Comparison of LightCycler PCR and culture for detection of group B streptococci from vaginal swabs

M. Convert, G. Martinetti Lucchini, M. Dolina and J.-C. Piffaretti

Istituto Cantonale di Microbiologia, Bellinzona, Switzerland

ABSTRACT

Group B streptococci (GBS) are an important cause of neonatal sepsis and meningitis. New rapid, sensitive and specific methods for detection of GBS in pregnant women are needed in order to provide timely treatment of neonates. The sensitivity, specificity and cost of a LightCycler PCR method was compared with selective culture for the detection of GBS from 400 vaginal swabs. In addition, two DNA extraction methods (simple boiling and automated DNA extraction by Roche MagNA Pure LC) were compared for a subgroup of 100 clinical samples. The sensitivity of the LightCycler PCR assay for the detection of GBS from vaginal swabs was significantly higher than that of culture. There were no culture-positive, LightCycler PCR-negative cases. The efficiencies of the two DNA extraction procedures were not significantly different. The detection of GBS from vaginal swabs by the molecular method (including simple boiling extraction) required the same hands-on time, but the procedure was completed in 1.5 h, compared with c. 48 h for the culture-based approach. Disadvantages of the molecular method are the increased costs (45%) and the absence of antibiogram data. The LightCycler PCR is a promising tool for sensitive, specific and rapid detection of GBS directly from clinical specimens of pregnant women.

Keywords Detection, group B streptococci, LightCycler, PCR, pregnant women, Streptococcus agalactiae

Original Submission: 19 January 2005; Revised Submission: 21 April 2005; Accepted: 2 June 2005

Clin Microbiol Infect 2005; 11: 1022–1026

INTRODUCTION

Group B streptococci (GBS; Streptococcus agalactiae) are a leading cause of sepsis, meningitis and death among newborn infants in developed countries. Approximately 10–40% of pregnant women are colonised by GBS, in either the vagina or the rectum, and 40–70% of these women transfer GBS to the neonate [1]. Neonatal infection usually results from vertical transmission during delivery, or acquisition in utero just before labour. Of the colonised neonates, 1–3% develop disease, almost always in the form of early-onset infection within 24 h of birth [2]. Early-onset disease in the neonate is defined as the clinical presentation of pathology, mainly sepsis and meningitis, within the first week of life [1]. Neonatal infection can be prevented in most cases by providing intrapartum antimicrobial prophylaxis to the colonised mother [1,3]. Hence, determination of colonisation by GBS at the time of labour is essential.

Screening all pregnant women for carriage of GBS between week 35 and week 37 of pregnancy is a recommended approach for avoiding the transmission of GBS from mothers to neonates [4]. The current standard methods for detection of GBS consist of selective broth cultures of combined vaginal and anal specimens. Although these methods are sensitive and specific, they are time-consuming, requiring 48–72 h, and also require the presence of viable organisms [1]. Many simple and rapid tests for GBS have been developed, but these are not useful for direct detection because they are neither sensitive nor specific enough to substitute for bacterial culture [5,6]; thus, only women with heavy colonisation can be identified readily with such methods. Hence, new rapid, sensitive and specific methods for direct detection of GBS from clinical specimens in pregnant women are needed for timely treatment of neonates.

© 2005 Copyright by the European Society of Clinical Microbiology and Infectious Diseases
Several PCR-based assays for the detection of GBS have been developed, but these require complicated procedures not applicable readily to clinical laboratories [7]. However, the development of real-time PCR (rt-PCR) and fluorescence-labelling technologies has provided new platforms for bacterial detection and identification. PCR assays specific for GBS are promising tools for rapid, sensitive and specific detection directly from clinical specimens. Ke et al. [8] developed an rt-PCR method based on amplification of a cfb gene fragment that is present in virtually every strain of GBS [9]. Bergeron et al. [10] showed that this rt-PCR was specific but, surprisingly, only of equivalent sensitivity to the conventional bacterial culture test. Details of a qualitative test based on amplification of the same cfb gene fragment have recently been published [11].

Quantification, in addition to identification of the pathogen, may provide important information about the potential evolution of an infection, or the course of the disease, by quantifying the amount of genomic DNA of a specific bacterium in a patient sample [12].

In the present study, the sensitivity, specificity and cost of a LightCycler PCR method were compared with those of selective culture for detection of GBS from 400 clinical samples. In addition, two DNA extraction methods (simple boiling and automated DNA extraction) were compared for a subgroup of 100 clinical samples.

MATERIALS AND METHODS

Collection of specimens and preliminary treatment

Between August and December 2003, 400 vaginal swabs were obtained from pregnant women at 33–37 weeks of gestation. All specimens were collected and transported to the Instituto Cantonale di Microbiologia (Bellinzona, Switzerland), using the Ames Clear Transystem (Verridial, Blonay, Switzerland). All swabs were first inoculated for standard culture, and then dipped into 500 μL of sterile distilled water for DNA extraction and LightCycler PCR assay.

Enrichment and selective culture

All 400 vaginal swabs were cultured in 5 mL of LIM broth (Todd–Hewitt broth with colistin and nalidixic acid; Chemie Brunschwig, Basel, Switzerland) and incubated overnight at room temperature. Columbia CNA plates (Columbia Agar Base, defibrinated sheep blood 5% v/v, selective supplement for the isolation of streptococci; Oxoid, Basel, Switzerland) were then inoculated with 50 μL of broth [4]. The plates were incubated at 37°C in CO2 5% v/v for 24–48 h. Species identification of β-haemolytic colonies was performed by agglutination with a streptococcal grouping kit (Streptex; Sodiag SA, Losone, Switzerland).

DNA extraction

DNA was extracted from all 400 vaginal swabs with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Rotkreuz, Switzerland), used according to the manufacturer’s instructions, and in addition, from a subgroup of 100 randomly selected clinical samples, by simple boiling of 250 μL of the 500-μL suspension for 10 min. Finally, the DNA preparations were stored at 4°C.

LightCycler PCR assay

The LightCycler (v. 3.5; Roche Diagnostics) was used to perform rt-PCR. Primers and probes were as described previously [8] and were synthesised by TIB MOLBIOL (Berlin, Germany). Briefly, primers StrepB1 (5’-TTTACCACGCTG-TATTAGAAGTA-3’) and StrepB2 (5’-GTTCCCTGAACAT-ATCCTTGAT-3’) amplify a 153-bp fragment of the cfb gene. The probes used were pStB-flu (5’-AACCCACGCAA-TGGCTCAA-FL-3’) and pStB-lcr (5’-LC Red640-GCTTGAC-CAACATAGCATTCA-3’). Each 20-μL reaction volume contained 2 μL of LightCycler DNA Master Hybridization Probes mix (Roche Diagnostics), 0.4 μM each primer, 0.2 μM each probe, 0.1 U of uracil-DNA glycosylase (UNG; Roche Diagnostics), 3.5 mM MgCl2 and 5 μL of DNA extract. One positive and several negative controls were included in each run. Strict precautions to prevent carryover of amplified DNA were used.

Thermocycling conditions were optimised to one cycle at 95°C for 10 min, followed by 55 cycles of 10 s at 95°C, 14 s at 57°C and 5 s at 72°C, with a temperature transition rate of 20°C/s. The fluorescence was measured at the end of each extension step. The melting curve program comprised 95°C for 10 s (20°C/s), 54°C for 15 s (20°C/s), and 78°C for 10 s (0.2°C/s), with continuous fluorescence acquisition.

Samples with discordant results (positive by LightCycler and negative by standard culture) were investigated further by DNA sequence analysis of the amplification product.

DNA sequencing

PCR products were purified using the Amicon Microcon Kit (Millipore, Volland, Switzerland), eluted in 20 μL of AE buffer and stored at 4°C. Cycle sequencing reactions were performed in total volumes of 15 μL, using an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems, Rotkreuz, Switzerland) on an ABI Prism 310 Genetic Analyser (Perkin-Elmer Applied Biosystems), according to the manufacturer’s instructions. DNA sequencing for GBS (cfb gene fragment of 153 bp) was performed in both directions with primers StrepB1 and StrepB2.

PCR specificity and sensitivity

The specificity of the rt-PCR assay was verified by testing genomic DNA from bacterial species related phylogenetically to GBS and from other species often present in the vaginal flora.
Table 1. Bacterial strains (n = 89) used to test the specificity of the PCR assay specific for group B streptococci

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species (no. of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>S. pneumoniae (5)</td>
</tr>
<tr>
<td></td>
<td>S. pyogenes (6)</td>
</tr>
<tr>
<td></td>
<td>Group C streptococcus (2)</td>
</tr>
<tr>
<td></td>
<td>Group D streptococcus (4)</td>
</tr>
<tr>
<td></td>
<td>Group F streptococcus (5)</td>
</tr>
<tr>
<td></td>
<td>Group G streptococcus (4)</td>
</tr>
<tr>
<td></td>
<td>Non-haemolytic streptococci (2)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Enterococcus spp. (10)</td>
</tr>
<tr>
<td>Listeria</td>
<td>L. monocytogenes (5)</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>Ent. aerogenes (3)</td>
</tr>
<tr>
<td></td>
<td>Ent. cloacae (4)</td>
</tr>
<tr>
<td>Escherichia</td>
<td>E. coli (5)</td>
</tr>
<tr>
<td>Gardnerella</td>
<td>G. vaginalis (1)</td>
</tr>
<tr>
<td>Haemophilus</td>
<td>H. influenzae (5)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>K. oxytoca (3)</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae (5)</td>
</tr>
<tr>
<td>Proteus</td>
<td>P. mirabilis (5)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Ps. aeruginosa (5)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Staph. aureus (5)</td>
</tr>
<tr>
<td></td>
<td>Coagulase-negative staphylococci (5)</td>
</tr>
</tbody>
</table>

(n = 89; Table 1). Clinical isolates of GBS (n = 40) were also tested to further validate the GBS-specific rt-PCR. The sensitivity of the method was determined as described below.

Cloning of the cfb amplicon and quantification of genomes of GBS

The 153-bp fragment of the cfb gene was amplified from genomic DNA of GBS with the use of primers StrepB1 and StrepB2. The amplicon was purified using the Amicon Microcon Kit and was cloned using the TOPO TA cloning kit (Invitrogen, Basel, Switzerland) according to the manufacturer’s instructions. The recombinant plasmid was isolated from transformed Escherichia coli with a QiAprep spin miniprep kit (Qiagen, Basel, Switzerland). The purified plasmid was serially diluted and the DNA concentration was determined using a PicoGreen dsDNA quantitation kit (Molecular Probes; Juro Supply, Lucern, Switzerland). Quantification of genomes of GBS from clinical samples was achieved using the standard curve generated by serial dilutions of reference plasmid DNA.

Statistical analysis

The sensitivities of the LightCycler PCR assay and the culture method were compared by McNemar’s chi-square test. The Wilcoxon rank sum test was used for comparison of sensitivity between the two DNA extraction methods.

RESULTS

Sensitivity and specificity of the real-time PCR assay

All 40 clinical isolates of GBS, but none of the non-group B streptococcal isolates, generated a clear positive signal with the rt-PCR assay (Table 1). The lower limit of the assay was found to be one group B streptococcal genome copy/PCR assay (data not shown).

Validation of the real-time PCR assay

The standard culture method was used as baseline in order to evaluate the effectiveness of the rt-PCR method. In total, 400 vaginal swabs were tested, of which 278 (69.5%) were negative by culture and LightCycler PCR, 75 (18.8%) were positive by both methods, and 47 (11.8%) were positive only by PCR. There were no vaginal swabs that were positive by culture only (Table 2). Thus, the LightCycler PCR generated significantly (p < 0.001; McNemar’s chi-square test) more positive results (30.5%) than did the culture method (18.8%).

To exclude PCR false-positives, the amplicons from the specimens with discrepant results (i.e., LightCycler PCR-positive and culture-negative (n = 47) were sequenced in both directions. Since the analysed sequences generally differed from each other by one or more nucleotides, carryover contamination between samples could be excluded. Thus, use of the rt-PCR method resulted in an increase of 62% in the number of GBS detected compared with culture.

Quantitative data for 250 clinical samples, selected randomly, were also generated. The results suggested that rt-PCR was more sensitive than the culture method. Thus, culture-positive specimens contained 1.4-fold more DNA than samples that were positive only by rt-PCR (data not shown).

Comparison of two DNA extraction methods

DNA was extracted from 100 vaginal swabs by both simple boiling and the MagNA Pure LC method. All swabs positive by MagNA Pure were also positive by boiling, even though comparison of the median rt-PCR results indicated a trend to higher sensitivity after extraction by MagNA Pure (MagNA Pure, 7.7 x 10^1 copies of the cfb gene/5 μL; boiling, 3.2 x 10^2 copies/5 μL; p = 0.085, Wilcoxon rank sum test).

Table 2. Comparison of positive and negative results between the real-time PCR assay and standard culture for detection of group B streptococci

<table>
<thead>
<tr>
<th>Standard culture</th>
<th>LightCycler PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>75 (18.75%)</td>
</tr>
<tr>
<td>Negative</td>
<td>47 (11.75%)</td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>278 (69.50%)</td>
</tr>
</tbody>
</table>

© 2005 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, CMI, 11, 1022–1026
DISCUSSION

The present study found that 122 (30.5%) of 400 women were colonised with GBS according to the rt-PCR assay. This percentage is consistent with other data obtained by similar molecular methods [10,13], and suggests, in contrast to the results of Bergeron et al. [10], that the LightCycler PCR assay is more sensitive than the standard culture method for detecting GBS from vaginal swabs. Indeed, a 62% increase in the number of samples positive for GBS was obtained by LightCycler PCR (n = 122) compared with culture (n = 75), and this was confirmed by sequencing the amplicons from the PCR-positive, culture-negative samples. Previous prospective clinical studies with various pathogens have consistently demonstrated increases in sensitivity for assays based on LightCycler PCR technology compared with standard culture methods [14,15]. In addition, the LightCycler technique provides same-day results, thereby allowing timely and appropriate antimicrobial therapy, and thus lessening the risks of undesirable sequelae.

Forty-seven (11.8%) of 400 vaginal samples were positive only by rt-PCR and were confirmed by DNA sequencing. This difference may be caused by the inability of culture to detect low bacterial numbers (which appeared to be the case in the present study), or by the presence of antagonistic microorganisms, such as Candida albicans [16]. Heavy colonisation with GBS correlates with a higher risk of neonatal sepsis, while, conversely, a low number of GBS is associated with a decreased risk of neonatal disease [17]. The rt-PCR also detects non-viable bacterial cells, which could, in part, also explain the higher detection rate by PCR.

As rt-PCR does not require viable organisms, but detects DNA, inappropriate storage or transport of specimens should not alter the efficiency of the method. The ability of culture to identify GBS declines quickly, or can be compromised, if antibiotics or hygiene products (which often contain antibacterial agents) are present locally when sampling is performed [16]. The risk of infection with GBS may be overlooked if culture is the only method of evaluating the maternal group B streptococcal colonisation status. However, culture is still required when an antibiogram is needed. Penicillin is the drug of choice for prophylaxis and treatment of disease caused by GBS, and to date, resistance to this agent has not been reported among GBS. However, macrolides are the recommended second-line agents, and the first alternative for mothers with a penicillin allergy [18]. Several studies have reported increasing resistance to macrolides among GBS [3,18]. Thus, PCR cannot replace culture if susceptibility testing is required.

The efficiency of detection of GBS by LightCycler PCR may be related both to the inherent qualities of the rt-PCR assay and to the DNA extraction technique used. Sample preparation is one of the most important steps when performing PCR. The present study demonstrated that the analytical sensitivity of detection of GBS by rt-PCR was similar after DNA extraction by either the MagNA Pure LC Kit or simple boiling. Qualitatively, no significant discrepancies were found. The time required for extraction of a comparable number of specimens was greater (1 h) for the MagNA Pure method than for boiling (15 min), and compared with culture, the boiling method was simple and provided sufficient PCR sensitivity.

Practical considerations, such as flexibility, workload and cost, are important when a method or instrument is being selected for use in the clinical laboratory. The total hands-on time required to analyse a comparable number of specimens by culture and by the LightCycler PCR assay was similar, but a longer period was required to obtain the results by culture (48–72 h) compared with the LightCycler assay (2 h). Including the cost of equipment (based upon an amortisation of 5 years and a test run of ten patient-samples/day/260 days/year), the total estimated costs and time (including pre-analytical processing and analysis) required to complete the analysis of a single sample were: CHF11 (7 Euro) and c. 48 h for culture; CHF16 (10 Euro) and 1.5 h for LightCycler PCR with boiling; and CHF41 (27 Euro) and 2.5 h for LightCycler PCR with the MagNA Pure kit. However, these costs will decrease with increasing numbers of samples.

The LightCycler PCR assay is relatively easy to perform and may be adapted to small laboratories. Thus, the final choice for a laboratory (rt-PCR vs. culture) will depend mainly on how urgently the results are required.

The present study obtained more positive results with the amplification test than with culture. In clinical microbiology, culture has,
historically, been considered to be the standard test. With the advent of nucleic acid amplification tests, this approach might now be considered inappropriate in certain cases. After exclusion of the possibility that nucleic acid amplification test-positive, culture-negative samples are false-positives (e.g., caused by laboratory contamination or non-specific amplification), it should be considered that culture might be hindered by the presence of inhibitors in the sample. Thus, the general statement that ‘a test cannot, by definition, be more sensitive than the standard of culture’ can be questioned. This has been debated for *Chlamydia trachomatis*, and to a lesser extent, for *Neisseria gonorrhoeae* infections [19–21]. In order to improve the development of new diagnostic tests, it is imperative to revisit the standard test concept, considering the advantages and disadvantages of nucleic acid amplification tests for each aetiological agent and in relation to each individual disease.

ACKNOWLEDGEMENT

We thank A. von Graevenitz for reading the manuscript.

REFERENCES


© 2005 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, CMI, **11**, 1022–1026