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Evaluation of a Sensitivity Real-time PCR assay for Group B Streptococcus Detection in Vaginal-Rectal Swab

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Abstract

Group B *Streptococcus* (GBS) is associated with invasive infections in newborns. The obstetric field requires a rapid method for obtaining GBS screening results. Our objective was to determine whether a sensitive real-time PCR assay demonstrates acceptable diagnostic performance. This study involved 301 pregnant women. Vaginal and rectal swabs were collected between 35 and 37 weeks of gestation. We report the diagnostic performance of the sensitive real-time PCR assay compared to composite reference method, along with 95% confidence intervals (CI). Positive results were detected in 35 cases (11.6%), 27 cases (8.9%), and 21 cases (6.9%), using real-time PCR, real-time PCR after enrichment, and microbial culture methods, respectively. Microbial culture and sequencing were applied as composite reference method, the sensitivity of real-time PCR was 96.15%, specificity was 96.36%, and diagnostic accuracy was 96.34%. Compared with the composite reference method, the sensitivity and specificity of the real-time PCR detection method after enrichment was 96.15% and 99.27%, with a diagnostic accuracy of 99.0%. Microbial culture demonstrated a sensitivity of 80.77%,

specificity of 100%, and diagnostic accuracy of 98.33%. This study demonstrates that the sensitive real-time PCR assay represents an acceptable rapid GBS screening method, offering clinicians a new option for early screening.

Keywords Group B Streptococcus, Pregnant women's, Real-time PCR assay, Microbial Culture

Introduction

Streptococcus agalactiae, also known as Group B *streptococcus* (GBS), is a facultative anaerobic Gram-positive coccus that can colonize the gastrointestinal and female genital tracts intermittently, transiently, or persistently[1]. GBS colonization in pregnant women refers to positive GBS culture results from vaginal, rectal, or perianal samples collected during pregnancy[2]. In addition to rectovaginal colonization, the isolation of GBS from a urine culture during pregnancy is also considered as heavy colonization. Without intervention, GBS colonization in pregnant women carries a 50% risk of vertical transmission, leading to severe neonatal infections such as neonatal sepsis and neonatal meningitis in approximately 1%–2% of infants born to colonized mothers[3–6]. Because GBS is a major cause of neonatal infection, the U.S. Centers for Disease Control and Prevention (CDC) has

implemented a guideline recommending GBS screening for all pregnant women at 35-37 weeks gestation, with prophylactic intrapartum antibiotic (IAP) treatment administered to those testing positive[2,6]. At least 10% of women who test negative in late-pregnancy GBS culture screening are found to be colonized at delivery[7]. Therefore, rapid, highly sensitive, and highly specific GBS testing is beneficial for pregnant women with negative prenatal screening results, and for women who have not undergone any prenatal GBS screening, particularly in the presence of risk factors such as preterm labor, premature rupture of membranes, or imminent delivery.

Currently, microbial culture remains the gold standard for detecting GBS, which has a sensitivity ranging from 54.3% to 83.3%[8,9] and a lengthy incubation period, requiring 18 to 72 hours to obtain results. The 2010 revised guidelines[6] from the U.S. CDC state that nucleic acid amplification tests (NAATs), such as polymerase chain reaction (PCR), can be used to assess antenatal GBS colonization, with the advantage of rapid turnaround time. Additionally, PCR methods have demonstrated a higher analytical sensitivity in direct comparative studies[9–11], leading to a relative reduction in false-negative results when compared to culture. In recent years, real-time PCR detection

methods for GBS have advanced rapidly, with commercialized GBS molecular detection kits available for prenatal screening. For routine prenatal screening, the standard of care involves incubating vaginal-rectal swabs in selective enrichment broth for 18-24 hours prior to testing. This enrichment step is recommended for most U.S. FDA-approved NAATs to optimize sensitivity and specificity by allowing the recovery of low levels of GBS[12,13]. In contrast, for intrapartum management of women with unknown GBS status, rapid NAAT platform are designed to process unenriched samples directly at the point of care to guide timely antibiotic prophylaxis. However, studies have found the sensitivity of PCR performed without enrichment to be lower than that of PCR performed after enrichment[11,14]. Against this backdrop, this study aims to evaluate the diagnostic performance of a sensitive real-time PCR commercial assay (GBS Nucleic Acid Detection Kit, PCR-Fluorescent Probe Method, Sansure Biotech Inc.) for detecting GBS in vaginal-rectal swabs from pregnant women. It compares this method with microbial culture techniques to assess its sensitivity and specificity, thereby providing a novel approach for rapid clinical diagnosis of GBS.

Methods

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Qingdao Municipal Hospital (approval No. 2024-KY-035) and was conducted according to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Study population

Pregnant women at 35 to 37 weeks of gestation who visited Qingdao Municipal Hospital between August 2024 and November 2024 were enrolled. Obstetricians collected samples from both the vaginal and lower third of the rectum of each patient using two sterile cotton swabs simultaneously. One swab was used for microbial culture, while the other was preserved and immediately sent to the clinical molecular biology laboratory for real-time PCR testing. Microbiological culture results served as the reference standard. Nucleic acid testing was performed by clinical laboratory technicians, with reference results processed blindly. A total of 303 pregnant women were enrolled, but two were excluded: one due to poor sample quality and another who declined participation after

sample collection. The final study cohort comprised 301 pregnant women.

GBS culture

Swab specimens were placed in GBS enrichment Lim broth tubes and incubated at 35°C in a 5% CO_2 incubator for 18–24 hours before transferred to Columbia blood agar plates. Plates were incubated at 35°C with 5% CO_2 for 18–24 hours. Suspicious colonies were screened using Gram staining and catalase testing. Gram-positive cocci and catalase-negative colonies were identified using the Vitek 2 Compact (bioMérieux, Marcy-l'Étoile, France) fully automated microbial analyzer. If no suspicious colonies were observed, incubation time was extended to 48 hours for re-examination and reporting of results.

Real time PCR instruments

The GBS Nucleic Acid Detection Assay (Sansure Biotech Inc., Hunan, China) is a real-time PCR *in vitro* diagnostic tests approved by National Medical Products Administration (NMPA) for the qualitative detection of GBS from vaginal-rectal swabs from pregnant women. DNA was purified using the Natch96 fully automated nucleic acid extraction system (Sansure

Biotech Inc., Hunan, China). GBS DNA amplification was performed using the QuantStudio™5 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The QuantStudio™5 Real-Time PCR System software was used to interpret results , with a detection limit of 39 Ct. The target amplification regions are the conserved domains of the *cfb* and *cps* genes. The kit's analytical sensitivity is no less than 200 copies/mL. The reagent batch used is C24003, with an expiration date of June 2025.

Real time PCR procedures

A volume of 1 mL sterile physiological saline was aseptically added to each specimen collection tube. The tube was then vortexed to ensure complete homogenization. From this suspension, 300 µL was aliquoted for nucleic acid extraction. For the Enrichment real time PCR, the swab was inoculated into selective enrichment broth and incubated. After 18-24 hours, a 300 µL aliquot of the broth culture was subjected to identical nucleic acid extraction and PCR procedures using the same real-time PCR assay. The PCR reaction conditions were as follows: 50°C for 2 min, 1 cycle; 94°C for 5 min, 1 cycle; 94°C for 15 s, 57°C for 30 s, 45 cycles; 37°C for 10 s, 1 cycle. Both DNA extraction and PCR amplification were

performed according to the manufacturer's instructions. For each batch of testing, positive and negative controls were performed. The coefficients of variation (CVs) for both intra- and inter-assay Ct values were less than 5% according to manufacturer.

Sanger Sequencing

Sanger sequencing was performed on 8 discordant samples to resolve discrepancies between the real-time PCR assay and the microbial culture. This was done to confirm the specificity of the PCR signal and to rule out amplification of non-target sequences. The 150 bp conserved region of the cfb gene was amplified using the primers the same with amplification primers according to manufacturer. Unpurified PCR products, along with PCR forward and reverse primers, were used for purification of PCR amplicons and bidirectional Sanger sequencing. The obtained nucleotide sequences were used for BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to perform species classification and identification.

Comparison to Composite Reference Method

A composite reference standard was employed to arbitrate the final classification of each sample as a true positive or true

negative. For samples with any discordant result among the three index tests, definitive classification was determined by Sanger sequencing. A sample was adjudicated as a true positive if sequencing confirmed the presence of GBS-specific DNA, and as a true negative if sequencing yielded a negative or non-specific result.

Statistical Analysis

Data analysis was performed using SPSS 26.0 statistical software. Count data were expressed as percentages. Detection results were summarized in 2×2 contingency tables, and sensitivity, specificity, agreement rate, and Youden index were calculated. Agreement was assessed using the Kappa test. 90-95% sensitivities and ≥95% specificities of the study method are generally considered consistent with the reference method[15]. A consistency Kappa value ≥0.75 indicates high agreement between the study method and the reference method. A Kappa value ≥0.40 indicates basic agreement, while a Kappa value <0.40 indicates no agreement. $P \leq 0.05$ is statistically significant.

Results

Basal Characteristics

The average age of pregnant women was 30 [20.0–45.0] years. Among them, the age of pregnant women in the GBS-positive group was 30 [28.8–31.0] years, while that in the GBS-negative group was 30 [28.0–41.0] years. Due to potential false negatives in microbial culture methods, this study employed sequencing techniques to validate samples where real-time PCR results differed from microbial culture findings. In this study, microbial culture and sequencing were employed as reference method, identifying 26 positive cases with a positivity rate of 8.6%. This indicates that the GBS carriage rate among women in late pregnancy is 8.6% of Qingdao China. Among 301 patients, 35 (11.6%), 27 (8.9%), and 21 (6.9%) demonstrate positive via real-time PCR, real-time PCR after enrichment, and microbial culture, respectively.

Real time PCR Assay vs. Composite Reference Method

The number of positive cases detected by real-time PCR was 35 (11.6%). Compared with the reference method, the sensitivity of real-time PCR was 96.15% (25/26) (95% CI: 78.4–99.7)(Table 1), and the specificity was 96.36% (265/275) (95% CI: 93.2–98.1), with the accordance rate of 96.34% [(25 + 265)/301], Youden index of 0.93, and consistency Kappa index of 0.821 ($P < 0.01$). Real-time PCR detection can detect all

culture-positive samples. However, it failed to detect one sample that were PCR-positive after enrichment, resulting in a false-negative rate of 3.8% (1/26). Ten positive samples were ultimately confirmed as negative, yielding a false-positive rate of 3.64% (10/275).

Real-Time PCR Assay After Enrichment vs. Composite Reference Method

Among 301 samples, 27 (8.9%) tested positive by real-time PCR after enrichment. The sensitivity was 96.15% (25/26) (95% CI: 78.4–99.7)(Table 2), the specificity was 99.27% (273/275) (95% CI: 97.1–99.8), with the accordance rate of 99.0% [(25 + 273)/301], Youden index of 0.95, and consistency Kappa index of 0.938 ($P < 0.01$). One negative sample was culture-positive and direct PCR-positive. Two positive sample results, one of which was confirmed consistent with the result of PCR without enrichment while the other was opposite, were both culture-negative and ultimately confirmed as negative, yielding a false positive rate of 0.72% (2/275).

Microbial Culture vs. Composite Reference Method

Among the 301 samples, microbial culture detected 21 positive samples, as shown in Table 3. Compared with the reference

method, the sensitivity of microbial culture was 80.77% (21/26) (95% CI: 60.0–92.6). Five positive specimens were not detected by microbial culture, resulting in a false-negative rate of 19.23% (5/26). Specificity was 100% (275/275) (95% CI: 98.2–100.0). The accordance rate was 98.33% [(21 + 275)/301], with the Youden index of 0.81 and consistency Kappa index of 0.885 ($P < 0.01$).

Discussion

Timely, rapid, and accurate detection of GBS is a critical component of perinatal prevention measures against neonatal GBS infection. Conventional laboratory testing primarily relies on microbiological culture methods, including direct culture, liquid chromogenic culture, and enrichment culture[15,16]. Enrichment culture remains the gold standard for GBS detection, which is a multi-step process that requires 24-72 hours to complete by trained laboratory technicians. Compared to enrichment culture, direct culture eliminates the enrichment step, offering advantages in reducing reporting time and lowering laboratory costs. However, reports indicate this method has a false-negative rate of 18.5% to 20%[6,17]. Liquid chromogenic culture methods offer advantages such as simple operation, automated detection, and reduced

susceptibility to operator bias. However, they still exhibit a certain rate of missed detection. Therefore, selecting a sensitive and rapid screening method for late-pregnancy GBS has become an urgent priority.

In this study, we demonstrate that a sensitive real-time PCR assay used without prior enrichment culture, exhibits good clinical sensitivity and specificity when compared to real-time PCR testing after enrichment and microbial culture methods. Due to the inherent false-negative rate of microbial culture methods, we performed sequencing confirmation on samples showing discrepancies between real-time PCR and microbial culture results. Cases identified as GBS-positive through microbial culture and those confirmed as GBS-positive via sequencing were both classified as true GBS-positive cases in this study. It is worth noting that in contrast to previous studies[11,12], we find direct real-time PCR without prior enrichment to have a comparable sensitivity to real-time PCR with enrichment. Several factors might have contributed to this finding. First, samples are likely to contain DNA also from non-viable GBS that will be diluted in the enrichment broth. This highlights the difference between detecting nucleic acid presence and viable bacterial growth. Second, the study

population may have had a high initial GBS colonization rate, which diminished the relative advantage of enrichment in improving detection rates. Furthermore, the sample size may have been insufficient to statistically reveal any potential minor differences between the two methods.

The detection rate of the real-time PCR method in this study was 14.38% higher than that of the culture method, which is slightly higher than the 3.7%–4.6% improvement reported in other studies[10,18–20]. The reasons are analyzed as follows: 1. The real-time PCR detection kit used in this study has high sensitivity, with a detection limit of 200 copies/mL. 2. The GBS colonization rate among pregnant women varies across different countries and regions. A 2017 systematic review incorporating studies from 85 countries indicated an overall GBS colonization rate among pregnant women of 18%, with rates of 12.5% and 11% in South Asia and East Asia, respectively[21]. Another meta-analysis incorporating 30 Chinese studies published between 2000 and 2018, involving a total of 44,716 pregnant women, indicated that the GBS colonization rate among Chinese pregnant women was 11.3%[22]. The lower GBS colonization rate observed in the Qingdao population was slightly lower than that reported in the

aforementioned studies, with fewer positive cases identified. This represents a limitation of the research; conducting a large-scale, multicenter study could yield more accurate results.

10 samples of this study initially detected as positive by direct real-time PCR were ultimately confirmed as negative by sequencing, resulting in a false-positive rate of 3.64%. Vaginal-Rectal Swab samples harbor a vast and diverse microbial community. Despite rigorous design and validation of the manufacturer's primer/probe sequences, unexpected homology may still occur, potentially leading to false-positive results. To avoid this situation, Sanger sequencing was performed on samples where PCR results did not match culture findings. Interestingly, only two of the positive samples detected by real-time PCR after bacterial enrichment were ultimately confirmed as negative, resulting in a false-positive rate of 0.72%. The lower false-positive rate of post-enrichment PCR is probably attributable to the dilution of complex vaginal-rectal microbiota during enrichment, which reduces non-target DNA and minimizes cross-reactivity as compared to non-enriched samples. Although handling-related contamination during direct sample processing like vortexing cannot be

entirely excluded. For samples that have undergone bacterial enrichment, operators consciously handle them gently, aware that rough handling could potentially cause contamination. While dilution of vaginal-rectal microbiota appears to be the dominant factor.

PCR methods have great potential to improve GBS detection strategies. The establishment of standard laboratory-based real-time PCR systems relies on qualified laboratories and trained technicians. They require separate nucleic acid extraction and batch analysis, have higher sensitivity and specificity, therefore applying more to antepartum screening. In contrast, despite the lower sensitivity and specificity, direct real-time PCR based on fully automated molecular systems, can deliver results within 60-90 minutes from sample loading and are designed for near-patient testing, applying to intrapartum screening in delivery[8,14]. For routine prenatal screening at 35-37 weeks gestation, where turnaround time is less critical, selective enrichment culture may be complemented in the future by high-throughput laboratory-based NAATs as the new gold standard to improve GBS detection[9,23]. For intrapartum management of women with unknown GBS status, longer turnaround time of standard real-

time PCR and lower sensitivity of direct real-time PCR can not be ignored. As described by Costa et al[24]. and Peng et al[11]., access to a local laboratory operating 24/7 can support the implementation of real-time PCR. Besides, establishing a fully automated PCR detection system based on the sensitive PCR evaluated in the present study might also contribute to diminish sensitivity issues in the future.

While the high sensitivity of the PCR assay is advantageous for detecting colonization, its clinical utility must be balanced against the risk of prompting unnecessary antibiotic prophylaxis due to false-positive results. In our evaluation, the assay demonstrated a specificity of 96.36% without enrichment and 99.27% with enrichment, which is designed to minimize this risk. Additionally, molecular methods have limitations in detecting GBS antibiotic resistance genes, making information regarding penicillin and β-lactam antibiotic allergies particularly important[25–28]. Therefore, microbial culture remains the primary method for GBS screening in most medical institutions. A reflexive diagnostic workflow is proposed here to optimize both rapidity and clinical utility. Initial testing with real-time PCR is performed first. For PCR-positive results—particularly in the context of

penicillin allergy—the same enriched broth (if available) or a parallel sample should undergo reflexive culture to isolate the organism for antimicrobial susceptibility testing (AST). Also, early studies often highlighted the high cost of direct real-time PCR for GBS. With technological maturation and economies of scale, the upfront expense has decreased. A true cost-effectiveness analysis should factor in local costing structures, laboratory workflow, and the clinical-economic benefit of faster time-to-result.

Studies indicate that many GBS-EOD cases involve newborns whose mothers tested negative for GBS colonization during late pregnancy[7]. Rapid diagnosis is beneficial when providing GBS results to pregnant women who have not undergone perinatal GBS testing but are at risk of preterm birth, premature rupture of membranes, or imminent delivery. Real-time PCR demonstrates considerable value, capable of being integrated into routine screening processes to enhance screening accuracy[11]. Point-of-care testing (POCT) based on real-time PCR will not require specialized PCR laboratories, offering shorter turnaround times, and fewer technical requirements[29], which supports clinical decision-making.

Conclusion

This study demonstrated that the sensitive real-time PCR detection assay (Sansure Biotech) exhibits favorable clinical parameters and strong overall consistency compared to GBS microbial culture (a laboratory standard) and even real-time PCR testing after enrichment. Combined with its short turnaround time and rapid detection speed, it possesses the characteristics required for rapid prenatal diagnostic testing. A critical next step is the large-scale and multi-center validation of both direct real-time PCR as a intrapartum reference standard and the development of consensus guidelines to ensure its standardized application in clinical practice.

Data availability

The data supporting the reported results are available on request from the corresponding author.

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Author contributions

XCW and JSY designed the study; XCW, KZ and SZ performed the experiments; XCW and NNW analyzed the data; JYW, KZ and NNW participated in the clinical specimen detection. XCW and JSY wrote the manuscript. JYW and KZ revised the manuscript. All authors have read the manuscript and approved to submit to this journal.

Competing interests

The authors declare no competing interests.

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Table 1. Diagnostic performance of High-sensitivity Real Time PCR Assay compared with Composite Reference Method.

Group B Streptococcus (GBS) Diagnostic performance		Composite Reference Method		Total
		positive	Negative	
High-sensitivity real time PCR assay	positive	25	10	35
	Negative	1	265	266
Total		26	275	301
Sensitivity (%, 95% CI)		96.15 (78.4–99.7)		
Specificity (%, 95% CI)			96.36 (93.2–	

			98.1)	
Accordance Rate (%, 95% CI)				96.34 (93.3-98.0)
Youden Index				0.93

Table 2. Diagnostic performance of Real-Time PCR Assay after enrichment compared with Composite Reference Method.

Group B Streptococcus (GBS) Diagnostic performance		Composite Reference Method		Total
		positive	Negative	
High-sensitivity real time PCR assay	positive	25	2	27
	Negative	1	273	274
Total		26	275	301
Sensitivity (%, 95% CI)		96.15 (78.4–99.7)		
Specificity (%, 95% CI)			99.27 (97.1–99.8)	
Accordance Rate (%, 95% CI)				99.00 (96.8-99.7)
Youden Index				0.95

Table 3. Diagnostic performance of Microbial Culture compared with Composite Reference Method.

Group B Streptococcus (GBS) Diagnostic performance		Composite Reference Method		Total
		positive	Negative	
Microbial Culture	positive	21	0	21
	Negative	5	275	280

Total	26	275	301
Sensitivity (%, 95% CI)	80.77 (60.0–92.6)		
Specificity (%, 95% CI)		100 (98.2–100.0)	
Accordance Rate (%, 95% CI)			98.33 □95.9-99.3□
Youden Index			0.81