

## RAPID DETECTION OF GROUP B STREPTOCOCCI IN PREGNANT WOMEN AT DELIVERY

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## ABSTRACT

**Background** Group B streptococcal infections are an important cause of neonatal morbidity and mortality. A rapid method for the detection of this organism in pregnant women at the time of delivery is needed to allow early treatment of neonates.

**Methods** We studied the efficacy of two polymerase-chain-reaction (PCR) assays for routine screening of pregnant women for group B streptococci at the time of delivery. We obtained anal, vaginal, and combined vaginal and anal specimens from 112 pregnant women; in 57 women, specimens were obtained before and after the rupture of the amniotic membranes. The specimens were tested for group B streptococci by culture in a standard selective broth medium, with a conventional PCR assay, and with a new fluorogenic PCR assay.

**Results** Among the 112 women, the results of the culture of the combined vaginal and anal specimens were positive for group B streptococci in 33 women (29.5 percent). The two PCR assays detected group B streptococcal colonization in specimens from 32 of these 33 women: the one negative PCR result was in a sample obtained after the rupture of membranes. As compared with the culture results, the sensitivity of both PCR assays was 97.0 percent and the negative predictive value was 98.8 percent. Both the specificity and the positive predictive value of the two PCR assays were 100 percent. The length of time required to obtain results was 30 to 45 minutes for the new PCR assay, 100 minutes for the conventional PCR assay, and at least 36 hours for culture.

**Conclusions** Colonization with group B streptococci can be identified rapidly and reliably by a PCR assay in pregnant women in labor both before and after the rupture of membranes. (N Engl J Med 2000;343:175-9.)

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**G**ROUP B streptococcus, or *Streptococcus agalactiae*, is a leading cause of sepsis, meningitis, and death among newborn infants in Western countries. Early-onset infections (those appearing within seven days after birth) with this organism account for approximately 80 percent of group B streptococcal infections in infants and are usually acquired by contact with the genital tract of the mother during labor and delivery.<sup>1</sup> In 1998, the incidence of disease caused by group B streptococci was 0.6 per 1000 live births, and there were about 2000 group B streptococcal infections among infants in the United States, approximately 100 of which

were fatal.<sup>2</sup> Infants who have such infections may require prolonged hospitalization, and those who survive may have mental retardation or visual loss. Among pregnant women, the prevalence of colonization with group B streptococci ranges from 15 to 40 percent.<sup>1</sup> Women who are carriers are also at risk for severe infections.<sup>3</sup>

The Centers for Disease Control and Prevention (CDC),<sup>4</sup> the American College of Obstetricians and Gynecologists,<sup>5</sup> and the American Academy of Pediatrics<sup>6</sup> recommend the use of either risk assessment or screening for group B streptococcal colonization in pregnant women to identify candidates for intrapartum prophylaxis. Screening consists of obtaining vaginal and anal specimens for culture at 35 to 37 weeks' gestation. Risk assessment is performed at the onset of labor, and the presence of fever, a prolonged interval between rupture of membranes and delivery, or imminent preterm delivery is considered indicative of the need for prophylaxis.

The standard method for the diagnosis of group B streptococcal colonization consists of culturing combined vaginal and anal secretions in a selective broth medium that inhibits the growth of other microorganisms.<sup>7</sup> However, this method requires at least 36 hours, because the broth must be incubated for 18 to 24 hours and then subcultured on agar plates, and group B streptococci must be identified by agglutination tests. Moreover, the cultures are negative in some women whose infants subsequently have group B streptococcal infections.<sup>8</sup> On the other hand, the use of antibiotic prophylaxis on the basis of risk assessment leads to unnecessary treatment in many women.<sup>2-5</sup>

A rapid screening test for group B streptococcus that could accurately identify pregnant women who are carrying the bacteria at the time of delivery would obviate the need for prenatal screening<sup>4</sup> and reduce the use of antibiotic prophylaxis in women who are not colonized. Therefore, we prospectively evaluated the speed and accuracy of the standard culture meth-

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od and two DNA-based tests — a conventional polymerase-chain-reaction (PCR) assay and a rapid PCR assay — in identifying group B streptococcal colonization in pregnant women at the time of delivery.

## METHODS

### Study Design

The study was approved by the research ethics committee of the Centre Hospitalier Universitaire de Québec, and all the women gave written informed consent. We studied 112 pregnant women who had been hospitalized for delivery. Specimens of anal, vaginal, and combined vaginal and anal secretions were obtained from all the women soon after admission. In the case of 57 women whose membranes were intact at the time of admission, a second specimen was obtained after the membranes ruptured.

### Collection and Culture of Specimens

All specimens were collected with a commercially prepared collection and transport system for aerobes and anaerobes (Culturette, Becton Dickinson Microbiology Systems, Cockeysville, Md.). For anal specimens, a swab was carefully inserted approximately 2.5 cm beyond the anal sphincter and then gently rotated to touch anal crypts. For vaginal specimens, excessive secretions or discharge was wiped away, and secretions from the mucosa of the lower third of the vagina were obtained with a swab. For the combined vaginal and anal specimens, a swab was inserted first into the vagina and then into the anus as described above. Ampules containing 0.5 ml of Stuart's bacterial transport medium were crushed, and the swab was soaked with the medium immediately after the sample was obtained. All three types of specimens were transported at room temperature and were cultured and tested by PCR assays at the Infectious Diseases Research Center of Laval University within 24 hours after collection. The same specimen was used for the standard culture and the PCR assays.

For the identification of group B streptococci, the specimens were incubated in GNS, a standard selective broth medium that consists of Todd-Hewitt broth supplemented with gentamicin (8 µg per milliliter) and nalidixic acid (15 µg per milliliter).<sup>4</sup> A minimum of 36 hours of incubation was required for group B streptococci to be identified.

### Preparation of Samples for PCR Assays

For the PCR assays, 10 µl of the transport medium was prepared as a crude lysate with a DNA-extraction kit (Infectio Diagnostic, Sainte-Foy, Canada) according to the manufacturer's instructions. Purified group B streptococcal genomic DNA, used as a positive control, was prepared with a commercial kit (G Nome, Bio101, Vista, Calif.) according to the manufacturer's instructions.

### Primers and Probes

The PCR reaction mixture contained primers specific for group B streptococci, Sag 059 and Sag 190, to amplify both the genomic DNA and the internal-control template.<sup>9</sup> Two pairs of fluorescently labeled adjacent hybridization probes, STB-F and STB-C and IC-F and IC-C, were also used for the rapid PCR assay to detect amplicons specific for group B streptococci and internal-control amplicons, respectively.<sup>9</sup>

### Conventional PCR Assay

For the conventional PCR assay, 2 µl of the crude lysate was added to the PCR reaction mixture. The internal control added to each PCR reaction allowed us to assess the efficiency of the amplification reaction and to ensure that PCR inhibition was absent. In addition, purified group B streptococcal genomic DNA was used as a positive control. Multiple blanks were also included as negative controls to verify that there was no cross-contamination between samples. For the amplification, the reaction mixtures under-

went denaturation at 94°C for 3 minutes, followed by 40 cycles of 1 second at 95°C and 30 seconds at 55°C for the hybridization and elongation step, with a final period of extension at 72°C for 2 minutes (model PTC-200 thermocycler, MJ Research, Watertown, Mass.). Subsequently, 10 µl of the amplified reaction mixture was analyzed by electrophoresis on a 2 percent agarose gel. The entire process required a minimum of 100 minutes.

### New PCR Assay

The group B streptococci-specific real-time PCR assay was developed with a new, rapid DNA-amplification apparatus (LC 32 LightCycler, Idaho Technology, Idaho Falls, Idaho) that combines air thermal cycling and a fluorescence-based system of detection.<sup>10</sup> For this purpose, two pairs of adjacent hybridization probes labeled with a fluorescent reporter molecule that hybridizes to the group B streptococci-specific amplicon or to the internal-control amplicon were used.<sup>9</sup> These adjacent probes, which are separated by one nucleotide, generate increased fluorescence during hybridization to their target sequences as a result of the transfer of fluorescence resonance energy.

For amplification, 1 µl of the crude lysate was added to 9 µl of the amplification reaction mixture. The reaction mixtures were subjected to a pre-denaturation step at 94°C for 3 minutes, followed by 45 cycles of 1 second at 94°C, 15 seconds at 56°C, and 3 seconds at 72°C, with a rate of change in the temperature of 20°C per second. Fluorescence was measured at each cycle in each capillary by the built-in fluorometer. Internal and positive controls were used.

Strict precautions were taken to prevent cross-contamination of amplified DNA.<sup>11</sup> Procedures performed before PCR manipulations and those performed afterward were conducted in separate rooms. The use of the LightCycler obviates the need for post-amplification analysis, thus minimizing the risk of cross-contamination.

Both PCR methods were specific and sensitive enough to detect a single genomic copy of group B streptococcus.<sup>9</sup> The internal control was always amplified when *S. agalactiae* DNA was absent, thereby showing that negative PCR results for group B streptococci were not attributable to the presence of inhibitors in the clinical samples. Examples of the results of amplification with both PCR tests are shown in Figure 1.

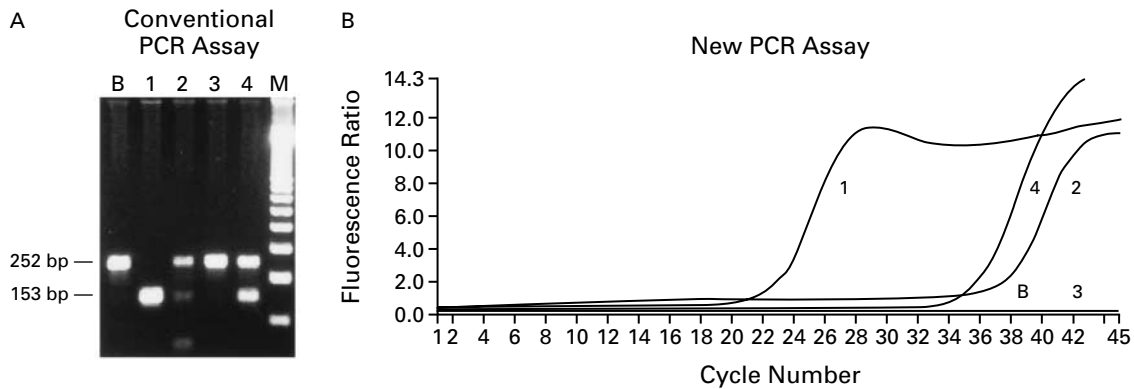
With the new PCR assay, as the bacterial load increases, the time needed to detect the microorganism diminishes. Although the preparation of samples, including bacterial lysis and extraction, required 10 minutes, the time required for amplification and detection ranged from 20 to 35 minutes, depending on the bacterial load. Therefore, the entire procedure required 30 to 45 minutes.

### Statistical Analysis

The rates of colonization calculated on the basis of the results of culture and PCR assays were estimated for each type of specimen. In the case of the women from whom two specimens were obtained (one before and one after the rupture of membranes), the results of the samples collected after the rupture of membranes were used to calculate the colonization rates. The sensitivity, specificity, and positive and negative predictive values of both PCR assays were estimated by comparing the results of these tests with the culture results. The consistency of results between each pair of samples collected before and after rupture of membranes was examined to evaluate the effect of the rupture of amniotic membranes on the rate of detection of group B streptococci. The 95 percent confidence intervals for sensitivity, specificity, and positive and negative predictive values were calculated according to the method of Blyth and Still,<sup>12</sup> since this method yields more accurate confidence limits than other methods when the proportion is close to 0 or 1.

## RESULTS

Among the 112 pregnant women, 33 (29.5 percent) were identified as carriers of group B strepto-



**Figure 1.** Results of the Conventional PCR Assay (Panel A) and the New PCR Assay (Panel B) for the Detection of Group B Streptococci in Combined Vaginal and Anal Specimens from Pregnant Women.

In the conventional PCR assay, the product of group B streptococci-specific amplification is 153 bp, whereas the 252-bp product represents the internal-control amplicon (Panel A). In the new PCR assay, the extent of group B streptococci-specific amplification is measured in terms of the increase in fluorescence during the amplification process (Panel B). In each panel, sample 1 was obtained from a heavily colonized woman; sample 2 was obtained from a lightly colonized woman; sample 3 was obtained from a woman with no colonization; sample 4 was a positive control, to which 10 fg of purified group B streptococcal genomic DNA had been added; and sample B was a negative control, to which no target DNA had been added. In Panel A, lane M shows a 100-bp molecular-size standard.

cocci on the basis of the results of culture of combined vaginal and anal specimens, as compared with 20 (17.9 percent) on the basis of culture of vaginal specimens and 30 (26.8 percent) on the basis of culture of anal specimens (Table 1). The results of both PCR assays of the combined specimens were also positive for 32 of the 33 women identified as carriers on the basis of the analysis of culture results. In the one woman with discordant results, the results of culture and the two PCR assays were negative in the specimen obtained before the rupture of the membranes, but the culture results were positive in the specimen obtained after the rupture of the membranes, whereas the PCR-assay results remained negative. Among the 57 women (of whom 16 had a positive culture) from whom combined vaginal and anal specimens were obtained before and after the rupture of amniotic membranes, this was the only discordant result.

Before the rupture of membranes in these 57 women, the sensitivity, specificity, and negative and positive predictive values of both PCR assays as compared with the culture were all 100 percent (Table 2). After rupture, the sensitivity of the PCR assays was 93.8 percent and the negative predictive value was 97.6 percent; the specificity and positive predictive value were both 100 percent (Table 2). In the four women, two with intact membranes and two with ruptured membranes, who had received antibiotics at least 90 minutes before the combined vaginal and anal specimens were obtained, the PCR assays and culture results were negative in three women and positive in one.

Overall, group B streptococci were detected slight-

**TABLE 1.** RATES OF DETECTION OF COLONIZATION WITH GROUP B STREPTOCOCCI ACCORDING TO THE METHODS USED AND THE SOURCES OF SPECIMENS AMONG 112 PREGNANT WOMEN.\*

| METHOD OF DETECTION    | ANAL SPECIMEN | VAGINAL SPECIMEN | COMBINED VAGINAL AND ANAL SPECIMEN |
|------------------------|---------------|------------------|------------------------------------|
|                        |               |                  | number positive (percent)          |
| Culture                | 30 (26.8)     | 20 (17.9)        | 33 (29.5)                          |
| Conventional PCR assay | 32 (28.6)     | 21 (18.8)        | 32 (28.6)                          |
| New PCR assay          | 31 (27.7)     | 21 (18.8)        | 32 (28.6)                          |

\*For 57 of the 112 women, two clinical specimens were obtained from each site (one before and one after the rupture of membranes); the results for the specimens collected after the rupture of membranes were used to calculate the rates of colonization.

ly more often by the PCR assays than by culture (Table 1). The sensitivity of both PCR assays was 97.0 percent and the negative predictive value of both was 98.8 percent, as compared with the culture results (Table 3). The specificity and positive predictive value of the PCR assays were both 100 percent (Table 3). The amount of time required to obtain results was 30 to 45 minutes for the new PCR assay, 100 minutes for the conventional PCR assay, and at least 36 hours for culture.

**DISCUSSION**

To prevent group B streptococcal disease in neonates, the current recommendation is to screen preg-

**TABLE 2.** INFLUENCE OF RUPTURE OF THE MEMBRANES ON THE SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE OF PCR ASSAYS FOR GROUP B STREPTOCOCCI IN COMBINED VAGINAL AND ANAL SPECIMENS FROM 57 PREGNANT WOMEN.

| STATUS OF THE MEMBRANES | SENSITIVITY |   | SPECIFICITY |   | POSITIVE PREDICTIVE VALUE |   | NEGATIVE PREDICTIVE VALUE |   |
|-------------------------|-------------|---|-------------|---|---------------------------|---|---------------------------|---|
|                         | PERCENT     | NO. OF BOTH CULTURE- AND PCR-POSITIVE RESULTS/NO. OF CULTURE-POSITIVE RESULTS | PERCENT     | NO. OF BOTH CULTURE- AND PCR-NEGATIVE RESULTS/NO. OF CULTURE-NEGATIVE RESULTS | PERCENT                   | NO. OF BOTH CULTURE- AND PCR-POSITIVE RESULTS/NO. OF PCR-POSITIVE RESULTS | PERCENT                   | NO. OF BOTH CULTURE- AND PCR-NEGATIVE RESULTS/NO. OF PCR-NEGATIVE RESULTS |
|                         |             | RESULTS   |             | RESULTS   |                           | RESULTS   |                           | RESULTS   |
| Before rupture          | 100         | 15/15   | 100         | 42/42   | 100                       | 15/15   | 100                       | 42/42   |
| After rupture           | 93.8        | 15/16   | 100         | 41/41   | 100                       | 15/15   | 97.6                      | 41/42   |

**TABLE 3.** SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE OF THE PCR ASSAYS FOR THE DETECTION OF GROUP B STREPTOCOCCI IN COMBINED VAGINAL AND ANAL SPECIMENS FROM 112 PREGNANT WOMEN.\*

| TYPE OF PCR ASSAY | SENSITIVITY      |   | SPECIFICITY      |   | POSITIVE PREDICTIVE VALUE |   | NEGATIVE PREDICTIVE VALUE |   |
|-------------------|------------------|---|------------------|---|---------------------------|---|---------------------------|---|
|                   | PERCENT (95% CI) | NO. OF BOTH CULTURE- AND PCR-POSITIVE RESULTS/NO. OF CULTURE-POSITIVE RESULTS | PERCENT (95% CI) | NO. OF BOTH CULTURE- AND PCR-NEGATIVE RESULTS/NO. OF CULTURE-NEGATIVE RESULTS | PERCENT (95% CI)          | NO. OF BOTH CULTURE- AND PCR-POSITIVE RESULTS/NO. OF PCR-POSITIVE RESULTS | PERCENT (95% CI)          | NO. OF BOTH CULTURE- AND PCR-NEGATIVE RESULTS/NO. OF PCR-NEGATIVE RESULTS |
|                   |                  | RESULTS   |                  | RESULTS   |                           | RESULTS   |                           | RESULTS   |
| Conventional      | 97.0 (82.5–99.8) | 32/33   | 100 (94.2–100)   | 79/79   | 100 (86.9–100)            | 32/32   | 98.8 (92.3–99.9)          | 79/80   |
| New               | 97.0 (82.5–99.8) | 32/33   | 100 (94.2–100)   | 79/79   | 100 (86.9–100)            | 32/32   | 98.8 (92.3–99.9)          | 79/80   |

\*For 57 of the 112 women, two clinical specimens were obtained (one before and one after the rupture of membranes); the results for the specimens collected after the rupture of membranes were used in this analysis. CI denotes confidence interval.

nant women by culture of combined vaginal and anal secretions at 35 to 37 weeks' gestation and to treat those with positive cultures or to treat women with risk factors for disease transmission empirically. We have developed two PCR assays for the detection of group B streptococci that have been shown to be specific and sensitive in tests with purified DNA.<sup>9</sup> One of these assays can identify group B streptococci in women in labor within 45 minutes. Whether we used cultures or PCR assays, the combined vaginal and anal specimen was the sample of choice for group B streptococcal screening, in accordance with the CDC recommendations.<sup>4</sup> The prevalence of colonization in our study is in keeping with that reported in the literature.<sup>1</sup> Overall, group B streptococcal colonization was detected more often by PCR assay than by culture.

The fact that rupture of membranes did not significantly influence the ability of the PCR assays to

identify carriers of group B streptococci is important, since the timing of the rupture of the amniotic membranes is unpredictable. Among the combined vaginal and anal specimens that we tested, the results were discordant in only one woman. Although PCR assays are extremely sensitive,<sup>9</sup> the bacterial load in that sample may have been very low or the sample could have been contaminated during processing for culture. Antibiotics could theoretically have decreased the specificity of the PCR assays, but at least in the four women who received antibiotics before specimens were obtained, the correlation between the results of the PCR assays and the results of culture was good.

Although the rate of carriage of group B streptococci among pregnant women does not change with the trimester of pregnancy, the duration of colonization varies, and therefore screening women at a certain point in pregnancy does not necessarily identify those who are carrying the organism at the time of

delivery.<sup>13-15</sup> Moreover, pregnant women who are not receiving adequate prenatal care are less likely to be screened for group B streptococci before delivery and thus have an increased probability of unknowingly transmitting the infection to their babies.<sup>4</sup> Approximately 18 percent of all pregnant women who are considered at risk according to a risk-based approach are empirically treated with antibiotics.<sup>16</sup> Of these, as few as 20 percent are actually carriers of group B streptococci.<sup>16</sup> Finally, implementation of screening-based antibiotic prophylaxis is not possible for women whose prenatal records are not available at the time of delivery.

To overcome these limitations, the CDC has called for the development of a test for the detection of group B streptococci in pregnant women that is sensitive, provides rapid results, and is easy to use.<sup>4</sup> The new PCR assay that we have developed appears to meet these requirements. It is sensitive (sensitivity, 97.0 percent) and can provide results within 45 minutes. Since, in normal circumstances, labor and delivery take 2 to 18 hours,<sup>17</sup> this PCR assay should allow the detection of group B streptococci quickly enough for intrapartum antibiotic prophylaxis to be given. For instance, in a sample of 2110 consecutive nulliparous and multiparous pregnant women who were admitted for delivery in our hospital, only 15 percent delivered their infants within four hours after admission. Even in the case of women whose infants were born before results of the PCR assay became available, the results could be used to identify infants at risk for group B streptococcal diseases. Therefore, intrapartum screening would eliminate the need for empirical antibiotic prophylaxis and reserve treatment for women and babies who actually need it. Since endometritis and chorioamnionitis can occur in pregnant women<sup>3</sup> who are carriers of group B streptococci, the use of this PCR assay could also help prevent these infections.

In conclusion, we found that group B streptococci can be detected rapidly and reliably by a PCR assay of combined vaginal and anal secretions from pregnant women at the time of delivery. The use of this test at the time of delivery should facilitate treatment and may eventually result in a reduction in morbidity and mortality due to group B streptococcal infections in both mothers and their infants.<sup>18</sup>

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