

# The performance of GeneXpert® PCR assay in detecting group B streptococcus colonization at labor

Mohammed Rohi Khalil<sup>1,\*</sup>, Jens Kjølhseth Møller<sup>2</sup>, Stine Yde Nielsen<sup>2</sup>

<sup>1</sup>Department of Gynecology and Obstetrics, Kolding Hospital, University Hospital of Southern Denmark, 6000 Kolding, Denmark

<sup>2</sup>Department of Clinical Microbiology, Vejle Hospital, University Hospital of Southern Denmark, 7100 Vejle, Denmark

\*Correspondence: [mohammed.khalil@rsyd.dk](mailto:mohammed.khalil@rsyd.dk) (Mohammed Rohi Khalil)

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**Background:** The aim of this study was to assess the performance of GeneXpert® group B streptococcus (GBS) PCR assays in a Danish setting, using rectovaginal GBS culture at labor as the gold standard. **Methods:** Three hundred and sixty-six (366) women with one or more of the following risk factors for GBS carriage—GBS during current pregnancy, prior infant with EOGBS, temperature  $>38.0^{\circ}\text{C}$  during labor, preterm labor  $<37$  weeks of gestation, rupture of membranes  $\geq 18$  hours—were included in the study. Intrapartum rectovaginal swab samples were tested at the bedside by GeneXpert® GBS PCR assay, and cultured on agar plates (Granada) with and without prior use of growth-selective enrichment broth. **Results:** The GeneXpert® GBS PCR assay showed a sensitivity of 91.7%, a specificity of 97.2%, a PPV of 92.6%, and a NPV of 96.8%. The turnaround time of the assay was 50 minutes. **Conclusions:** The GeneXpert® has a high performance, indicating that the assay can be used in a clinical setting.

## Keywords

Group B streptococcus; Early-onset neonatal infection; Polymerase chain reaction; Rapid intrapartum assay; GeneXpert®

## 1. Introduction

Group B streptococcus (GBS) is the most frequent cause of early-onset GBS infection (EOGBS), which is associated with significant morbidity and mortality among newborns. The incidence rate of EOGBS ranges from 0.5 to 3.0 per 1000 live births, with 4 to 10% mortality [1–4]. In Denmark, where the risk-based strategy is recommended, the incidence ranges from 0.1 to 0.3 per 1000 live births [5]. Intrapartum antibiotic prophylaxis (IAP) is administered to women at risk. The five risk factors for EOGBS are GBS during current pregnancy, prior infant with EOGBS, temperature  $>38.0^{\circ}\text{C}$  during labor, preterm labor  $<37$  weeks of gestation, and/or rupture of membranes  $\geq 18$  hours. However, a large fraction of women in labor with the risk factors are actually GBS-negative, whereas half of EOGBS cases are newborns of mothers colonized by GBS without any of the risk factors [6–8].

Rapid nucleic acid amplification tests are increasingly performed during labor [9, 10]. The sensitivity and specificity of GeneXpert® PCR have been reported in several studies to be between 85.7% and 100% and between 82.6% and 96.6%, re-

spectively [8, 9, 11, 12].

The aim of this study was to assess the performance of GeneXpert® group B streptococcus (GBS) PCR assays in a Danish setting, where the risk-based approach is still the recommended strategy, using rectovaginal GBS culture at labor as the gold standard.

## 2. Materials and methods

### 2.1 Study population and sample collection

Three hundred and sixty-six (366) women were included in the study, which was conducted between December 2018 and July 2019. Participants with one or more of the following risk factors for GBS carriage were tested at the labor ward of Lillebaelt University Hospital, Denmark: GBS during current pregnancy, prior infant with EOGBS, temperature  $>38.0^{\circ}\text{C}$  during labor, preterm labor  $<37$  weeks of gestation, rupture of membranes  $\geq 18$  hours.

The study was approved by the Danish Data Protection Agency (j.nr. 2012 58-0018). According to Danish legislation, quality assessment studies do not require approval from an ethics committee.

Intrapartum rectovaginal swab samples (ESwab, Copan diagnostics, Brescia, Italy) were taken during labor, collected by a midwife, tested by GeneXpert® (Cepheid Ltd., Sunnyvale, CA, USA) GBS PCR assay, and cultured on Granada agar plates with and without prior growth of material in a selective enrichment broth. The swab sample was taken from the lower part of the vagina and 2 cm beyond the anal sphincter.

### 2.2 GeneXpert® PCR

The test was designed for use at the point of care in a labor ward and is run on the GeneXpert® molecular diagnostic system. The test identifies GBS DNA from rectovaginal swab specimens, using fully automated real-time PCR with fluorogenic detection of the amplified DNA.

Training was given by the manufacturer during installation, which included training in sample collection, preparing the cartridge(s), and analyzing results. Training took about 30 minutes and additional training materials were provided to staff.

After sampling, the swab was transferred to the designated chamber of the GeneXpert® (Cepheid Ltd., Sunnyvale, CA, USA) GBS assay cartridge. The swab was snapped at the score mark, and the cartridge was loaded into the Cepheid GeneXpert® system for automated sample preparation and PCR. All results were reported either as positive or negative based on detection of *S. agalactiae* *cfb* gene. The turnaround time of the assay was 50 minutes. The results are reported by the GeneXpert® software as a qualitative answer, i.e., either positive or negative for GBS, or as invalid, i.e., “Error”, when the presence or absence of GBS cannot be determined, a system component fails, maximum pressure is reached, or the probe check fails.

The GeneXpert® processing unit was placed at the labor ward, and all analyses were carried out by midwives.

### 2.3 Culture

All swabs were sent to the Department of Clinical Microbiology at Lillebaelt University Hospital, Denmark. The samples were cultured as soon as possible, otherwise they were kept at 4 °C until the next morning. Semi-quantitative evaluation to grade the presence of GBS as few (+), moderate (++), or numerous (+++) was performed.

Fifty microliters from the ESwab were cultured directly on the Granada agar and examined after incubation under anaerobic atmosphere for 24 and 48 hours.

Another 200 µL from the ESwab were inoculated into separate tubes with 5 mL of Todd-Hewitt broth with 1% yeast extract, 15 µg/mL nalidixic acid, and 10 µg/mL colistin (Lim broth, Becton Dickinson). These were inoculated aerobically at 37 °C overnight, and 10 µL were then subcultured on Granada agar (BioMérieux) plates and examined after 24- and 48-hour incubation under anaerobic atmosphere.

All GBS-like colonies were routinely confirmed as *Streptococcus agalactiae* using MALDI-TOF (Bruker Daltonik, Bremen, Germany). They were identified by the orange color on Granada plates and from the enrichment broth.

### 2.4 Statistical analyses

We determined the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the GeneXpert® assay. The rectovaginal culture result was used as a gold standard reference. For statistical analyses we used, STATA software (version 16; Stata Corp LP, TX, USA).

## 3. Results

Among the 366 women in labor tested, 99 were positive by culture and 95 were positive by GeneXpert®. The results were reported by the GeneXpert® software as a qualitative answer, i.e., either positive or negative for GBS. Table 1 shows the distribution of the results.

Among the GBS culture-positive samples, 88.9% (88/99) were positive using GeneXpert®; among the GBS culture-negative samples, 2.7% (7/255) were GeneXpert®-positive.

Among the 366 GeneXpert® results, 4.1% (N = 15) were primary invalid results/errors, out of which 3 were culture

**Table 1. Distribution of GBS test results (N = 366).**

Number	GeneXpert®	Culture
88	+	+
8	-	+
3	Error	+
7	+	-
12	Error	-
248	-	-

+, positive result; -, negative result; Error, inconclusive result due to technical error.

positives and 12 were culture negatives. The GeneXpert® assay had a sensitivity of 92% (88/96) and a specificity of 97% (248/255) (Table 2).

**Table 2. The performance characteristics with culture as reference. Errors are not included.**

	GeneXpert® GBS PCR	
	% (n/N)	(95% CI)
Sensitivity	91.7 (88/96)	84.2–96.3
Specificity	97.3 (248/255)	94.4–98.9
PPV	92.6 (88/95)	85.8–96.3
NPV	96.9 (248/256)	94.1–98.4

The risk factors and corresponding GBS PCR results are shown in Table 3. The PCR test was positive in 26% (95/366) of all cases with one or more risk factors for EOGBS. Among women with GBS bacteriuria during their current pregnancy, 67% were positive. Among women with ROM for more than 18 hours, only 16% were positive (Table 3).

There was very little inconsistency between cultures with and without enrichment broth. Three percent (3/99) of swab samples were only positive in the enrichment broth, indicating that this broth pre-enrichment step is of limited value in a clinical setting. The two culture methods were therefore not evaluated separately. There was 100% consistency between the results from MALDI-TOF performed on direct culture and those from culture with the enrichment broth.

Total hands-on time required from the midwife who performed the testing (i.e., time from inserting the swab into the chamber S Insert Cartridge to starting the assay) was less than 1 minute.

## 4. Discussion

The study was designed to assess the performance of GeneXpert® GBS assay, as compared to intrapartum rectovaginal GBS culture, which was considered as the gold standard. Compared to culture, the sensitivity of GBS detection by GeneXpert® was 91.7% and the PPV was 92.6% (Table 2).

One strength of our study is that the testing was conducted on fresh specimens in order to avoid cycles with freeze-thawing on a stored material. Another strength of our study is that the rectovaginal swabs were prospectively collected

**Table 3. Intrapartum PCR test results among women with one or more risk factors for GBS.**

Risk factors	Intrapartum PCR GBS test			Total N
	Negative N (%)	Positive N (%)	Missed test N (%)	
GBS in urine	17 (28%)	41 (67%)	3 (5%)	61
Preterm delivery <37 weeks	72 (86%)	10 (12%)	2 (2%)	84
ROM >18 hours	135 (80%)	26 (16%)	7 (4%)	168
Temperature >38 °C	28 (62%)	15 (33%)	2 (5%)	45
Previous EOGBS	4 (50%)	3 (38%)	1 (12%)	8
Total	256 (70%)	95 (26%)	15 (4%)	366

from women who fulfilled one or more of the risk factor criteria. A third strength is that the PCR and the two versions of GBS culture (with and without broth pre-enrichment) were tested on the same set of samples.

It could be considered a weakness that midwives performed the PCR analysis, and the fact that they are typically less experienced in this than trained lab technicians could be considered a bias. The PCR assay was performed in the delivery ward by midwives, who had received a brief introduction to the equipment by the local distributor in Denmark. Based on the methods described, the swab is placed directly in the machine and a result is delivered in 50 min. Thus, there is minimal user interference and the machine is suitable for midwives to perform. The user interface is very simple, even for a non-technical person. There were no issues encountered with midwives using the technology; they were generally positive towards the rapid testing. Total hands-on time (i.e., time from inserting the swab into the chamber S Insert Cartridge to starting the assay) was less than 1 min. With adequate training, this simple procedure can be incorporated as a clinical routine.

The results were reported by the GeneXpert® software as a qualitative answer, i.e., either positive or negative for GBS. We found that 4.1% (N = 15) of the samples had primary inconclusive results. If the PCR assay presented with an inconclusive result (technical error), a second (repeat) test was conducted when possible. However, IAP treatment was always based on the first test result; it was administered only if there was a primary inconclusive PCR result.

Helmig *et al.* [13] found that less than 1% of results were inconclusive; however, the PCR analysis of the swabs were not performed, as in our study, by midwives at a labor ward, but instead were performed by trained lab technicians. Mueller *et al.* [11] reported that 55.3% of their test results were initially inconclusive, but this reduced to 13.4% after the midwives were trained for two hours. Håkansson *et al.* and Helali *et al.* [8, 14], who also ran their PCR tests in labor wards, recorded about 15% and 9% invalid test results, respectively.

Invalid results are an important issue when assessing the feasibility of point-of-care technology. In a busy clinical setting, an inconclusive test result may very well result in IAP, since there is often no time to wait for a new test.

There was very little inconsistency between cultures with

or without enrichment broth; only three samples that were positive only in the enrichment broth. Therefore, the two culture methods were not evaluated separately. The difference in detection rates between direct plating on the Granada medium and plating after prior Lim broth enrichment had been earlier found by El Aila NA *et al.* [15] to be 4%. Granada medium cannot detect non-hemolytic GBS, thereby potentially decreasing the sensitivity of this culture medium for GBS screening [2]. However, the frequency of non-hemolytic GBS isolates is only 1% among invasive GBS strains [16].

Our hypothesis for the inconsistencies between culture and PCR is primarily informed by a failure to detect vaginal colonization with low numbers of GBS, which may be of less risk to the newborn during birth [17]. False negative results obtained by PCR were reported in samples with low colony growth, which were probably below the detection limit of PCR techniques [18]. Also, Tickler *et al.* [19] noted four types of chromosomal deletions in the region of the *cfb* gene in GBS isolates that resulted in negative Xpert GBS tests, which could possibly explain our culture-positive but GeneXpert®-negative findings.

El Helali *et al.* [14] performed a large cost-effectiveness study of intrapartum PCR compared to antenatal cultures and concluded that the final costs for both techniques were similar. They reported a significant decrease in the prevalence of EOGBS in the intrapartum PCR negative group. They also found a significant reduction in the use of IAP. The GeneXpert® GBS assay can be used to identify GBS colonization at the onset of labor as a supplement to the risk factor-based approach, and could potentially reduce the unnecessary use of intrapartum antibiotic prophylaxis.

## 5. Conclusions

We conclude from this study that the GeneXpert® has a high performance, which makes the assay applicable in a clinical setting.

## Abbreviations

EOGBS, early-onset GBS infection; GBS, group B streptococcus; IAP, intrapartum antibiotic prophylaxis; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; ROM, rupture of membranes.

## Author contributions

MRK was in charge of the collection of samples; SYN was in charge of the culture of all samples. JKM supervised the project and was in charge of the data management. All authors participated in the writing process. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved by the Danish Data Protection Agency (j.nr. 2012 58-0018). According to Danish legislation, quality assessment studies do not require approval from an ethics committee.

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## Conflict of interest

The authors declare no conflict of interest.

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