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Research

Performance Assessment of the GenomEra™ Assay in Detecting Group B Streptococcus in Vaginal and Rectal Samples

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Abstract

Background

Group B streptococcus (GBS), is one of the principal causes of severe neonatal infections. The most important risk factor for EOGBS is the vaginal colonization causing vertical transmission of bacteria to the infant during labor and delivery. Identification of pregnant women colonized with GBS is essential in the prevention of early onset neonatal sepsis (EOGBS). The current culture-based methodfor detection of GBS is less sensitive and time-consuming. Multiple assays have been developed in order to establish rapid and efficient screening test for detection of GBS.

Objective

To evaluate the performance of a PCR assay, the GenomEra™ GBS assay (Abacus Diagnostica, Finland), as a direct and rapid method for detection of GBS in vaginal or rectal samples, by using the culture-based method as reference.

Methods

One hundred fifty-nine (159) unidentified vaginal and rectal samples were selected on the basis of culturing-results obtained from the clinical department of microbiology at Karolinska University Hospital. Samples were directly (without prior enrichment) analyzed with the GenomEra™ GBS assay.

Results

The PCR assay resulted inthe sensitivity of 83.9% and the specificity of 94.9%, with aPPV and the NPV of 91.2% and 90.2%, respectively. The assay had a turnaround time of 1 hour.

Conclusion

The PCR assay provides a rapid alternative for screening of women for GB Sduring the delivery, thus enabling targeted prophylaxis of GBS positive mothers.

Keywords: Group B Streptococci; Early-onset Neonatal Infection; Polymerase Chain Reaction; Rapid Intrapartum Assay; GenomEra®

Introduction

Streptococcus agalactiae (group B streptococcus, GBS) is one of the main causes of serious neonatal infections with high morbidityand mortality [1]. In adults, GBS is found in the normal microbiota of the urinary, genital, and lower gastrointestinal tract [1]. The prevalence of asymptomatic colonization with GBS among pregnant women ranges from 15 to 40% [2-4]. GBS colonization during pregnancy is recognized as one of the principal causes of neonatal sepsis and meningitis among the newborns [5].

Manifestation of a neonatal infection with GBS can occur during two different periods: the early onset disease (EOD) appears in the first week of life (<7 days), and the late onset disease (LOD) after the first week to the third month after birth (>7–90 days). In EOD, the newborns are contaminated during birth by the bacteria present in their mother's vagina. Sepsis and pneumonia are the most common clinical syndromes of EOD. In contrast to EOD, in LOD the source of GBS infection cannot

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always be clarified; about 50% of infants with LOD however carry the same GBS serotype as their mother [2,6].

In the 1980s, several studies showed that intrapartum antibiotic prophylaxis (IAP) during labor is very effective in preventing neonatal GBS infections and for reducing the incidence of EOD [7,8]. In 1996, the Centers for Disease Control and Prevention (CDC) recommended the use of IAP for women identified as carriers of GBS by antepartum cultures [9]. After wide spread introduction of this prophylaxis, the incidence of EOD in the U.S. decreased from 1.7 cases/1000 live birthsto 0.34–0.37 cases/1000 live births [10,11].

Since 2002, CDC recommends the screening of pregnant women at 35-37 weeks of gestation and IAP for any women carrying GBS [9]. Antepartum screening is thus limited to women who deliver after 35 weeks. However, newborns that are born earlier are at higher risk for neonatal GBS infection [10]. Another limitation of screening several weeks antepartum is that GBS colonization can be transient or intermittent [12]. Consequently, screening results might not reflect the mother's status during delivery and thus antibiotic prophylaxis cannot always be properly targeted.

In addition to U.S., the screening strategy is applied in most European countries and Australia [13,14]. Another strategy is risk-based prophylaxis, which is used in some countries with low prevalence of EOD, such as Sweden, the Netherlands, and the United Kingdom. Risk factors include preterm birth (<37 weeks), rupture of membranes ≥18 h prior to delivery, GBS growth in the urinary tract during pregnancy, previous infant with invasive GBS disease, or fever during labor [13]. Although cost-effective, the risk-based strategy has been proved to be significantly poorer compared with screening strategy in preventing EOD [15].

The gold standard for GBS screening is culture in selective enrichment broth [9], which may require up to 72 hours for detection of GBS thus making it unsuitable for screening during labor. Therefore, several different PCR assays targeting GBS-specific genes have been developed [16-21]. A direct method that could be performed at time and place of labor and without enrichment step could be beneficial to rapidly screen women and to start appropriate prophylaxis for GBS-positive mothers. An optimal method would detect low-level colonization of GBS, be specific for GBS and have a short turnaround time.

In this study, we evaluated the performance of the GenomEra™ GBS assay for the detection of GBS directly from vaginal/rectal samples without prior enrichment in culture. The gold standard culture-based detection method was used as a reference.

Material and Methods

Samples

This study was performed at the clinical microbiology at Karolinska University Hospital; Huddinge, Sweden. During a period of 7 weeks, rectal and vaginal samples from women sent in eSwab (Copan Diagnostics, Murrieta, CA, USA) were first screened with the culture in selective culture-based method and then stored at -20°C before PCR analysis. Based on the culture results, all GBS positive samples (n=62) as well as 97 negative samples were selected for this study.

Culture-based Method

Vaginal and rectal samples were routinely cultured on selective Crystal Violet blood agar plates (Columbia Blood agar (Alpha Biosciences, Baltimore, MD, USA) with 1.65 µg/ml Crystal Violet (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and 7.5% sheep blood). The samples were also inoculated into a chromogenic GBS agar plate (CHROMagar, Paris, France) and into selective Todd-Hewitt GBS enrichment broth (THB; Becton, Dickinson and Company) containing antibiotics (1 µg/ml gentamicin and 15 µg/ml nalidixic acid) and 5% horse blood. Cultures were incubated for 2 day at 35°C in 5% CO_2. Suspected GBS colonies were identified either by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS; Bruker, Millerica, MA, USA) or with an agglutination test (PathoDxtra, Thermo Scientific, Waltham, MA, USA). In GBS-negative samples, the THB was sub cultured after 1-day incubation onto another Crystal Violet blood agar plate and incubated for 1 day at 35°C in 5% CO_2. Colonies were identified by MALDI-TOF MS.

GenomEra GBS™ Assay

Frozen vaginal/rectal swab samples (n=159), that were previously screened with the culture-based method, were tested with the GenomEra™ GBS assay kit (Abacus Diagnostica, Turku, Finland). The assay is based on the lanthanide chelate label technology [22] and detects a 100 bp-sequence of the GBS-specific *cfb* gene. An internal amplification control (IAC) is included in each run.

Each sample was quick-thawed before sample preparation. An aliquot of 60µl of each homogenous swab sample was transferred into a test tube and vortexed for 5 min. There after, 35µl was transferred onto the test chip and immediately runin the GenomEra CDX™ instrument. Results were automatically reported as *positive* (+) or *negative* (-) by the instrument. In some cases, the results showed borderline or PCR-inhibition. For cases of *PCR-inhibition*, the PCR-assay was repeated with a smaller, 40 µl-sample volume from the same Swab. GenomEra™ GBS assay results were compared to the results from the culture-based method.

Statistical Analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined for the molecular assay by using the culture result as a reference method and they were calculated by using the MedCalc Software Version 16.2.1 (MedCalc Software, Ostend, Belgium).

Results

Of the total 159 clinical swab samples routinely analyzed by the culture method, 62 samples were GBS-positive and 97 samples were GBS-negative **(Table 1).**

Table 1: Agreement between the GenomEra GBS assay and the GBS culture

	GBS Culture		
GenomEra GBS PCR assay	Positive	Negative	Total
Positive	52	5	57
Negative	6	92	98
Borderline	4	0	4
Total	62	97	159

The *cfb* gene was detected in 57 samples while there was no detection of *cfb* gene in 98 samples. Five PCR-positive samples were negative in the culture-based method, regarded as false positive. Of the 62 culture-positive samples, 52 samples were also positive by PCR. Six PCR-negative samples were positive by culture and thus regarded as false negative. These samples remained negative in repeated tests. Of the 97 culture-negative samples, 92 samples were also negative by PCR. In some cases (n=4), the result was reported as borderline. These samples were re-culturedon selective GBS Chromagar plate and GBS were isolated after enrichment. All borderline results were thus culture-positive and, accordingly, regarded as false negative. In some cases (n=3) the PCR reaction was inhibited, but after diluting the samples and repeating the PCR assay, the results were reported as positive.

The sensitivity of the PCR assay was 83.9% and the specificity was 94.9%. The PPV and the NPV were 91.2% and 90.2%, respectively (**Table 2**).

Table 2: Performance of PCR test using vaginal and rectal samples for GBS as the reference standard.

	PCR-GBS if Risk-factor present (N=159)	
	% (n)	95% CI
Sensitivity	84% (52/62)	72.33% to 91.98%
Specificity	95% (92/97)	88.38% to 98.31%
PPV	91% (52/57)	81.48% to 96.09%
NPV	90% (92/102)	83.88% to 94.21%

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value

The preparation time for each sample was approximately 6 min and the runtime for the PCR assay was 50 min, which results in a turnaround time of approximately 1 hour.

Discussion

The main purpose of this study was to evaluate the performance of the GenomEra GBS assay for the detection of GBS directly from vaginal and rectal swab samples, making it more suitable to use during a labor as a point-of-care test. The test performed a sensitivity and specificity of 83.9% and 94.9%, respectively. The GenomEra™ GBS assay has an easy and rapid sample preparation and had a turnaround time of less than 1 hour.

It is a limitation that the PCR was inhibited in few samples (3/159, 1.9%). Valid PCR results were obtained after diluting these 3 samples, however, doubling the turnaround time. Freezing of samples before PCR analysis may also have affected negatively in the sensitivity of PCR. However, twenty frozen positive GBS samples had been tested on the PCR-assay by the laboratory before the study, and all these samples were positive with PCR and no negative freezing effect was observed (data not shown). For discrepant results, a third method (e.g. another PCR method) could have been used for confirmation of the false positive or negative samples.

The 4 cases reported in PCR test as borderline were re-cultured, and all were thus culture-positive. If we considered these as PCR positive, the test would performed a sensitivity of 90% and PPV of 98%.

European consensus guidelines from year 2015 recommend intrapartum point-of-care GBS screening [13]. GenomEra™ GBS assay with short turnaround time and easy-to-use application meets these criteria and could be an option for detection of GBS during labor. Its performance was comparable to other available PCR tests [19,23-26]. Using PCR screening during labor, the inaccuracy of culture screening strategy due to intermittent colonization during pregnancy would be avoided thus enabling targeted prophylaxis to prevent EOD.

Conclusion

The PCR assay provides a rapid alternative for screening of women for GBS during the delivery, thus enabling targeted prophylaxis of GBS positive mothers.

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