# PRENATAL GROUP B *STREPTOCOCCUS* TEST USING REAL-TIME POLYMERASE CHAIN REACTION

Chi-Feng Wei, Bo-Ching She, Hung-Shuo Liang, Qing-Dong Ling[1], Chen-Yuan Tsai[2], Chih-Wei Yen[3], Hsueh-Yin Wu[2], Ming-Song Tsai[4]*

*Department of Obstetrics and Gynecology, Cathay General Hospital, Si-Jhih, [1]Cathay Medical Research Institute, Taipei, Departments of [2]Laboratory Medicine and [3]Pediatrics, Cathay General Hospital, Si-Jhih, and [4]Obstetrics and Gynecology, Cathay General Hospital, Taipei, Taiwan.*

**SUMMARY**

**Objective:** The aim of this study was to evaluate the percentage of pregnant women with negative Group B *Streptococcus* (GBS) screening results by culture at 35 weeks' gestation, who subsequently had positive GBS test results after 39 weeks' gestation.

**Materials and Methods:** From 2006 to 2007, we recruited 150 pregnant women who received routine GBS culture screening at 35 weeks' gestation with negative results, and who had repeat cultures and real-time polymerase chain reaction (RT-PCR) tests for GBS after 39 weeks' gestation.

**Results:** Two percent of pregnant women with GBS-negative results by culture screening at 35 weeks' gestation were GBS-positive at 39 weeks' gestation.

**Conclusion:** It is necessary to perform a GBS test 4 weeks after an initial negative GBS culture at 35–37 weeks of gestation. RT-PCR provides a simple and rapid alternative method for detecting rectovaginal GBS colonization at the time of labor. [*Taiwan J Obstet Gynecol* 2009;48(2):116–119]

**Key Words:** group B streptococci, maternal screening, pregnant women, real-time polymerase chain reaction

## Introduction

In 1996, the Center for Disease Control and Prevention (CDC), the American Academy of Pediatrics, and the American College of Obstetricians and Gynecologists recommended intrapartum antimicrobial prophylaxis for women with late antenatal group B *Streptococcus* (GBS) rectovaginal colonization or, alternatively, with maternal risk factors for transmitting the infection [1–3]. The CDC Active Bacterial Core Surveillance system reported that the incidence of early-onset GBS disease (0–6 days of life) declined by 65% as result of implementation of these recommendations. Adoption of a universal screening strategy for women at 35–37 weeks' gestation in 2002 resulted in a further reduction of 31% in 2004. For preterm infants, the case-fatality rates were 23% and 9% for early-onset and late-onset cases, respectively, compared with 4% and 0%, respectively, for term infants. At Parkland Memorial Hospital, Dallas, Texas (USA), the incidence of early-onset GBS disease in 2004 was 0.4 cases per 1,000 live births, down from 2.85 cases before the introduction of screening and prophylaxis [4].

GBS is a leading cause of illness and death among newborns in the United States. Approximately 10–30% of pregnant women are found to be colonized with GBS when tested using the culture method [1,5]. This percentage is consistent with other results obtained using similar molecular methods [6,7]. GBS can be transmitted to the newborn if antibiotics are not administered 2–4 hours prior to delivery [8]. Infants infected with GBS may suffer mental retardation and hearing or vision loss.

The 2002 CDC guidelines call for prenatal GBS screening of all pregnant women at 35–37 weeks' gestation [9]. The current standard methods for detection of GBS consist of selective broth cultures of combined vaginal and anal specimens. Although these methods

*Correspondence to: Dr Ming-Song Tsai, Department of Obstetrics and Gynecology, Cathay General Hospital, 280, Jen-Ai Road, Taipei 106, Taiwan.
E-mail: mstsai@cgh.org.tw
Accepted: November 6, 2008

are sensitive and specific, they are time-consuming, requiring 48–72 hours, and also depend on the presence of viable microorganisms. However, many women deliver preterm, lack prenatal care, or become colonized with GBS between 35 weeks' gestation and delivery. For these women, culture-based results will not be available in time, resulting in a risk of infection or unnecessary treatment with antibiotics. Hence, an alternative, rapid, sensitive and specific method for detection of GBS from clinical specimens in pregnant women is needed to allow timely treatment of neonates and to reduce unnecessary worry about GBS infection between 35 weeks' gestation and delivery. We evaluated pregnant women who had negative results using a culture-based method at 35 weeks' gestation, and had cultures and real-time polymerase chain reaction (RT-PCR) tests after 39 weeks' gestation.

## Materials and Methods

This prospective, observational, pilot study was conducted at Cathay General Hospital with institutional board approval.

During prenatal visits to the obstetrics clinic of the Si-Jhih branch of Cathay General Hospital, pregnant women were informed about the study and asked about their willingness to participate. From 2006 to 2007, we recruited 150 pregnant women who had negative results on culture-based GBS screening at 35 weeks' gestation. They received another rectovaginal culture and RT-PCR GBS test at 39 weeks' gestation.

Two swabs were used and both were first inserted in the vaginal mucosa and then the anal sphincter. One swab was used for culture and one for polymerase chain reaction (PCR). The same swab was inserted approximately 2.5 cm beyond the anal sphincter and gently rotated to sample the anal crypts. All rectovaginal swabs were cultured in 5 mL of LIM broth (Todd-Hewitt broth containing 10 μg of colistin per milliliter and 15 μg of nalidixic acid per milliliter) and incubated overnight at room temperature. Blood agar plates were then inoculated with 50 μL of broth. The plates were inoculated at 37°C in 5% carbon dioxide (v/v) for 24–48 hours. Species identification of β-hemolytic colonies was performed by agglutination with a streptococcal grouping kit (Prolex Streptococcal Grouping Latex Kit; Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada). The procedures were performed according to the manufacturer's instructions.

For PCR, the second swab was placed in a sample preparation buffer tube and cut. This was allowed to stand for 5 minutes and then vortexed at high speed for 15 seconds. A total of 50 μL of solution was added to the lysis tube, vortexed at high speed for 5 minutes, centrifuged for 2–5 seconds, and heated at 95°C for 2 minutes. The lysis tube was placed on ice or a cooling block for DNA extraction. A total of 25 μL of diluents, which were provided in the IDI-Strep B kit (Becton Dickinson & Co., Franklin Lakes, NJ, USA), were added, and 1.5 μL of the lysate solution was transferred to the master mix tube, centrifuged for 5–10 seconds, and vortexed for 2–5 seconds. The tube was placed on a SmartCycler cooling block (Cepheid, Sunnyvale, CA, USA) at 2–8°C until ready to load and reconstituted with one master mix for each specimen. One positive control and one negative control were reconstituted by adding 25 μL of diluents to each tube, and the positive control/negative control tubes were centrifuged for 5–10 seconds, vortexed for 2–5 seconds and placed on a SmartCycler cooling block at 2–8°C until ready to load. Each reaction tube was inserted in a SmartCycler I-Core module and the results were available in less than 45 minutes. A Cepheid SmartCycler DNA detection system was used for RT-PCR analysis. We use the IDI-Strep B test kit with a sensitivity of 93.9%, specificity of 95.8%, positive predictive value (PPV) of 83.8%, and negative predictive value (NPV) of 98.5%, with FDA approval [10].

The IDI-Strep B test kit genetic target for GBS identification is the *cfb* gene. This gene encodes the CAMP factor, which is present in virtually all GBS isolates. Detection of the CAMP factor is used for presumptive identification of GBS by biochemical methods. The *cfb* gene is well conserved within this species. Primers amplify a conserved region of 154-bp fragment of the *cfb* gene. The amplified DNA targets are detected using the molecular beacons FAM at the 5′ end and quencher DABCYL at the 3′ end.

Results of the culture and RT-PCR methods were compared for each specimen. For discrepant results, RT-PCR was repeated twice to confirm the initial findings. The sensitivity, specificity, and PPV/NPV were calculated for the RT-PCR assay.

## Results

A total of 150 pregnant women with negative GBS findings at 35 weeks' gestation using the culture-based method gave informed consent for the study and were enrolled. They received repeat rectovaginal GBS culture and RT-PCR tests after 39 weeks' gestation.

The median maternal age was 26 years, median gestational age was 39.3 weeks, median gravida 3, and median parity 1 (Table 1).

**Table 1.** Patient demographics

| Characteristic | Value |
|---|---|
| Maternal age, median (range) (yr) | 26 (18–44) |
| Gestational age, median (range) (wk) | 39.3 (39–40) |
| Gravidity, median (range) | 3 (1–5) |
| Parity, median (range) | 1 (0–3) |

**Table 2.** Sensitivity, specificity, and positive and negative predictive values of the culture method compared with real-time polymerase chain reaction (RT-PCR) for detection of group B *Streptococcus* (GBS)

| GBS detection methods | Culture method | |
|---|---|---|
| | Positive | Negative |
| RT-PCR method | | |
| Positive | 2 | 1 |
| Negative | 0 | 147 |
| Sensitivity | 2/2 (100%) | |
| Specificity | 147/148 (99.3%) | |
| Positive predictive value | 2/3 (66.7%) | |
| Negative predictive value | 147/147 (100%) | |

Three of the 150 women were found to be positive for GBS after 39 weeks. Two of these were positive using both the culture and RT-PCR methods, while the other had a negative culture result, but a positive RT-PCR result. The RT-PCR result was repeated twice for confirmation.

Table 2 shows the comparison between the culture method and RT-PCR. The sensitivity of the RT-PCR assay was 100% and specificity was 99.3%. The PPV was 66.7%, and the NPV was 100%.

## Discussion

We demonstrated that 2% of pregnant women with negative results on GBS culture screening at 35 weeks' gestation had positive results 4 weeks later, demonstrated by culture or RT-PCR.

According to CDC guidelines [9], pregnant women with preterm labor need a GBS culture every 4 weeks until delivery, and term pregnant women with negative GBS cultures who are within 4 weeks of delivery do not require intrapartum antibiotic prophylaxis. However, these pregnant women are at risk of newly colonized GBS, meaning that some neonates will still be at risk of GBS infection if their mothers have only one negative GBS culture screening at 35–37 weeks gestation.

The total hands-on time for the two tests is similar, but it takes longer to obtain the results of culture-based screening (48–72 hours) than of RT-PCR (2 hours). In emergencies such as preterm delivery, rapid results are needed to lower infant morbidity and mortality, and RT-PCR provides a simple, rapid result with sufficient sensitivity for detecting GBS colonization in pregnant women.

Our study produced one discrepant result, with a negative culture result and a positive RT-PCR result. This could have occurred because RT-PCR detects only bacterial genes, not viable bacteria colonies. The inability of a culture to detect small bacterial colonies or the presence of antagonistic microorganisms can also cause discrepant results. Specimens can sometimes be culture-positive and RT-PCR-negative, if rare mutant GBS colonies are present.

A bacterial culture is always required, especially when an antibiotic is needed. Penicillin is the drug of choice for prophylaxis and treatment of GBS infection, and no resistance to this agent has so far been reported among GBS isolates. One disadvantage of RT-PCR is that it cannot indicate antibiotic susceptibility [11,12]. However, even with penicillin-resistant or rare GBS colonies, PCR can be used to confirm the DNA sequence to identify the organism and so aid with the choice of appropriate antibiotics.

According to our results, 2% of pregnant women with negative GBS cultures at 35 weeks' gestation had positive results based on either culture or RT-PCR findings 4 weeks later. We suggest that GBS testing should be repeated 4 weeks after an initial negative screening result at 35–37 weeks' gestation. RT-PCR can be considered suitable for use at the time of labor, because it can provide simple, rapid results and is sufficiently sensitive to detect GBS.

## References

1. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR Recomm Rep* 1996;45:1–24.
2. American College of Obstetrics and Gynecologists Committee Opinion. Prevention of early-onset group B streptococcal disease in newborns. Number 173, June 1996. Committee on Obstetric Practice, American College of Obstetrics and Gynecologists. *Int J Gynaecol Obstet* 1996; 54:197–205.
3. American Academy of Pediatrics. Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. *Pediatrics* 1997;99:489–96.

4. Wendel GD Jr, Leveno KJ, Sánchez PJ, Jackson GL, McIntire DD, Siegel JD. Prevention of neonatal group B streptococcal disease: a combined intrapartum and neonatal protocol. *Am J Obstet Gynecol* 2002;186:618–26.
5. Schrag SJ, Zell ER, Lynfield R, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *N Engl J Med* 2002;347: 233–9.
6. Bergeron MG, Ke D, Menard C, et al. Rapid detection of group B streptococci in pregnant women at delivery. *N Engl J Med* 2000;343:175–9.
7. Overman SB, Eley DD, Jacobs BE, Ribes JA. Evaluation of methods to increase the sensitivity and timeliness of detection of *Streptococcus agalactiae* in pregnant women. *J Clin Microbiol* 2002;40:4329–31.
8. de Cueto M, Sanchez MJ, Sampedro A, Miranda JA, Herruzo AJ, Rosa-Fraile M. Timing of intrapartum ampicillin and prevention of vertical transmission of group B *streptococcus*. *Obstet Gynecol* 1998;9:112–4.
9. Centers for Disease Control and Prevention (CDC). Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. *MMWR Recomm Rep* 2002;51:1–22.
10. Davies HD, Miller MA, Faro S, Gregson D, Kehl SC, Jordan JA. Multicenter study of a rapid molecular-based assay for the diagnosis of group B *Streptococcus* colonization in pregnant women. *Clin Infect Dis* 2004;39:1129–35.
11. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000;342:15–20.
12. de Azavedo JC, McGavin M, Duncan C, Low DE, McGeer A. Prevalence and mechanisms of macrolide resistance in invasive and noninvasive group B streptococcus isolates from Ontario, Canada. *Antimicrob Agents Chemother* 2001;45: 3504–8.