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Original Article

Applicability of an in-house extraction protocol in a Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for the identification of *Streptococcus agalactiae* from broth-enriched vaginal/rectal swab specimens

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Carrot broth;
LIM broth

Abstract *Background and purpose:* Early laboratory identification of group B *Streptococcus* (GBS, *Streptococcus agalactiae*) in the birth canal of pregnant women is critical for prompt administration of antimicrobial therapy and may further reduce the mortality rate due to GBS neonatal infection.
Methods: A total of 164 vaginal/rectal swab specimens collected from pregnant women at 35—37 weeks of gestation were screened for GBS vaginal colonization. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Biotyper, Bruker Daltonik GmbH, Bremen, Germany) system was used to detect GBS from Carrot broth

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and LIM broth enrichment using an in-house extraction protocol. The results were compared to those by conventional broth-enriched culture/identification methods as the gold standard. BD MAX™ GBS assay (Becton Dickinson, Sparks, MD, USA) was also performed for Carrot broth-enriched specimen. Discordant results were investigated using the GeneXpert® GBS PCR assay (Cepheid Inc., Sunnyvale, CA, USA).

Results: Using the extraction protocol, 33 (20.1%) of the 164 specimens were positive in Carrot broth, and 19 (11.6%) were positive in LIM broth. Using the culture protocol, 38 (23.2%) samples in Carrot broth and 35 (21.3%) in LIM broth were positive. The sensitivity, specificity, and positive and negative predictive values using the extraction protocol in Carrot broth and LIM broth compared to the gold standard conventional culture/identification method were 86.8% and 50.0%, 100% and 100%, 100% and 100%, and 96.2% and 86.9%, respectively.

Conclusions: The extraction protocol with MALDI-TOF MS from Carrot broth-enriched samples provides a more rapid turnaround time, lower cost, and acceptable sensitivity and specificity to correctly identify pathogens when compared to conventional culture/identification methods.

Introduction

Group B *Streptococcus* (GBS, *Streptococcus agalactiae*) is the primary pathogen causing severe perinatal infection in pregnant women,[1–7] which results in premature rupture of membranes, chorioamnionitis, and other postpartum infections. Group B *Streptococcus* can also cause septicaemia, meningitis, pneumonia, and nervous system sequelae in the foetuses.[3–5,8–16]

Since 1970, GBS screening has been implemented among pregnant women in the United States, and the mortality rate due to neonatal infection decreased from 50% to 4% by 1990. According to the official documents (Morbidity and Mortality Weekly Reports, MMWR) of the US Centers for Disease Control and Prevention (US CDC) in 2002, GBS screening should be performed between weeks 35 and 37 of pregnancy with vaginal/rectal swabs.[3,5,17] Prophylactic antibiotic therapy during pregnancy significantly reduces severe infections caused by GBS in newborns.[4,18] According to statistical data from the National Taiwan University Hospital (NTUH) in Taiwan in 2020, the GBS-positive rate in the birth canal of pregnant women was 25%.

The gold standard for the detection of GBS colonization with vaginal/rectal swabs is to culture samples in a group B selective broth and incubate for 18–24 h. After incubation, the broth is sub-cultured on a blood agar plate and incubated for 18–24 h. The potential β-haemolytic GBS colonies are identified using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) system.[19] However, the turnaround time (TAT) of this method is at least 36 h (4, 15). Recently, both PCR-based detection and TOF peak-based detection of MALDI-TOF MS have been shown to be sensitive and specific alternative methods.[2,5,6,9,10,18,20–24]

Previous studies have used an in-house saponin-based extraction protocol to evaluate the performance of the MALDI-TOF MS system in the identification of bacterial and fungal pathogens in positive blood cultures.[25,26] The overall rate of identification using an in-house saponin-based extraction protocol was 89.9% (364/405) for genus-level identification and 73.1% (296/405) for species-level identification in positive blood cultures.[25] In positive paediatric VersaTREK® blood culture bottles, 83.5% and 92% of the isolates were accurately identified to the species and genus levels, respectively.[21] The results reveal that using our protocol also helps to identify bloodstream infection pathogens 18–24 h earlier than when using sub-cultured colonies. Therefore, in this study, the extraction protocol was compared with the culture method after enrichment by Carrot broth or LIM broth. Two types of commercial quantitative PCR (qPCR) assays were also compared to our routine culture method. The objective of this study was to evaluate the accuracy and efficiency of accelerating the reporting time of results.

Materials and methods

Vaginal/rectal sample collection

A total of 164 vaginal/rectal screening swab specimens collected from women at 35–37 weeks of pregnancy to screen for GBS vaginal colonization were evaluated at National Taiwan University Hospital (NTUH), a tertiary medical centre with 2400 beds located in northern Taiwan, from October to November 2021. The 164 vaginal-rectal specimens were evaluated simultaneously by extraction protocol and conventional culture/identification methods followed by analysis using the MALDI-TOF MS system and a PCR-based assay for the presence of GBS in Carrot broth or LIM broth (Fig. 1).

Conventional culture/identification methods

GBS was detected using vaginal/rectal swabs cultured in a Group B selective broth such as LIM broth (Todd-Hewitt broth with yeast extract, colistin and nalidixic acid; Hardy Diagnostics Inc.; Santa Maria, CA, USA) or Carrot broth

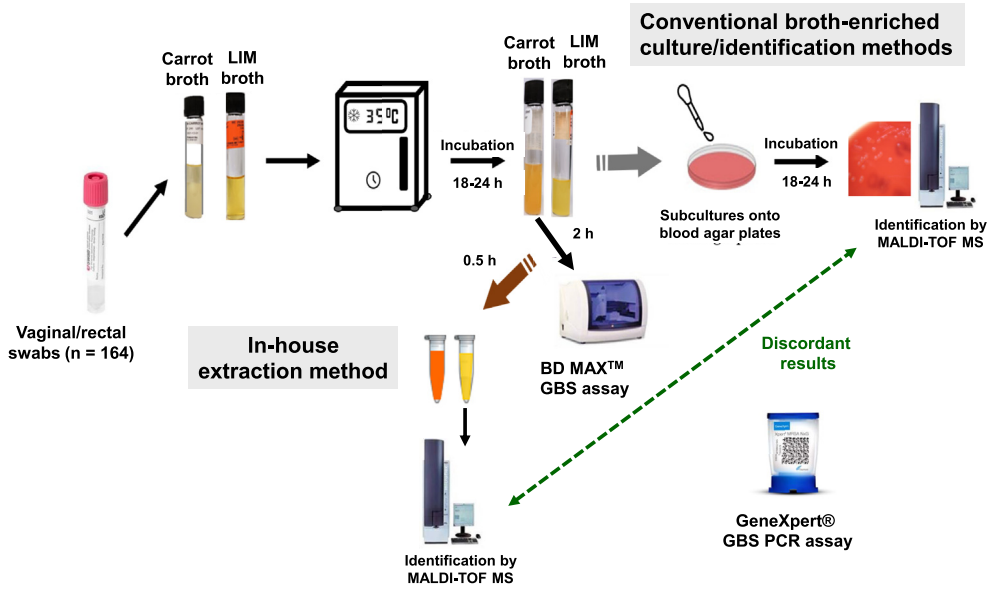

Figure 1. Laboratory workflow of 164 vaginal-rectal screening specimens cultured for the identification of group B *Streptococcus* (GBS, *Streptococcus agalactiae*) by using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The identification results using the conventional broth-enriched culture/identification methods and an in-house extraction protocol were compared. Molecular methods by BD MAX™ GBS assay (for Carrot broth only) and GeneXpert® GBS PCR assay (for discrepant identification results) were applied.

(Hardy Diagnostics Inc.). We added 400 μL of vaginal-rectal specimens into LIM broth and the same amount into Carrot broth. The selective broths were cultured in a 35 °C incubator for 18–24 h, sub-cultured on blood agar plates and incubated overnight. The identification of potential beta-haemolytic GBS colonies was performed by the MALDI-TOF MS (Bruker Biotyper, Bruker Daltonik GmbH, Bremen, Germany) system. Identification scores ≥2.000 indicated species-level identification. Scores ranging from 1.700 to 1.999 indicated genus-level identification. Scores <1.700 indicated no reliable identification.

In-house extraction protocol

After being cultured for 18–24 h in Carrot broth or LIM broth, a 1-mL sample was added to a 1.5-mL microtube (Eppendorf, Hamburg, Germany). The tube was thoroughly centrifuged for 2 min at 13,000×*g*. The supernatant was discarded. The pellet was washed by pipetting with 1 mL of deionized water, and then the solution was centrifuged for 2 min at 13,000×*g*. The supernatant was discarded, and the pellet was subjected to a formic acid extraction protocol for the MALDI-TOF MS system.

BD MAX™ GBS assay

The BD MAX™ GBS assay (Becton Dickinson, Sparks, MD, USA) was performed, an FDA-approved nucleic acid amplification test (NAAT) was used for routine GBS screening, which amplifies a section of the *cfb*-gene target sequence of the GBS chromosome. BD MAX™ GBS assay implemented using the BD MAX™ system (Becton Dickinson, Sparks, MD, USA) is a PCR-based alternative. The system dispenses 15 μL of the specimen enriched by Carrot broth into a microfluidic chamber where real-time PCR amplification and detection are performed.[23] A cycle threshold (Ct) value of ≤37 indicated a positive result. The BD MAX™ GBS assay was designed and licensed to detect GBS DNA in LIM broth only.

GeneXpert® GBS PCR assay

The GeneXpert® GBS assay (Cepheid Inc., Sunnyvale, CA, USA) is indicated for the rapid identification of antepartum and intrapartum GBS colonization; it uses fully automated real-time PCR with fluorogenic detection of a target within a 3′ DNA region adjacent to the *cfb* gene of *S. agalactiae*. In this study, vaginal/rectal swab specimens were transferred to Carrot broth or LIM broth and then cultured in a 35 °C incubator for 18–24 h. The swab was soaked in the selected broth for 1 min and then transferred into the Xpert GBS cartridge and snapped at the scored mark. The test process takes approximately 50 min. A Ct value of ≤41.0 indicated a positive result.

Results

Conventional culture/identification methods

Among the 164 vaginal-rectal specimens collected prenatally and tested by conventional culture/identification methods followed by analysis using the MALDI-TOF MS system, GBS was detected in 38 (23.2%) and 35 (21.3%) from the Carrot broth- and LIM broth-enriched specimens, respectively (Table 1).

Table 1 Comparison of various Group B *Streptococcus* detection methods for 164 vaginal/rectal screening swab specimens from pregnant women at 35—37 weeks. All the specimens were tested simultaneously by an in-house extraction protocol and conventional culture/identification methods followed by analysis using the MALDI-TOF MS system after Carrot broth or LIM broth enrichment. BD MAX™ GBS assay was performed for the presence of GBS in enriched Carrot broth only.

Methods	No. (%) of positive specimens	
	Carrot broth	LIM broth
Conventional culture/identification method	38 (23.2)	35 (21.3)
In-house extraction protocol GBS		
Score values $\geq$ 2.0	22 (13.4)	15 (9.1)
Score values $\geq$ 1.7	33 (20.1)	19 (11.6)
Organisms detected other than GBS	95 (57.9)	137 (83.5)
No any organisms detected	36 (22)	8 (4.9)
BD MAX™ GBS assay	43 (26.2)	NA

NA, Not applicable.

In-house extraction protocol

Using the extraction protocol for Carrot broth, 33 (20.1%) of the 164 specimens were identified as GBS positive (score values $>$ 1.7), 95 (57.9%) were identified as positive for other organisms, and 36 (22.0%) were not any organisms unidentified (Table 1). Using the extraction protocol for LIM broth, only 19 (11.6%) specimens were GBS positive, 137 (83.5%) specimens were positive for other organisms, and 8 (4.9%) specimens were unidentified.

BD MAX™ GBS assay

A total of 43 specimens were found to be positive by BD MAX™ GBS assay in Carrot broth-enriched specimens (Ct values, 12—37) (Table 1). In total, 38 specimens were culture-positive and 33 specimens were extraction-positive in Carrot broth. For two specimens, BD MAX™ GBS assay did not detect any microorganisms whereas the extraction protocol detected *Enterococcus* spp. In total, 119 specimens were BD MAX™ GBS assay-negative; the same was found in extraction and culture methods.

Performance of various GBS detection methods compared to the conventional culture/identification method

The results from the extraction protocol and BD MAX™ GBS assay were compared to the gold standard culture method. Discrepant results were further tested by GeneXpert ® GBS PCR assay. Among 164 samples, thirty-eight (38/164, 23.2%) were detected as positive when using Carrot broth culture as gold standard, but only thirty-five (35/164, 21.3%) were positive when using LIM broth culture (Table 1).

The sensitivities, specificities, and positive and negative predictive values of the extraction protocols for Carrot broth and LIM broth were 86.8% and 50%, 100% and 100%, 100% and 100%, and 96.2% and 86.9%, respectively. The performance of all methods is summarized in Table 2. Extraction protocols performed exceptionally well with a specificity of 100%. BD MAX™ GBS assay had 100% sensitivity. The TAT of the gold standard culture took 48—72 h, the longest time in this study. The TATs of the extraction protocols from Carrot broth or LIM broth were 18—24 h.

Discrepancy analysis

Five conflicting results were found between the culture method and BD MAX™ GBS assay (Table 3). These five samples were further tested by FDA-approved GeneXpert ® GBS assay for the detection of GBS in antepartum women. GeneXpert® GBS PCR assay revealed that four specimens were negative in both Carrot and LIM broth, but one was positive (Ct value of 40.3) in LIM broth.

Table 4 shows the results of 19 specimens that were positive according to both culture methods with Carrot broth and BD MAX™ GBS assay but showed different results between Carrot broth and LIM broth when using the extraction protocol or culture method. Carrot broth had 14 positive results (scores $>$1.7) by the extraction protocol. LIM broth had 16 positive results (scores $>$2) by the culture method, but the extraction protocol detected no positive samples.

Discussion

The gold standard for GBS screening recommended by the US CDC in 2002 is the culture-based method.[17] Although this method requires 48—72 h for the identification of suspected GBS colonies, the benefit of this method is that it is easy for susceptibility testing. The US CDC guidelines also provide

Table 2 Performance of the identification of group B *Streptococcus* (GBS, *Streptococcus agalactiae*) from 164 vaginal-rectal screening specimens by an in-house extraction protocol from Carrot broth and LIM broth enrichment compared to the conventional culture/identification methods (see Fig. 1).

	Carrot broth			LIM broth	
Performance	Culture	Extraction	BD MAX™ GBS	Culture	Extraction
Sensitivity	100%	86.8%	100%	92.1%	50%
Specificity	100%	100%	96%	100%	100%
Positive predictive value	100%	100%	88.4%	100%	100%
Negative predictive value	100%	96.2%	100%	97.7%	86.9%

Table 3 Discrepancy analysis for BD MAX GBS assay-positive and conventional culture-negative samples.

Carrot broth		LIM broth		BD MAX™ GBS assay in Carrot broth (Ct value)	GeneXpert® GBS assay (Ct value)	
Identification by MALDI-TOF MS	Score value	Identification by MALDI-TOF MS	Score value		Carrot broth	LIM broth
E faecalis	2.17	*E. faecalis*	1.78	Positive (37)	Negative	Positive (40.3)
E. avium	1.72	*E. avium*	2.06	Positive (27)	Negative	Negative
S. gallolyticus	1.78	*E. faceium*	1.96	Positive (31)	Negative	Negative
E. faecalis	2.25	*E. faecalis*	2.04	Positive (28)	Negative	Negative
E. faecalis	2.23	*E. faecalis*	2.06	Positive (31)	Negative	Negative

Ct, cycle threshold.

Table 4 Discrepancies of identification results using an in-house extraction protocol and conventional culture/identification method in enriched Carrot broth and LIM broth.

	Carrot broth				LIM broth			
	In house Extraction		Conventional culture/identification		In house Extraction		Conventional culture/identification	
	Identification	Score value	Identification	Score value	Identification	Score value	Identification	Score value
1	GBS	1.79	GBS	2.24	*S. vestibularis*	1.84	–	
2	GBS	2.0	GBS	2.27	*E. faecalis*	2.1	GBS	2.34
3	GBS	1.97	GBS	2.28	*E. faecalis*	2.2	GBS	2.42
4	GBS	1.7	GBS	2.42	*E. faecalis*	2.37	GBS	2.29
5	GBS	2.16	GBS	2.14	*E. faecalis*	2.11	GBS	2.28
6	GBS	1.78	GBS	2.3	*E. faecalis*	1.84	GBS	2.26
7	GBS	1.78	GBS	2.37	*E. faecalis*	1.94	GBS	2.35
8	GBS	2.05	GBS	2.38	*E. faecalis*	2.18	GBS	2.43
9	*E. faecalis*	1.85	GBS	2.26	*E. faecalis*	1.98	–	
10	*E. faecalis*	2.32	GBS	2.36	*E. faecalis*	2.19	GBS	2.31
11	GBS	1.93	GBS	2.42	*E. faecalis*	2.19	GBS	2.37
12	*E. faecalis*	2.03	GBS	2.41	*E. faecalis*	2	GBS	2.35
13	GBS	2.02	GBS	2.37	*S. lutetiensis*	1.82	GBS	2.3
14	GBS	1.97	GBS	2.17	*L. garvieae*	1.75	GBS	2.33
15	GBS	2.11	GBS	2.31	*L. garvieae*	1.81	GBS	2.19
16	*E. faecalis*	2.17	GBS	2.33	*E. faecalis*	2.1	–	
17	GBS	1.83	GBS	2.25	-		GBS	2.27
18	*E. faecalis*	2.15	GBS	2.32	*E. faecalis*	2.26	GBS	2.26
19	GBS	1.96	GBS	2.37	*E. coli*	1.91	GBS	2.37

GBS, group B *Streptococcus*.

molecular testing methods utilizing NAAT for GBS detection.[9,23,27–31] Although PCR testing may enhance the detection rates of GBS screening, the specificity and costs must also be considered. To reduce the overall diagnostic processing time for GBS screening and detection, the extraction protocol and a high-volume commercial real-time PCR were compared as potential alternatives to the standard culture method after initial broth enrichment.

Clinical results of the extraction protocol demonstrated the identification of GBS in Carrot broth (n = 33), with slightly lower results than the culture method (n = 38). The performance of Carrot broth versus LIM broth for GBS detection was also compared. The results of the extraction protocol indicated that Carrot broth enrichment was much more sensitive (86.8%) than LIM broth enrichment (50%). Church et al. demonstrated that Carrot broth performance was similar to that of LIM broth but more rapidly detected and differentiated GBS because of the production of an orange–red pigment (i.e., within 24 h).[4] Schreckenberger et al. indicated that the overall the sensitivity and specificity for Carrot Broth were 96.8% and 100%, respectively, and for the LIM Broth method were 93.2% and 99.4%, respectively. One specimen was correctly identified by the Carrot Broth as a non-GBS and considered as false-positive result on LIM Broth since it was latex agglutination positive for GBS.[32] Many studies have also indicated that Carrot broth performed better than LIM broth based on an evaluation of their sensitivity, specificity, positive predictive value, and negative predictive value.[4,30,32–34]

Previous studies have shown that BD MAX™ GBS assay exhibits acceptable sensitivity and specificity.[2,23,29,31] In the present study, forty-three (43/164) women were GBS

positive according to BD MAX™ GBS assay. Two invalid results were obtained from the BD MAX™ GBS assay, but both were identified as *Enterococcus faecalis* by the MALDI-TOF MS system using the extraction protocol in both Carrot and LIM broth (score >1.7). Five conflicting results were found for BD MAX™ GBS assay-positive and culture-negative patients. Since only BD MAX™ GBS assay was performed with Carrot broth enrichment, this could be a recognized limitation of Carrot broth enrichment. To confirm the conflicting data, we used the alternative GeneXpert ® GBS assay for the 5 specimens with conflicting results.[9,20,27,28,35] Four specimens we BD MAX™ GBS assay re negative according to both the standard culture method and the GeneXpert® GBS PCR assay but were positive according to BD MAX™ GBS assay. These BD MAX™ GBS assay results were considered to be "false-positives." According to the BD MAX™ GBS assay package insert, this assay is designed to detect GBS DNA in LIM broth. Additionally, Riedlinger et al. and Andreasen et al. performed BD MAX™ GBS assay, and positive samples in LIM broth were regarded as false-positives.[23,29] Riedlinger et al. emphasized that "false-positive" results were due to nonspecific amplification, and Andreasen et al. noted this as a weakness of BD MAX™ GBS assay, especially in samples with low GBS DNA load or poor DNA integrity.[23,29] In the present study, we observed that false-positive samples from Carrot broth by BD MAX™ GBS assay showed similar results to those mentioned above. One case that was detected as both positive by BD MAX™ GBS assay (Ct of 37) and GeneXpert® GBS PCR assay was considered a positive case. This case was identified as *E. faecalis* in LIM and Carrot broth by both the extraction protocol and the culture-based method. Several studies found that some *Enterococcus* species might mimic *S. agalactiae* when they exhibited a clearly β-hemolytic phenotype (e.g., *Enterococcus gallinarum*, *E. faecalis*, and *Enterococcus durans*).[35,36] However, Shin et al. mentioned that nucleic acid amplification tests had significantly higher sensitivity than culture and potential false-positive results on GeneXpert GBS assay might correlate to relatively high Ct values. False-positive results can lead to unnecessary antibiotic treatment which can have harmful effects.[9]

The extraction protocol identified 22 (22/33) specimens with spectral scores ≥2.0 in Carrot broth and 15 (15/19) specimens in LIM broth. Confidence scores between 1.70 and 2.00 were obtained from 11 (11/33) specimens by the extraction protocol in Carrot broth and 4 (4/19) in LIM broth for GBS detection. None of the samples using the culture method in Carrot broth yielded scores <2.00. In 11 cases with Carrot broth extraction, only 2 of the samples with the extraction assay in LIM broth detected GBS, and 10 of those using the culture method in LIM broth were positive. We also evaluated 43 positive cases of BD MAX™ GBS assay in which the extraction and culture methods were performed in Carrot and LIM broth, and discrepancies were shown in the results data. In total, 19 positive results were determined using the standard culture method in Carrot broth, but only 16 specimens were positive in LIM broth. In the GBS extraction protocol, 14 (14/19) specimens in Carrot broth identified GBS, but none of the specimens in LIM broth were GBS positive. The results showed that Carrot broth enrichment was better than LIM broth enrichment by both the extraction and culture methods, as previously described by Church et al.[30] The results of the extraction protocol showed acceptable confidence scores by the MALDI-TOF MS system both in Carrot and LIM broth, but more GBS pathogens were detected in specimens extracted in Carrot broth.[28] The extraction protocol in the present study showed good performance for GBS identification after Carrot broth enrichment. However, the extraction protocol in the MALDI-TOF MS system identification provided results from 16 to 24 h earlier than that of the sub-cultured colonies and allowed early appropriate antimicrobial therapy towards the GBS pathogen, which may decrease the mortality rate of neonatal infection.

In conclusion, the GBS extraction protocol is an excellent alternative that identifies pathogens in enriched Carrot broth within 24 h, which may reduce medical costs, TAT and the number of personnel needed. Early identification allows antibiotics to be used in the early stage of GBS infection and may prevent early onset of disease. As such, it has the potential to further reduce the incidence of GBS infection.

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Declaration of competing interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.05.003.