Modified use of real-time PCR detection of group B Streptococcus in pregnancy

Ali A. Rabaan, 1,* Justin V. Saunar, 2 Ali M. Bazzi 2 and Joan L. Soriano 2

Abstract

The CDC recommends antenatal screening of vaginal/rectal samples for *Streptococcus agalactiae* at 35–37 weeks’ gestation, with intra-partum antibiotic prophylaxis for positive cases. We tested a modified use of the Cepheid Xpert GBS real-time PCR kit on enrichment cultures from 554 vaginal/rectal swabs compared to the current subculturing gold standard method. Swabs were inoculated on polymyxin nalidixic acid agar plates, and Todd–Hewitt enrichment broth cultures were examined daily for growth. Todd–Hewitt broth culture was also used for Xpert GBS. There was 92.06 % agreement between the subculture and PCR methods. Sensitivity of Xpert GBS was 100 %, specificity was 89.40 %, positive predictive value was 75.96 % and negative predictive value was 100 %. Colonization incidence was higher with younger (≤24 years) or older (≥35 years) maternal age. Modified use of the Cepheid Xpert GBS would assist rapid diagnosis of *S. agalactiae* colonization and facilitate timely and appropriate assignment to intra-partum antibiotic prophylaxis.

*Streptococcus agalactiae* (Lancefield Group B Streptococcus; GBS) is the most common cause of neonatal morbidity and mortality, affecting between 0.5 and 3 live births per 1000 [1–5]. Approximately 30 % of pregnant women have vaginal and/or rectal GBS colonization, which can increase the risk of stillbirth or premature delivery [1–4]. In infants, colonization with GBS via vertical transmission from the mother can cause early-onset disease (EOGBS) if it presents within the first seven days after birth. Between 1 and 3 % of babies born to GBS-colonized mothers develop severe EOGBS, with a fatality rate of approximately 12 % [1–5]. Maternal GBS vaginal colonization during labour is a prerequisite for EOGBS. Other risk factors include GBS bacteriuria during pregnancy, premature delivery, prolonged membrane rupture, previous birth of an infant with GBS disease, and intra-partum fever, black race, young maternal age and high body mass index [1, 3].

Intra-partum antibiotic prophylaxis (IAP) significantly reduces the incidence of GBS neonatal colonization and EOGBS [1, 2, 6]. In 2002, the Centers for Disease Control and Prevention (CDC) issued guidelines recommending GBS antenatal screening at 35–37 weeks of gestation, with IAP given to women who tested positive [7]. Revised guidelines were issued in 2010 [2]. Improved laboratory detection methods, management of women with known risk factors, updating of IAP regimens to deal with penicillin allergy and management of at-risk newborns were emphasized [2].

Culture-based detection of GBS involves the collection of combined vaginal and rectal specimens from the mother and enrichment culture in selective broth medium such as LIM or Todd–Hewitt (TH) broth, then subculturing onto blood agar and/or chromogenic agar [2]. However, results generation takes 24–72 h and a low positive predictive value has been noted in some studies [8]. As it is usually used on women at 35–37 weeks’ gestation according to CDC guidelines, those who have not been receiving pre-natal care or who deliver pre-term are missed, while colonization status often changes between testing and time of delivery [9, 10]. Consistent with the emphasis on improvement of laboratory detection methods in the 2010 CDC guidelines, real-time PCR-based screening methods have been developed. These have the advantage of delivering results within 1–2 h and have high specificity and sensitivity. One such method is based on use of the Cepheid Xpert GBS kit (Cepheid, Sunnyvale, CA) run on the GeneXpertR Dx System. Studies comparing the performance of this method to culture methods directly on intra-partum, antenatal or amniotic fluid samples showed that sensitivity ranged from 85 to 98.3 %,
and specificity from 87 to 99% [2, 11–18]. The test may be of particular potential benefit as a rapid, point-of-care test where GBS status is unknown, for example pre-term deliveries or for women who have not been receiving pre-natal care [11, 13, 18].

There is no nationally recommended screening procedure for GBS in pregnancy in Saudi Arabia. In our institution, the Johns Hopkins Aramco Healthcare Centre in the Eastern Province of Saudi Arabia, routine GBS screening of vaginal/rectal swabs from women at 35–37 weeks’ gestation using culture-based detection is carried out in a College of American Pathologists (CAP)-accredited laboratory. CAP standards state that if PCR is used for screening for GBS from direct swab samples, all negative results must be confirmed by culture analysis. In this study, we aimed to test the performance of a modified method in which enrichment-culturing of swab samples and real-time PCR detection were combined. Cepheid Xpert GBS real-time PCR was thus performed on a sample of enrichment TH broth culture, allowing CAP standards to be observed while reducing the turnaround time associated with subculturing.

A total of 554 women presenting for routine antenatal screening at 35–37 weeks’ gestation to OB/GYN clinics in the Eastern Province of Saudi Arabia were included: 43 samples were from Abqiq City (AB), 453 from Dhahran City (DH), two from Al-Hasa (AH), 54 from Ras Tanura City (RT) and two from Udhailiya City (UC). Ethical approval was obtained from the institutional review board (IRB). Vaginal swabs and concurrently collected rectal swabs were used. Samples were transported immediately in Amies non-nutritive transport medium (Thermo Scientific, Hampshire, UK) at ambient temperature to the Johns Hopkins Aramco Healthcare facility for testing.

For microbiological testing, according to CDC recommendations and established laboratory procedures, a small area of one quadrant of a Columbia polymyxin nalidixic acid agar (PNBA) plate was inoculated and streaked for colony isolation. Both swabs were then placed in a single tube of TH enrichment broth. All cultures were incubated at 35–37°C for 24 h in a 5% CO2 incubator. After 24 h, the PNBA plates were examined for the presence of beta-haemolytic streptococci and re-incubated for an additional 24 h if no growth occurred or too little occurred for examination. The plate was discarded if no growth occurred after 48 h. The catalase test was performed on suspect colonies to provide presumptive identification of any beta-haemolytic streptococci. GBS were identified by any one of the Phadebact rules or for women who have not been receiving pre-natal care [11, 13, 18].

If no GBS were isolated from Columbia PNBA plates, the TH enrichment broth culture was examined daily for evidence of growth. Positive cultures were subcultured onto 5% sheep blood agar plate (BAP) (Thermo Scientific, Hampshire, UK). Negative cultures were retained for five days and subcultured to BAP as soon as growth was apparent. If no growth occurred for five days, the culture was discarded. BAP subcultures were incubated at 35–37°C for 24 h in a 5% CO2 incubator, then presumptive presence of beta-haemolytic streptococci and identification of GBS was carried out as before.

In order to compare the performance of a modified use of the Cepheid Xpert GBS kit assay to subculturing, a Cepheid sample collection swab was immersed in the TH enrichment culture after 24 h and used directly for Cepheid Xpert GBS real-time PCR identification. The PCR target sequence is the GBS cfb gene. The kit was used according to the manufacturer’s instructions for bacterial cultures. Samples were tested on the GeneXpert Dx instrument according to the manufacturer’s instructions.

For the Cepheid Xpert GBS Assay multiplex real-time PCR test, a true positive was defined as positive for both PCR and subculture. A true-negative was defined as negative for both PCR and subculture. A false-positive was defined as positive by PCR and negative by subculture. A false-negative was defined as negative by PCR and positive by subculture. These definitions were used in calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the PCR test compared to the subculture standard method.

We calculated the concordance between the Cepheid Xpert GBS Assay multiplex real-time PCR (Cepheid) and identification from subcultures using the Kappa statistic, which can range from −1 to +1. Results were interpreted based on the guideline that negative Cohen’s kappa means no agreement between methods, between 0 and 0.20 is slight, 0.21–0.40 is fair, 0.41–0.60 is moderate, 0.61–0.80 is substantial, and 0.81–1 is almost perfect agreement [19, 20]. To compare the distribution of true positive and true negative results with respect to age group, nationality or location, Chi-squared analysis was used, with $P ≤ 0.05$ accepted as significant. Statistical analysis was performed using Social Sciences Statistics and GraphPad QuickCalcs online software.

There was 92.06% agreement between the methods, i.e. for the 554 samples the same result was obtained by both tests in 510 cases (Table 1); 58.45% would be expected by chance. The Kappa statistic was 0.81 (SE=0.03; 95% confidence interval (CI) 0.76 to 0.86), considered to indicate ‘almost perfect’ agreement.

<table>
<thead>
<tr>
<th>Subculture</th>
<th>GBS Assay</th>
<th>POS</th>
<th>NEG</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid Xpert</td>
<td>(n=139)</td>
<td>(n=415)</td>
<td>(n=554)</td>
<td></td>
</tr>
<tr>
<td>POS</td>
<td>139</td>
<td>44</td>
<td>183</td>
<td></td>
</tr>
<tr>
<td>NEG</td>
<td>0</td>
<td>371</td>
<td>371</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>139</td>
<td>415</td>
<td>554</td>
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If the current subculturing method was taken as the standard, the sensitivity (true positive rate) of GBS detection by the Cepheid Xpert GBS Assay was 100 % (Table 1) (100 % agreement). The specificity (true negative rate) for GBS detection was 89.40 % (Table 1). Positive predictive value (PPV) of the Cepheid Xpert GBS Assay compared to the subculture method was 75.96 %, while the negative predictive value (NPV) was 100 % (Table 1). The mean real-time PCR threshold cycle (Ct) did not differ significantly between samples that were positive by both methods (true positives) (mean ±SD: 23.04±4.71; n=149) and those which were positive for the Cepheid Xpert GBS Assay but negative for subculture (‘false’ positives) (24.76±5.04; n=44).

To determine whether colonization with GBS was more likely to occur in some age groups than in others, we compared the distribution of results that were positive or negative by the gold standard subculture method by Chi-squared analysis (Table 2). The Chi-square statistic was 10.44, P=0.005, confirming that there was a significant variation in GBS occurrence with age group, primarily due to GBS positive by both methods (true positives) (24.76±5.04; n=44).

Real-time PCR systems are recognized to be highly sensitive and specific for direct GBS detection from vaginal/rectal swab samples [11–18]. Our modification of the Cepheid Xpert system was aimed at satisfying CAP standards, which state that if PCR is used for screening for GBS directly from swab samples, negative results must be confirmed by culture analysis. We combined enrichment-culturing of swab samples and real-time PCR detection. The 100 % NPV for the Cepheid Xpert GBS PCR Assay suggested that negative results obtained by PCR from TH enrichment culture samples were reliable without recourse to confirmation by culture analysis. Furthermore, the turnaround time from receipt of sample was reduced from typically two to three days for the subculturing method to 24 h for the Xpert GBS real-time PCR method. The medico-economic impact is therefore that as results will be available within 24 h, physicians can start the appropriate treatment immediately rather than waiting for the culture. The reduced turnaround time also means that there would be a reduced workload in the microbiology laboratory, reducing staff costs. Similar to our results, in a study on 826 specimens from women undergoing prenatal screening (35–37 weeks’ gestation), the sensitivity and specificity of the Xpert GBS assay performed on LIM broth-enriched cultures were 99.0 and 92.4 %, respectively with respect to the gold standard culture [21]. This sensitivity was higher than for direct testing of swab specimens with the Xpert GBS assay, which gave sensitivity and specificity of 85.7 and 96.2 %, respectively [21]. Others found that both overnight carrot broth-enhanced PCR and LIM broth-enhanced PCR using the BD GeneOhm StrepB assay generated more true-positive results than visualization of pigment after one day of carrot broth culture [22]. This suggests that the use of well-controlled and validated PCR analysis on broth-enriched cultures could limit the need to carry out follow-up testing on samples that do not develop pigmentation in chromogenic broth [22]. Furthermore, use of BD GeneOhm StrepB PCR on carrot broth cultures incubated for less than six hours gave greater sensitivity for GBS detection than direct swab PCR [23]. Possible future studies could address the possibility of reducing the culture time in our modified method.

In the context of our laboratory, the GeneXpertR Dx System generally is available 24 h per day, seven days per week for a range of tests. Trained personnel are available who routinely run this system and have validated the GBS detection test, so no further set-up costs are required.

There is limited published information on the prevalence of EOGBS and GBS colonization in Saudi Arabia. In a study of 326 women in a Jeddah hospital, the prevalence of GBS vaginal and/or rectal colonization was increased from 17.3 % at 33.5 weeks’ gestation to 31.6 % during labour (mean 39.2 weeks’ gestation) [24]. Results of another study from Saudi Arabia on maternal and neonatal risk factors suggested that neonates with EOGBS were significantly more likely to have mothers with GBS bacteriuria, while a recent study using data from 3863 women showed that the prevalence of asymptomatic bacteriuria in Saudi Arabia is 2.1 % [25, 26]. Other risk factors for EOGBS were identified as lack of antenatal attendance, prolonged membrane rupture and use of antibiotics during labour [25]. In our study, the frequency of positive GBS results detected by the subculture gold standard was higher in women aged 24 years or younger and particularly in those aged greater than 35 years, compared to those aged between 25 and 34 years. Younger maternal age has been identified as a risk factor for GBS colonization in some studies, although in many other cases maternal age has not been identified as predictive of GBS colonization [1, 3, 27–29]. Our results suggest that, in the Eastern Province of Saudi Arabia, older maternal age may

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Total (n=554)</th>
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<tr>
<td></td>
<td>≤24 (n=143)</td>
</tr>
<tr>
<td>GBS POS</td>
<td>39 (27.27 %)</td>
</tr>
<tr>
<td>GBS NEG</td>
<td>104 (72.73 %)</td>
</tr>
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Table 2. Distribution of GBS status by age group

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be a risk factor for GBS colonization in women that should be followed up.

Use of real-time PCR is considered to be of particular potential benefit when women present at labour with unknown GBS status. Having a Cepheid Expert real-time PCR method available for use would allow more rapid delivery of results and the application of evidence-based management of the mother and newborn. Limitations of our study include the fact that although subculture methods are currently recommended as the gold standard for GBS testing, low positive predictive value has been noted in some studies [8]. In practice, the PCR method may be more likely to detect the presence of GBS than the subculture method, so apparent ‘false-positives’ in which PCR gave a positive result when subculture gave a negative result may have contained GBS, which was not followed up [11].

In summary, we have tested a modified use of the Cepheid Xpert GBS Assay for the detection of GBS by real-time PCR on TH-enrichment cultures from vaginal/rectal swabs of pregnant women at 35–37 weeks’ gestation, compared to subculturing. Results suggest that switching to a PCR-based screening system would provide for accurate, more rapid diagnosis of GBS during pregnancy. This would facilitate timely assignment of women to appropriate IAP, reduce the risk of unnecessary assignment of GBS-negative women to IAP and reduce the transmitted risk of EOSGBS in infants.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study was approved by the Johns Hopkins Aramco Healthcare Institutional Review Board.

References


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