

Group B *Streptococcus* detection: comparison of PCR assay and culture as a screening method for pregnant women

ABSTRACT

Streptococcus agalactiae or group B *Streptococcus* (GBS) is one of the most important causal agents of serious neonatal infections. Numerous assays have been evaluated for GBS screening in order to validate a fast and efficient method. The aim of this study was to compare the culture technique (established as the gold standard) with the molecular method of polymerase chain reaction (PCR) with specific primers (*atr* gene). Two hundred and sixty-three samples were analyzed. Vaginal samples were collected, according to the Centers for Disease Control and Prevention (CDC) recommendations, from women over 35 weeks of pregnancy at *Hospital de Clínicas de Porto Alegre* (HCPA). Two different extraction methods were tested in all samples collected. PCR technique yielded 71 (26.99%) positive results. Sensitivity and specificity for PCR were 100% and 86.88%, respectively. PCR demonstrated a shorter turnaround time than the culture. The molecular methodology proved to be a useful screening for GBS, allowing effective treatment to be initiated in shorter time to prevent newborn infection.

Keywords: *Streptococcus agalactiae*; polymerase chain reaction; culture; pregnancy.

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INTRODUCTION

Streptococcus agalactiae, or Lancefield group B *Streptococcus* (GBS), is one of the most important causal agent of serious infections and neonatal sepsis.¹⁻³ As many as 40% of all pregnant women present rectal and/or vaginal GBS colonization.⁴ The incidence of neonatal GBS infection is 0.5 per 100 live births. Vertical transmission from mother to the neonate by either pathway accounts for up to 75% of cases of neonatal GBS colonization, and 1% to 2% of these infants will develop early-onset GBS infection.^{5,6}

Since 2002, the Centers for Disease Control and Prevention (CDC) recommend GBS screening for pregnant women by culture-based method. This approach is preferable, resulting in more effective prevention than risk-based chemoprophylaxis (previous CDC recommendation).⁷⁻⁹ The standard method for the diagnosis of GBS colonization consists of culturing combined vaginal and anal swab in a selective broth medium that inhibits the growth of non-GBS microorganisms. However, this method requires at least 48h for fully

GBS identification. Moreover, negative culture results are observed in some women whose infants subsequently develop GBS infection.¹⁰

An ideal screening test for GBS colonization is which could accurately identify pregnant women who carry the bacteria (even low-count bacteria carriers) and presenting a short turnaround time. Many techniques have been tested in order to validate a fast and efficient method of GBS screening to replace the culture.¹¹⁻¹³ Nowadays, molecular biology based assays, such as PCR (polymerase chain reaction) tests, have become the focus of investigation of detection of GBS colonization in pregnant women.^{14,15} In these tests, samples preparation and amplification targets are decisive to performance assay. A good target for GBS amplification is the *atr* gene because it is well studied in this species. Moreover, the *atr* is an essential gene, which means that it has to be expressed/present in all cells of this species. The gene encodes a protein amino acid transporter *gs0538* which is extremely specific to *S. agalactiae* species. Because it is a housekeeping gene, probability of mutations in *atr* is comparatively rather low.^{16,17}

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The aim of study was compare this *atr* gene PCR with the gold standard (culture-based method) to evaluate the PCR performance as GBS colonization screening in pregnant women. Additionally, we tested two different extraction methods to amplify *atr* gene: a commercial kit and thermal lysis protocol, searching more cost-effective molecular tests.

MATERIALS AND METHODS

Samples

Combined rectal/vaginal specimens were collected to conduct this study according to the CDC recommendations.¹⁰ Samples collected were sent to the laboratory for microbiology and molecular tests for group B *Streptococcus* identification. We analyzed 263 samples of women at 35 or more weeks of pregnancy, in labor or not, attending the emergency obstetric room at *Hospital de Clínicas de Porto Alegre* (HCPA) from September 2007 to September 2008.

Microbiology tests

The swabs were inoculated into Todd Hewitt (Himedia Laboratories, India) selective medium supplemented with gentamicin (8 µg/mL) and nalidixic acid (15 µg/mL). The selective medium was incubated at 36°C in 5% CO₂ for 18h, and then subcultured onto blood agar plates (BioMérieux, Marcy l'Étoile, France), which were incubated at 36°C in 5% CO₂ for 24h. After incubation the plates were inspected for β-hemolytic colonies. When no β-hemolytic colonies were observed after 24h, plates were reincubated for another 24h and inspected again. The β-hemolytic colonies whose morphology was consistent with group B *Streptococcus* were subcultured in broth and submitted to the CAMP (Christie, Atkins, Munch, Petersen) test. The colonies positive for the CAMP test were presumptively considered GBS.

Polymerase chain reaction (PCR)

Sample preparation and DNA extraction

The swabs were incubated for 15 to 18h into Todd Hewitt selective medium. After centrifugation of broth, the precipitate was washed with 1X PBS solution and resuspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5). This solution was submitted to two different DNA extraction protocols: thermal lysis and thermal lysis followed by silica DNA extraction by commercial kit.

The thermal lysis was performed using TE solution for 10 min at 100°C to lyse bacterial cell wall.¹⁸ The second DNA extraction protocol was performed using the commercial kit QIAmp (Qiagen, Valencia, USA), according to manufacturers instructions. This additional step in DNA extraction was conducted for ensuring the PCR inhibitors elimination.

Polymerase chain reaction (PCR) gene *atr*

For the PCR reaction we used the *atr* primers 5'-CAA CGA TTC TCT CAG CTT TGT TAA-3' and 5'-TAA GAA ATC TCT TGT GCG GAT TTC-3', producing a 780-bp fragment that is specific for the gene *atr*.¹⁹ The amplified PCR product of one clinical isolate was sequenced and the *atr* sequence obtained was compared in GenBank using the Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information, Bethesda, MD (<http://www.ncbi.nlm.nih.gov/BLAST/>). This isolate sample was used as a positive control in all PCR reactions.

The volume of reaction was 25 µL and was prepared as follows: 67 mM Tris-HCl, 16 mM (NH₄)₂SO₄, 0.01% (w/w) Tween-20 (pH 8.8 at 25°C), 1.5 mM MgCl₂, 0.25 mM dNTP (ABgene®, Epsom, UK), 0.4 mM of each primer, 1.25 U of enzyme Super-Term DNA polymerase (JMR Holdings, London, UK) and 5 µL of each DNA sample. The amplification was performed with an initial denaturation at 94°C for 1 min followed by 30 cycles of 1 min at 94°C, 45 s at 55°C for primer annealing, 1 min at 72°C for elongation and a final period of extension at 72°C for 10 min.

The amplification products were detected by electrophoresis using 10 µL of the amplified reaction mixture in agarose gel 2%, containing 0.4 mg/mL ethidium bromide. Visualization was performed with ultraviolet light transilluminator. A 100-bp molecular weight marker (Invitrogen®, Calbad, USA) and a positive control of *S. agalactiae* were used to evaluate the PCR products. The samples presenting a 780-bp amplicon were considered positive for GBS.

Statistical analysis

Sensitivity, specificity, PPV and NPV were calculated for the PCR technique using culture as gold standard. The concordance between assays was determined using the Kappa coefficient. The statistical analysis was performed in SPSS® version 14.0.

RESULTS

In the molecular method, all samples were tested with both DNA extractions (thermal lysis only and thermal lysis + Qiagen extraction) before PCR reaction. We observed four discrepancies (4/263) between the two methods. PCR reactions with and without Qiagen extractions were compared, yielding a Kappa of 0.961, which indicates excellent agreement.²⁰ Thus, the PCR method was used irrespective of the process of DNA extraction used.

Altogether, the culture method turned out positive in 42 (15.96%) samples, while the PCR technique was

Table 1. Comparison of PCR and Todd Hewitt culture medium results

PCR	Culture		Total
	Positive	Negative	
Positive	42	29	71
Negative	0	192	192
Total	42	221	263

positive in 71 (26.99%) (Table 1). All culture-positive samples were also positive with the PCR technique, therefore resulting in 100% PCR sensitivity (95% CI: 91.62-100). Of the 221 culture-negative samples for GBS, 29 were positive with PCR and 192 were negative with both methods, which indicate a specificity of 86.8% (95% CI: 81.79-90.71) of the molecular method. The negative predictive value was 100% and positive predictive value was 59% (Figure 1). The agreement beyond chance (Kappa) between the techniques was 0.68, indicating substantial agreement.²⁰

DISCUSSION

Despite CDC recommendations to use culture as GBS screening in pregnant women, many routine clinical situations require a faster and more efficient method than culture.¹¹⁻¹³ Therefore, this study reports results for two different extraction protocols and the use of *atr* gene as amplification target by PCR and its potential use as GBS screening test. Thermal lysis is the cheapest extraction method and has good performance for this samples when compared to the Qiagen kit. This extraction protocol could present the best cost-effectiveness ratio when applied in clinical laboratory routine.

GBS colonization rates were 15.96% using the culture and 26.99% using PCR. In Brazil, the GBS colonization rate was estimated by Borger and co-workers to be between 15% to 25%. However, the prevalence of GBS colonization could vary widely with geographic location, age, parity and socio-economic status.²¹ Furthermore, a review conducted in 1998 on the prevalence of colonization by GBS in developing countries suggested that colonization rates may be underestimated in many studies due to inadequate culture techniques employed.²² Recent Brazilian studies using PCR to detect GBS have reported 21.6%

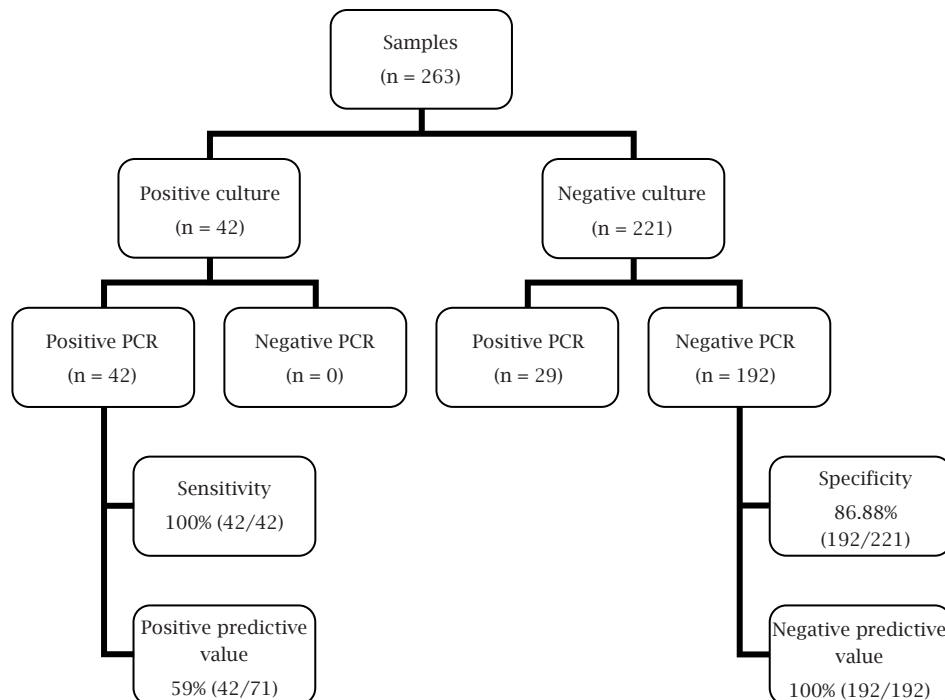


Figure 1: Diagram of sensitivity, specificity and predictive values of identifying the GBS presence in pregnant women by PCR assay.

and 20.4% as colonization rates.^{7,21} These rates are very similar to those observed in this study.

Great sensitivity, high negative predictive value and rapid results are desirable parameters of a screening test. In our study, the sensitivity of PCR was 100%, higher than the value observed in similar studies conducted by Gavino & Wang¹⁵ and Alfa²³, where the sensitivity was 95.8% and 90.5%, respectively. Probably, the high sensibility is due to use of selective and enriched broth previous to performing the PCR. The negative predictive value was 100%. This finding is important because it indicates that all samples with negative results are truly negative, which affords to safely withhold treatment from women presenting PCR negative samples. This is so important in clinical routine because false negative results in a screening test may lead to serious consequences for the patient, considering that this test is used to take a decision about antibiotic prophylaxis.

The specificity found was 86.88%, greater than the 64.5% found by Gavino & Wang.¹⁵ However, even being considered the gold standard, culture results can be false negative. It is known that culture may not be absolutely effective in the detection of GBS, since other bacteria of the vaginal/genital tract can inhibit the growth of GBS even when using the selective broth. So, the supposedly false positive results in PCR may actually indicate the presence of GBS in the studied material, since this is an analytical technique whose sensitivity could be greater than the bacteriological examination. The gold standard performance affects the positive predictive value parameter. Consequently, in our study the positive predictive value was 59%.

Moreover, the culture is a time-consuming method requiring at least 48h for fully GBS identification. The PCR proposed in this study, even using a previous incubation in selective broth, requires 24h to give the final result. Therefore, GBS PCR has a considerable shorter turnaround time than the culture.

In summary, since the publication of the Guidelines of GBS colonization by the CDC in 2002, the incidence of neonatal infections decreased by more than 60%. The use of new techniques with higher accuracy and faster results for detection of this microorganism could contribute even further to this improvement.² Accordingly, our study indicates that the PCR technique has proven to have high sensitivity, and that it should be a useful screening method. Such methodology can be a diagnostic tool for GBS, allowing effective treatment and decreasing newborn morbidity and mortality. In this scenario, cost-effectiveness studies are necessary to assess the feasibility of implementing this method as a routine in centers with maternity wards.

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