weeks gestation, or prolonged rupture of membranes for >18 h.

Studies have demonstrated that maternal intrapartum antibiotic prophylaxis (IAP) significantly reduces the rate of neonatal GBS colonization and the incidence of early-onset GBS disease [2–4]. Two approaches to prevent early-onset GBS disease have been used: (1) universal culture-based screening at 35–37 weeks gestation for all pregnant women, with IAP given to those with positive results, and (2) IAP given to all women with obstetric risk factors for GBS transmission, without prior screening. But a multistate, retrospective cohort study demonstrated that the culture-based screening strategy was >50% effective in prevention of neonatal infection [5]. Thus, new guidelines were issued in the United States (in 2002) and France (in 2001) that endorsed universal antenatal GBS culture screening at 35–37 weeks gestation, with the risk-based approach
GBS culture remains the reference standard for the detection of GBS colonization. However, this method requires 18–72 h. Therefore, culture-based testing cannot be performed intrapartum and is feasible only for antepartum screening, which leads to 2 limitations. First, antepartum screening at 35–37 weeks gestation excludes women who have preterm delivery at <35 weeks gestation. Moreover, ~7%–11% of women deliver before screening, and this group has the highest risk of serious neonatal GBS infection [8]. This set of women accounts for 32%–38% of early-onset GBS cases [9]. When looking at intrapartum risk factors, a study reported that up to 65% of early-onset GBS cases did not have any risk factors [10]. Second, GBS colonization can be intermittent during pregnancy; therefore, there is poor correlation between antenatal screening results and intrapartum maternal GBS colonization [11–14]. Consequently, some women may not be appropriately treated for GBS colonization or may be unnecessarily treated with antibiotics. Thus, in the era of IAP, most remaining cases of early-onset GBS occur in newborns whose mothers had negative results of screening for GBS colonization at 35–37 weeks gestation [15].

Rapid intrapartum tests based on immunological or hybridization methods have been used; they showed poor sensitivity and specificity, compared with culture [16, 17]. Real-time polymerase chain reaction (PCR)–based assays have been shown to be sensitive and specific tests [18–22]. But their practical use is limited because they require manual DNA extraction by technically skilled operators using specific laboratory equipment and are batch based.

Recently, the Xpert GBS assay (Cepheid), a commercial real-time PCR test for the rapid detection of GBS, became available. The aim of our study was to evaluate the diagnostic accuracy of this test at the onset of labor and to compare it with the current screening by culture at 35–37 weeks gestation.

**METHODS**

**Participants.** This prospective study was conducted at a 680-bed general hospital in Paris, France. The maternity ward manages only deliveries at >33 weeks gestation. From April 2007 through March 2008, all women giving birth at the maternity ward were considered for inclusion, regardless of whether they received prenatal care. Exclusion criteria were planned cesarean deliveries, deliveries at <35 weeks gestation, and women who had received antibiotic therapy in the week before admission. Data collection was planned before study onset and included the following characteristics: previous infant with early-onset GBS disease, bacteriuria during current pregnancy, parity, gestational age at delivery, duration of rupture of membranes, duration of labor, and whether newborns received a diagnosis of early-onset GBS disease. Proven early-onset GBS sepsis in newborns was defined by positive results of blood, cerebrospinal fluid, urine, and/or pulmonary cultures, in the presence of clinical signs and laboratory abnormalities consistent with infection [7]. Probable early-onset GBS infection in newborns was defined by positive results of GBS culture of gastric fluid aspiration, deep ear swab specimens, or both, in the presence of clinical signs and laboratory abnormalities consistent with infection [7].

**Study approval.** The local institutional review board approved the study protocol. A waiver of informed consent was granted because the study results were blinded—that is, obstetricians and midwives were not informed of the intrapartum results. Only the current GBS screening at 35–37 weeks gestation was used for decisions about IAP.

**Specimen collection.** In accordance with French screening policy, a vaginal specimen was obtained at 35–37 weeks gestation for each parturient receiving prenatal care. Obstetricians or midwives collected secretions from the lower one-third of the vagina by using a cotton swab (swab with Stuart liquid; Copan), without the use of a speculum [7]. Specimens were transported at ambient temperature to the microbiology laboratory for routine culture. At the time of delivery and before the start of IAP, a second vaginal specimen was collected using a double swab (swab with Amies liquid; Copan). These specimens were immediately transferred to the laboratory to be processed. One of the swab specimens was used in the Xpert GBS molecular test, whereas the other was processed by microbiological culture. The laboratory is located near the delivery rooms.

**GBS culture.** According to the recommendations of the French National Agency for Accreditation and Evaluation of Health Care (Agence Nationale d’Accréditation et d’Evaluation en Santé), antenatal (at 35–37 weeks gestation) swab specimens were cultured directly on Columbia ANC sheep blood agar (bioMérieux) that was incubated at 37°C in 5% CO2 for 18 h [7]. The intrapartum swab specimens were also cultured on Columbia ANC sheep blood agar and were subsequently placed in a broth medium that was incubated at 37°C for 18 h. GBS colonization was defined as negative in the absence of growth and was defined as positive in the case of GBS growth on at least 1 quadrant of the agar plate or when only the broth medium was positive for GBS. β-Hemolytic colonies and suspect nonhemolytic colonies were identified as GBS by using a commercial latex agglutination test (bioMérieux) in accordance with the manufacturer’s instructions.

**Molecular-based in vitro assay.** The real-time PCR was performed using the GeneXpert system (Cepheid), which integrates the complete process of DNA extraction, amplification, and detection in a completely automated fashion. The overall process is completed in <75 min. The DNA target sequence is
the 3′-adjacent region of the GBS *cfb* gene. A single-use cartridge holds the sample and all necessary reagents and hosts both the extraction and PCR process. Each test cartridge contains a sample processing control and an internal control. The sample processing control (ie, *Bacillus globigii*) validates the sample nucleic acid extraction step, and the internal control monitors the PCR reaction for inhibitors. A computer collects amplification data to detect the presence or absence of the GBS target. In our study, GBS colonization was defined as negative when the cycle threshold was 0 or >42 and was defined as positive otherwise. Antenatal and intrapartum GBS culture results and molecular test results were read independently of each other.

**Statistical methods.** Assuming a sensitivity of 92% for the molecular GBS test (data from Cepheid [23]), we calculated that a sample size of 865 patients would provide a precision inferior to 5% around the observed sensitivity. Taking into account an expected rate of unavailable molecular GBS test results of 10%, the planned sample size was increased to 960 patients.

Because the molecular GBS test results were not available for some specimens (because of PCR inhibition, significant presence of mucus, or manipulation errors), we first calculated the overall molecular GBS test yield (the probability of obtaining either a positive or a negative result—ie, whether the molecular GBS test results are false positive or false negative) and the positive yield (the probability of obtaining a positive or negative molecular GBS test result when GBS colonization is present according to GBS culture results) [24].

We estimated the sensitivity, specificity, predictive values, and likelihood ratios of the molecular GBS test, including unavailable molecular GBS test results in a 6-cell matrix as described elsewhere [24]. The 95% confidence intervals (CIs) of proportions were calculated using the Wilson method [25], and the 95% CIs of likelihood ratios were calculated using the “score test” method [26].

For the subset of women with an available result of GBS culture screening at 35–37 weeks gestation, we calculated the predictive values, compared with intrapartum test results. Statistical analyses were performed using SAS, version 9.1 (SAS Institute).

## RESULTS

We prospectively enrolled 968 pregnant women who presented for intrapartum care from April 2007 through March 2008. They all underwent both intrapartum GBS culture and molecular testing. Characteristics of the study population are presented in Table 1.

The intrapartum GBS colonization rate according to culture was 14.8% (95% CI, 12.7–17.1). Molecular GBS test results were not available for 105 (10.8%) women. Of these 42 (40%) were because of PCR inhibition, 39 (37%) because of a significant presence of mucus, and 24 (23%) because of manipulation errors in loading the cartridge at the beginning of the study. Consequently, the overall molecular GBS test yield was 89.2% (95% CI, 87.0–91.0), and the positive test yield was 95.8% (95% CI, 91.1–98.1). Among the 863 women with available results, the molecular GBS test had a sensitivity of 98.5%, a specificity of 99.6%, a positive predictive value of 97.8%, and a negative predictive value of 99.7% (Table 2).

Three specimens tested positive by the molecular GBS test and negative by the Columbia ANC sheep blood agar and enriched agar method. For these 3 specimens, the amplicon was

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**Table 1. Characteristics of the 968 Women in the Study Group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td>40.0 (39.1–40.6)</td>
</tr>
<tr>
<td>Preterm labor &lt;37 weeks</td>
<td>25 (2.6)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>562 (58.1)</td>
</tr>
<tr>
<td>2</td>
<td>279 (28.8)</td>
</tr>
<tr>
<td>&gt;3</td>
<td>127 (13.1)</td>
</tr>
<tr>
<td>Prenatal care</td>
<td>954 (98.6)</td>
</tr>
<tr>
<td>Time from rupture of membranes to delivery, h</td>
<td>4.5 (2.6–8.5)</td>
</tr>
<tr>
<td>Membranes rupture &gt;12 h before delivery</td>
<td>172 (17.8)</td>
</tr>
<tr>
<td>GBS bacteruria during pregnancy</td>
<td>14 (1.4)</td>
</tr>
<tr>
<td>Previous infant with GBS disease</td>
<td>7 (0.7)</td>
</tr>
<tr>
<td>Duration of labor, h</td>
<td>5 (3.5–6.5)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of women or median (25th–75th percentiles). GBS, group B streptococcus.

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**Table 2. Cross-Tabulation of Intrapartum Molecular Group B Streptococcus (GBS) Test Results by Intrapartum GBS Culture Results**

<table>
<thead>
<tr>
<th>Intrapartum GBS culture result</th>
<th>Intrapartum molecular GBS test result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>723</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>825</td>
</tr>
</tbody>
</table>

**NOTE.** The sensitivity was 98.5% (95% confidence interval [CI], 94.8%–99.6%); specificity was 99.6% (95% CI, 98.8%–99.9%); positive predictive value was 97.8% (95% CI, 93.8%–99.3%); negative predictive value was 99.7% (95% CI, 99.0%–99.9%); positive likelihood ratio was 238.5 (95% CI, 81.6–700.8); and negative likelihood ratio was 0.015 (95% CI, 0.004–0.052).

* Invalid molecular GBS test results because of polymerase chain reaction inhibition.

* Unavailable molecular GBS test results because of significant presence of mucus or manipulation errors in loading the cartridge at the beginning of the study.
A total of 933 women underwent antenatal screening, and 115 had positive results. Of these, 65 remained positive for GBS intrapartum and 63 received IAP; 3 infected and 2 colonized newborns were delivered among these 63 women.

Of 818 women who had negative results of screening at 35–37 weeks gestation, 63 were positive for GBS intrapartum but did not receive IAP; among these women, 4 infected and 9 colonized neonates were documented. Among the 664 women who had negative results of screening at 35–37 weeks gestation and were negative for GBS intrapartum, 3 infected newborns were recorded. Their mothers had received antibiotic therapy because of membrane rupture for >18 h and fever (temperature >38°C).

Of the 35 women who did not undergo antenatal screening, 14 had GBS bacteriuria during the current pregnancy and 7 had a previous infant with early-onset GBS disease. For such cases, antenatal screening is not indicated, and the women received IAP at the time of labor or rupture of membranes [6, 7]. The remaining 14 women had an unknown GBS status at 35–37 weeks gestation because of inadequate prenatal care; of these 14, 6 had obstetric risk factors at delivery and 4 received IAP. However, 1 GBS-infected newborn was delivered from a mother who had tested positive at intrapartum screening. She did not have any risk factor and did not receive IAP.

### DISCUSSION

IAP is currently the only effective method to prevent early-onset GBS disease. A key element of screening-based prevention strategies is the accurate identification of colonized parturients, to select them as appropriate candidates for IAP. Thus, the screening of pregnant women should use optimal microbiological methods in an attempt to identify real carriers of GBS at the time of delivery.

Our study showed that GBS carriage in pregnant women may be consistent or intermittent, as supported by previously published reports. In accordance with other studies [11, 19, 27, 28], we also found a poor positive predictive value (58.7%) for screening by culture at 35–37 weeks gestation, which resulted in IAP being given to women with very low risk of GBS trans-

#### Table 3. Cross-Tabulation of the Antepartum and Intrapartum Group B Streptococcus (GBS) Culture Results

<table>
<thead>
<tr>
<th>Antepartum GBS culture result</th>
<th>Intrapartum GBS culture result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>753</td>
<td>65</td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td>67</td>
</tr>
<tr>
<td>Missing</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>825</td>
<td>143</td>
</tr>
</tbody>
</table>

#### Table 4. Cross-Tabulation of the Antepartum Group B Streptococcus (GBS) Culture Results and the Intrapartum Molecular GBS Test Results

<table>
<thead>
<tr>
<th>Antepartum GBS culture result</th>
<th>Intrapartum molecular GBS test result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>664</td>
<td>37</td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>65</td>
</tr>
<tr>
<td>Missing</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>725</td>
<td>138</td>
</tr>
</tbody>
</table>

* Unavailable molecular GBS test results because of polymerase chain reaction inhibition.

* Unavailable molecular GBS test results because of significant presence of mucus or manipulation errors in loading the cartridge at the beginning of the study.
Figure 1. Flow diagram providing the results of the antenatal group B streptococcus (GBS) culture screening, the numbers of women who received intrapartum antibiotic prophylaxis (IAP), and outcomes for newborns. *Fourteen had GBS bacteriuria during pregnancy, and 7 had a previous infant with GBS disease. Of 5 with obstetric risk factors, 3 received IAP. One received IAP and had obstetric risk factors. Of 15 with obstetric risk factors, 2 received IAP. The mother had no obstetric risk factor and did not receive IAP. Nine other newborns were colonized with GBS. Two other newborns were colonized with GBS. “GBS infection” indicates probable early-onset GBS disease in newborns, which was defined by positive results of GBS culture of gastric fluid aspiration, deep ear swab specimens, or both and negative results of cultures of blood, cerebrospinal fluid, pulmonary, and/or urine specimens, in the presence of clinical signs and laboratory abnormalities consistent with infection. NA, not available.

mission, and an imperfect negative predictive value (92.1%), which means that some women who are colonized at the time of labor are not candidates for IAP. Similar results led Goodman et al [11] to conclude that, regardless of when antenatal GBS cultures are performed, they serve as a poor predictor of maternal GBS carriage at the time of delivery.

A substantial portion of early-onset GBS cases occur among infants of lightly colonized mothers [17] and among infants of mothers who test negative by antenatal culture screening [15]. Therefore, a sensitive but rapid intrapartum test is needed. These issues seemed to be overcome by PCR-based tests. However, there are discrepancies regarding their sensitivity for GBS screening. A method that amplified the cfb gene showed sensitivities of 97% and 94% [18, 19]. However, it was shown that, after initial enrichment in selective broth, the method that used the scpB gene was more sensitive than the method that used the cfb gene (99.6% vs. 75.3%) [20]. Later, it was suggested that the cfb method without prior broth enrichment is not sensitive enough for routine use [21]. Besides, it was shown that both vaginal and rectal specimens are required to achieve good sensitivity [22]. Subsequently, all these PCR methods needed either prior broth medium enrichment or recto-vaginal sample to be sensitive enough.

Among these sensitivity controversies, the major objection to all these developed real-time PCR assays is that they need well-trained operators, specific laboratory equipment, and consequently, batch testing. Therefore, the next step toward bringing PCR technology to the point-of-care setting was to find a test that is easy to use by any operator and one by which the results would be available 24 h/day, 7 days/week. Our current study showed that identification of GBS-positive women in an intrapartum setting can be accurately achieved by the use of the Xpert GBS assay. When compared with culture as the reference standard, the Xpert GBS test using intrapartum samples showed very high sensitivity of 98.5% and specificity of 99.6%.

The complete process using the GeneXpert takes <75 min. In this study, 75% of the women delivered their infant in >3.5 h, and the results could have been available in time for IAP to be given. However, a systematic review of duration of IAP did not identify any evidence that infants whose mothers delivered before the 4 h threshold of IAP (ie, 2 doses) are at higher risk for GBS sepsis [29].

In our study, 4 cases of neonatal infection and 9 cases of neonatal colonization, documented among the newborns of the 63 women who tested negative by screening at 35–37 weeks gestation who became positive for GBS intrapartum but did not receive IAP, could have been avoided. Considering the characteristics of our study population and the diagnostic accuracy of the Xpert GBS test, we assessed the projected outcomes from universal intrapartum screening using the molecular test; we assumed that the risk-based approach would be used for women with unavailable molecular GBS test results (Fig. 2). Probability estimates of infection and relative risk estimates of IAP for early-onset GBS used in this analysis are from Haberland et al [30] and Benitz et al [31], respectively. We projected that, if 100,000 women present to our maternity ward for intrapartum care, 16.57% of women would receive IAP and 0.188% of newborns would be infected with GBS. In our study population,
Projected outcomes of universal intrapartum screening and risk-based approach for women with unavailable results of molecular group B streptococcus (GBS) testing. Calculations were as follows. The overall GBS test yield equals $887/968 = 91.6\%$. The intrapartum GBS colonization prevalence equals $138/968 = 15.99\%$. The probability of infant infection given intrapartum maternal GBS colonization is $1.47\%$ [30], and the relative risk of IAP for early-onset GBS is $0.20$ [31], so the probability of infant infection given intrapartum maternal colonization and intrapartum antibiotic prophylaxis (IAP) equals $1.47\% \cdot (1 - 0.20) = 1.18\%$. The probability of infant infection given no intrapartum maternal colonization is $0.0017\%$ [30]. The probability of infant infection given maternal risk factors is $1.4\%$ [30], and the relative risk of IAP for preterm premature rupture of membranes in early-onset GBS is $0.64$ [31], so the probability of infant infection given maternal risk factors and IAP equals $1.4\% \cdot (1 - 0.64) = 0.50\%$. The probability of infant infection given no maternal risk factors is $0.12\%$ [30]. The estimated number of newborns equals $100,000 \cdot (887/968) = 102,066$ (taking into account twin deliveries), so the estimated GBS infection rate equals $192/102,066 = 0.188\%$.

we observed that, when using the approach of culture screening at 35–37 weeks gestation, $14.77\%$ of women received IAP and $1.11\%$ of newborns were infected with GBS. The universal intrapartum screening using the molecular test would correspond to an absolute risk reduction for GBS infection of $0.925\%$, which means that $108$ more women would require (universal) intrapartum screening to prevent a single case of early-onset GBS disease that would be missed using the current strategy of culture screening at 35–37 weeks gestation. However, this projection of outcomes is subject to errors propagation, because the probability estimates come from different studies. Thus, the results must be analyzed cautiously, and a larger randomized, controlled study is needed to confirm these preliminary results. A mathematical modeling comparing the antenatal culture and the intrapartum PCR test strategies showed a potential benefit per birth [30]. Future trials should also include a cost-benefit analysis, in which direct costs, diagnostic accuracy, and outcome measures are translated into long-term health benefits and costs.

The real-time PCR assay presented here is an accurate test to identify intrapartum GBS carriers at point of care. This new tool, which is easy to use, could enhance the exact identification of candidates for IAP, including women with preterm rupture of membranes or preterm labor. The use of this test at the time
of delivery could result in a reduction of early-onset GBS disease in neonates, and our findings question the relevance of the current guidelines and their impact in terms of appropriate use of IAP.

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Potential conflicts of interest. All authors: no conflicts.

References