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# Detection of Group B *Streptococcus agalactiae* from Anorectal and Vaginal Screening Tests

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## Abstract

**Objective:** The aim of the present study was to analyze two DNA extraction methods for use in molecular GBS diagnostics and compare them to the results of culture method.

**Materials and methods:** Two hundred vaginal samples were collected during the antenatal period, as per CDC recommendations, and *atr* gene polymerase chain reaction (PCR) was performed.

**Results:** Comparison of the two DNA extraction methods demonstrated 45% concordance. Sensitivity and specificity for 5 M Guanidine DNA extraction were 100% and 86.5%, respectively. Sensitivity and specificity for the commercial DNA extraction kit were 50% and 95%, respectively.

**Conclusion:** This study demonstrated that 5 M Guanidine DNA extraction was superior to the commercial kit, with PCR presenting a shorter turnaround time than culture. PCR could improve sensitivity and, therefore, may be a useful screening method. Sensitive GBS diagnosis allows for an effective treatment, with decreased newborn morbidity and mortality; therefore, cost-effectiveness studies are necessary to assess the feasibility of implementing PCR in routine laboratories, together with maternity ward collaboration.

**Keywords:** *Streptococcus agalactiae*; Polymerase chain reaction; Pregnancy

## Introduction

*Streptococcus agalactiae* (group B Streptococcus) has been described as an important pathogen in neonates and pregnant women [1]. During pregnancy, vaginal colonization with GBS is associated with significant newborn infection, which requiring investigation in view of its considerable morbidity and mortality [2-5].

Evidence shows that intrapartum antimicrobial chemoprophylaxis can prevent neonatal *S. agalactiae* colonization, sepsis and mortality. The Centers for Disease Control and Prevention (CDC) published recommendations in 1996 promoting both maternal risk-based strategies and microbiological surveillance, with the aim of identifying candidates for chemoprophylactic intervention [6,7]. The microbiological screening data outperformed the risk-based strategies in identifying at-risk mothers. The CDC, therefore, published guidelines in 2002, which defend screening in the late antenatal period, 35 to 37 weeks gestation, for *S. agalactiae* colonization [4].

There are, however, limitations in terms of specimens collected at delivery, which are cultured for 24 to 72 hours, and the guidance for antimicrobial prophylaxis. A test that gives rapid results, which could accurately detect GBS carriage at the time of labor, may enhance the accuracy of such screening [8].

Many techniques aimed at validating a fast and efficient method to replace the culture-screening test in the identification of GBS colonization in pregnant women (even in low-count bacteria carriers), with a short turnaround time, have been investigated [9-11].

Molecular biology-based assays, such as the polymerase chain reaction (PCR), have become the test of choice for detection of GBS colonization in pregnancy [12,13]. Different methods for DNA extraction, DNA amplification and detection of PCR products have been reported in the literature [8,14,15].

The *atr* gene presents a good target for GBS amplification. Having been extensively studied, the *atr* gene has been shown to be an essential gene, which is expressed in all *S. agalactiae* cells. The gene encodes for the amino acid transporter protein gs0538, which is highly specific to the *S. agalactiae* species. It is a housekeeping gene; therefore, the probability of mutation is low [16,17].

The Laboratory of Molecular Biology and Mycobacteria (LMBM) at the Federal University of Santa Catarina (UFSC), Brazil, has been using the 5M Guanidine method for GBS DNA extraction as an alternative to the in-house method for GBS diagnosis. The present study used PCR to analyze GBS isolates from clinical samples of pregnant women and compared DNA extraction methods, in order to improve molecular diagnosis.

## Materials and Methods

### Samples

The present study comprised 203 women at a minimum of 35 weeks gestation, whose antenatal care was based at a private or outpatient

clinic in Tubarão, Santa Catarina. Combined rectal/vaginal specimens were collected, as per the recommendations of the CDC [4].

All pregnant women agreed to participate in the study and signed an informed consent form.

The study was approved by the Research Ethics Committee of the University of Southern Santa Catarina (UNISUL) under no. 10.503. 4.01.III.

Each Sample was first collected with a rayon swab that was placed into a Todd Hewitt broth (Himedia Laboratories®, India) and then with a specific kit swab and placed into a ThinPrep PreservCyt vial (The ThinPrep, Cytic Corporation, 250 Campus Drive Malborough, MA 01752 USA®) and were subsequently sent to the LMBM at the UFSC, for molecular diagnosis of group B Streptococcus.

## Culture

To get the samples for culture, a swab was introduced in the lower third of the vagina and in the rectum, according to CDC [21]. The swab was placed into Todd Hewitt (Himedia Laboratories®, India) selective medium supplemented with gentamicin (8 µg/mL) and nalidixic acid (15 µg/mL). The medium was incubated at 36°C in 5% $CO_2$ for 18 hours. Subculture was then performed, using blood agar plates (BioMerieux®, Marcy l`Etoile, France) incubated at 36°C in 5% $CO_2$ for 24 hours. The β-hemolytic colonies are suggestive of the presence of GBS. When there was no growth, the plates were reincubated for additional 24 hours and checked again. The suspected colonies were submitted to the CAMP test and the positive samples were considered GBS [21].

## Sample preparation and DNA extraction

Molecular techniques were performed using samples collected in the ThinPrep PreservCyt vial (Corporation, 250 Campus Drive Malborough, MA 01752 USA®). The samples were centrifuged and the precipitate washed with 1X phosphate buffered saline (PBS) solution and suspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5). The solution was then submitted to two different DNA extraction protocols: the QIAmp mini kit for DNA extraction (Qiagen®, Valencia, USA), according to the manufacturer's instructions and the 5M Guanidine (*in-house*) method [18].

The *in-house* method consisted of 200 µL of sample with 1 mL of 5 M Guanidine (guanidine isothiocyanate 5 M, Tris 0.1 M pH 6.4, EDTA 0.5 M pH 8.0, Triton X 100), which was kept shaking overnight. 50 µL of silica solution was added and subsequently mixed by inverting for five minutes, followed by centrifugation at 12500×g for one minute. The supernatant was discarded, and the pellet washed twice in 500 µL of washing solution (guanidine isothiocyanate 5 M and Tris 0.1 M pH 6.4), twice in 500 µL of 70% ethanol and once in 500 µL of acetone. Between each wash, the sample was centrifuged at 12500×g for one minute. After washing, the pellet was dried at 56°C, rehydrated in 25 mL of TE buffer (pH 7.4) and centrifuged at 85000×g for five minutes. The supernatant was then transferred to an RNase-DNase-free microtube [18]. The two methods were compared with the culture, which is the gold standard for GBS identification.

## *atr* gene Polymerase chain reaction (PCR)

PCR were named PCR-Guanidine and PCR-kit, as per the extraction method used.

The PCR reactions were performed using GBS specific primers *atr*F (5'-CAA CGA TTC TCT CAG CTT TGT TAA-3') and *atr*R (5'-TAA GAA ATC TCT TGT GCG GAT TTC-3'), that amplified a 780 base pair (bp) fragment. This housekeeping gene encodes a glutamine transporter protein (gbs0538) of *S. agalactiae* [19,25,26].

A PCR mixture (20 µL) consisting of 1.5 mM $MgCl_2$ (Invitrogen®-USA), 1 U of recombinant Taq DNA polymerase (Invitrogen®-USA) and 10 picomoles of each primer. Amplification was performed at an initial denaturation of 94°C for 1 min, followed by 30 cycles of 1 min at 94°C, 45 seconds at 55°C for primer annealing, 1 min at 72°C for elongation and a final period of extension for 10 min at 72°C. The PCR products were resolved in 2% agarose gel and visualized using ethidium bromide staining under a UV light. A 100 bp molecular weight marker (InvitrogenR®, Calbad, USA) and a negative and positive control of *S. agalactiae* were used to evaluate the PCR products.

## Statistical analysis

Statistical analysis was performed using SPSS® version 20.0. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for PCR, using the culture as the gold standard.

# Results

This study was performed between December 2010 and August 2011. Two hundred and three samples were obtained from women at a minimum of 35 weeks gestation. Forty women (19.7%) were identified as GBS carriers by culture. All samples were tested using PCR for both DNA extraction methods (5 M Guanidine and Qiagen mini kit DNA extraction). PCR was positive for GBS in 30.5% (62/203) of the samples, with a positivity of 30.5% in the samples submitted to the 5 M Guanidine method and positivity of 13.8% (28/203) for the commercial kit samples.

In terms of culture, PCR-Guanidine presented a sensitivity of 100% and a specificity of 86.5% (CI95% 0.813-0.917), whilst PCR-kit demonstrated a sensitivity and specificity of 50% (CI95% 0.345-0.665) and 94.6% (CI95% 0.910-0.983), respectively. The NPV and PPV for PCR-Guanidine were 100% and 64.5% (CI95% 0.526-0.764), respectively, whilst for PCR-kit the NPV was 87.6% (CI95% 0.824-0.997) and the PPV was 71.4% (CI95% 0.546-0.881) (Table 1).

| Test | Accuracy (CI95%) | Sensitivity* (CI95%) | Specificity (CI95%) | Positive Predictive Value (PPV) (CI95%) | Negative Predictive Value (NPV)* (CI95%) |
|---|---|---|---|---|---|
| **PCR-Guanidine** | 0.892 (0.848-0.934) | 1.000 (1.000-1.000) | 0.865 (0.813-0.917) | 0.645 (0.526-0.764) | 1.000 (1.000-1.000) |
| **PCR-Kit** | 0.852 (0.801-0.902) | 0.500 (0.345-0.655) | 0.946 (0.910-0.983) | 0.714 (0.546-0.881) | 0.876 (0.824-0.997) |

| * p<0.01 |
|---|

**Table 1:** Comparison analysis between DNA extraction methods

## Discussion

Infection by GBS is the most common cause of neonatal infection in developed and developing countries, resulting in high levels of mortality and serious complications, among them sepsis and meningitis [21,22]. The mortality rate can reach 10% of all infected newborns, reflecting the importance of prevention of neonatal infection [23,24].

Many routine clinical situations demand a faster and more efficient GBS screening method than the culture method, as per CDC recommendations [9-11]. In the present study, results are reported for two different extraction protocols, with the use of the *atr* gene, as an amplification target for PCR as a GBS screening method. The commercial kit was more expensive and faster than the high performance Guanidine method. Although Guanidine method needs an overnight incubation, this method could, therefore, present the best cost-effectiveness ratio when applied to routine clinical laboratories, especially at low incoming countries. GBS colonization rates were 19.7% and 30.5% when using culture and PCR methods, respectively. In Brazil, recent studies report a prevalence ranging from 15.9% to 22.5%, when using the culture method [27-29], and from 26.9% to 35.9% when using the PCR method [28,29]. These rates are very similar to observed in this study. However, GBS colonization prevalence could vary widely according to geographic location, age, parity and socio-economic status [20].

In the present study, specificity was 86.5% for 5 M Guanidine DNA extraction, which is greater than that found in the literature and the gold standard. It should be highlighted that cultures can give false negative results; therefore, it may not be sufficiently sensitive for GBS detection, since other bacteria of the vaginal/genital tract can inhibit the growth of GBS, even when using a selective broth [13]. Furthermore, the GBS culture method is time-consuming, requiring at least 48 hours to fully identify GBS. Despite the PCR method used in the present study requiring incubation in selective broth prior to PCR, therefore, necessitating 24 hours to give the final result, the GBS PCR requires a considerably shorter time than the culture method. The 5 M Guanidine proved has better sensitivity and specificity when compared with the commercial extraction kit, although has the disadvantage of shaking overnight.

Since 2002, the incidence of neonatal infections has decreased by more than 60% and new GBS detection techniques, with increased accuracy and faster results, could further contribute to this improvement [4].

## Conclusion

This study demonstrated that 5 M Guanidine DNA extraction was superior to the commercial kit, with PCR with presenting a shorter turnaround time than culture. PCR with 5 M Guanidine DNA extraction could improve sensitivity and, therefore, may be a useful screening method. As perspective it is possible optimize the 5 M Guanidine DNA extraction to obtain a good DNA concentration with about 2 hours of Guanidine shaking and circumvent the loss of time with overnight shaking. Sensitive GBS diagnosis allows for an effective treatment, with decreased newborn morbidity and mortality; therefore, cost-effectiveness studies are necessary to assess the feasibility of implementing PCR in routine laboratories, together with maternity ward collaboration.

## References

1. Palmeiro JK, Dalla-Costa LM, Fracalanzza SEL, Botelho CAN, Nogueira KS, et al. (2010) Phenotypic and Genotypic Characterization of Group B Streptococcal Isolates in Southern Brazil. J Clin Microbiol 48: 4397-4403.
2. Baker CJ (1997) Group B streptococcal infections. Clin Perinatol 24: 59-70.
3. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, et al. (2008) Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. JAMA 299: 2056-2065.
4. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC) (2010) Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep 59: 1-36.
5. Schuchat A (1998) Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev 11: 497-513.
6. Lim DV, Morales WJ, Walsh AF, Kazanis D (1986) Reduction of morbidity and mortality rates for neonatal group B streptococcal disease through early diagnosis and chemoprophylaxis. J Clin Microbiol 23: 489-492.
7. Centers for Disease Control and Prevention (1996) Prevention of perinatal group B streptococcal disease: a public health perspective. MMWR Morbidity and Mortality Weekly Report. 45: 1-24.
8. Bergseng H, Bevanger L, Rygg M, Bergh K (2007) Real-time PCR targeting the sip gene for detection of group B Streptococcus colonization in pregnant women at delivery. J Med Microbiol 56: 223-228.
9. Aziz N, Baron EJ, D'Souza H, Nourbakhsh M, Druzin ML, et al. (2005) Comparison of rapid intrapartum screening methods for group B streptococcal vaginal colonization. J Matern Fetal and Neonatal Med 18: 225-229.
10. Carroll KC, Ballou D, Varner M, Chun H, Traver R, et al. (1996) Rapid detection of group B streptococcal colonization of the genital tract by a commercial optical immunoassay. Eur J Clin Microbiol Infectious Dis 15: 206-210.
11. Park CH, Ruprai D, Vandel NM, Hixon DL, Mecklenburg FE (1996) Rapid detection of group B streptococcal antigen from vaginal specimens using a new Optical ImmunoAssay technique. Diagn Microbiol Infect Dis 24: 125-128.
12. Wang E, Richardson H (1990) A rapid method for detection of group B streptococcal colonization: testing at the bedside. Obstet Gynecol 76: 882-885.
13. Gavino M, Wang E (2007) A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B Streptococcus colonization. American Journal of Obstetrics and Gynecology. 197: 388e1-388e4.
14. Salloum M, van der Mee-Marquet N, Valentin-Domelier AS, Quentin R (2011) Diversity of prophage DNA regions of Streptococcus agalactiae clonal lineages from adults and neonates with invasive infectious disease. PLoS One 6: e20256.
15. Wernecke M, Mullen C, Sharma V, Morrison J, Barry T, et al. (2009) Evaluation of a novel real-time PCR test based on the ssrA gene for the identification of group B streptococci in vaginal swabs. BMC Infect Dis 9: 148.

16. Glaser P, Rusniok C, Buchrieser C, Chevalier F, Frangeul L, et al. (2002) Genome sequence of Streptococcus agalactiae, a pathogen causing invasive neonatal disease. Mol Microbiol 45: 1499-1513.
17. Elbaradie SM, Mahmoud M, Farid M (2009) Maternal and neonatal screening for Group B streptococci by SCP B gene based PCR: a preliminary study. Indian J Med Microbiol 27: 17-21.
18. Lippke JA, Strzempko MN, Raia FF, Simon SL, French CK (1987) Isolation of intact high-molecular-weight DNA by using guanidine isothiocyanate. Appl Environ Microbiol 53: 2588-2589.
19. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, et al. (2003) Multilocus sequence typing system for group B Streptococcus. J Clin Microbiol 41: 2530-2536.
20. Borger IL, D'Oliveira REC, Castro ACD, Mondino SSB (2005) Streptococcus agalactiae in pregnant women: prevalence of colonization and antimicrobial susceptibility evaluation. Revista Brasileira de Ginecologia e Obstetrícia 27: 575-579.
21. Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC) (2010) Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. MMWR Recomm Rep 59: 1-36.
22. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A (2002) Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep 51: 1-22.
23. Honest H, Sharma S, Khan KS (2006) Rapid tests for group B Streptococcus colonization in laboring women: a systematic review. Pediatrics 117: 1055-1066.
24. Johri AK, Paoletti LC, Glaser P, Dua M, Sharma PK, et al. (2006) Group B Streptococcus: global incidence and vaccine development. Nat Rev Microbiol 4: 932-942.
25. Jolley KA, Chan MS, Maiden MC (2004) mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics 5: 86.
26. Streptococcus agalactiae Multi Locus Sequence Typing (MLST) website.
27. Costa AL, Lamy Filho F, Chein MB, Brito LM, Lamy ZC, et al. (2008) [Prevalence of colonization by group B Streptococcus in pregnant women from a public maternity of Northwest region of Brazil]. Rev Bras Ginecol Obstet 30: 274-280.
28. Munari FM, De-Paris F, Salton GD, Lora PS, Giovanella P, et al. (2012) A Combined Enrichment/Polymerase Chain Reaction Based Method for the Routine Screening of Streptococcus agalactiae in Pregnant Women. Braz J Microbiol 43: 253-260.
29. de-Paris F, Machado AB, Gheno TC, Ascoli BM, Oliveira KR, et al. (2011) Group B Streptococcus detection: comparison of PCR assay and culture as a screening method for pregnant women. Braz J Infect Dis 15: 323-327.