Journal of Microbiology, Immunology and Infection 57 (2024) 957–960

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Short Communication

Validation of a modified enrichment broth for efficient screening of group B *Streptococcus* in pregnant women

Daiki Tanno [a,b,c,*], Kyoichi Saito [b,c], Yasuaki Tomii [d], Yukari Nakatsuka [e], Kohei Uechi [f], Kazutaka Ohashi [b], Yukio Yamadera [b], Atsuko Hata [g], Masahiro Toyokawa [a,b,c], Hiroki Shimura [b,c]

[a] *Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, Fukushima, Japan*
[b] *Department of Clinical Laboratory, Fukushima Medical University Hospital, Fukushima, Japan*
[c] *Department of Laboratory Medicine, School of Medicine, Fukushima Medical University, Fukushima, Japan*
[d] *Department of Clinical Laboratory, Aiiku Hospital, Tokyo, Japan*
[e] *Department of Laboratory Medicine, Medical Research Institute KITANO HOSPITAL, PIIF Tazuke-kofukai, Osaka, Japan*
[f] *Division of Clinical Laboratory and Blood Transfusion, University of the Ryukyus Hospital, Okinawa, Japan*
[g] *Department of Pediatrics, Division of Infectious Diseases, Medical Research Institute KITANO HOSPITAL, PIIF Tazuke-kofukai, Osaka, Japan*

Received 9 March 2024; received in revised form 18 June 2024; accepted 25 July 2024
Available online 3 August 2024

KEYWORDS
Streptococcus agalactiae;
GBS screening in pregnant women;
Selective enrichment broth

Abstract We validated a modified enrichment broth that changes its color when group B *Streptococcus* (GBS) grows. No GBS was detected in any of the non-yellow samples. Thus, the non-yellow samples were considered GBS-negative without conducting further examinations, potentially reducing medical costs and workload.

* Corresponding author. Department of Clinical Laboratory Sciences, School of Health Sciences, Fukushima Medical University, 10-6 Sakae-machi, Fukushima City, Fukushima 960-8516, Japan.
E-mail address: dtanno@fmu.ac.jp (D. Tanno).

https://doi.org/10.1016/j.jmii.2024.07.015

Introduction

Streptococcus agalactiae, group B *Streptococcus* (GBS), is one of the causes of severe infections in neonates. As the Centers for Disease Control and Prevention (CDC) stated in 2010 that universal screening for vaginal—rectal GBS colonization in pregnant women using a selective enrichment broth is important,[1] some Asian countries have adopted the universal GBS screening program and have started administering intrapartum antibiotics for high-risk pregnancies to prevent the development of early-onset GBS disease.[2,3] Efficient determination of the GBS colonization status of pregnant women is crucial; therefore, GBS detection methods have been modified and developed worldwide.[4] One of these is Kyokuto enrichment broth (Kyokuto Pharmaceutical Industrial, Tokyo, Japan) developed in 2016, which is widely used in Japan. Kitagawa, one of the researchers affiliated with the manufacturer, validated the GBS detection method using Kyokuto enrichment broth, which fully agreed with the results obtained using the conventional Lim broth.[5] Although based on culture-based testing with small sample size, it was reported that Kyokuto enrichment broth indicates the growth of GBS when its color changes from purple to yellow. However, no GBS was detected from any samples that did not change to yellow.[5,6] In this study, we confirmed that all non-yellow cultured samples were GBS-negative using nucleic acid amplification test (NAAT)-based assay, with a large sample size of specimens from multiple facilities in Japan. Although a subculture is required for each sample when using the conventional Lim broth, the use of Kyokuto enrichment broth can eliminate the subculture procedure or further testing for non-yellow samples, which may lead to efficient GBS screening and reduction of cost and workload.

Methods

Sample collection

Overall, 1839 vaginal—rectal swabs were collected from pregnant women at 35—37 gestational weeks from March 1, 2020 to August 30, 2020, at Fukushima Medical University Hospital, Aiiku Hospital, and Kitano Hospital in Japan. Each specimen was directly inoculated into a Kyokuto enrichment broth and incubated aerobically at 37 °C for 18—24 h. The study protocol was approved by the Ethics Committee of Fukushima Medical University, Fukushima, Japan (approval no. 2019-209).

Enrichment broth and experimental procedure

The Kyokuto enrichment broth is based on the Lim broth supplemented with 10 mg/L colistin, 15 mg/L nalidixic acid with bromocresol purple, and sugar (undisclosed by the manufacturer) that is 100% metabolizable by *S. agalactiae*. Therefore, this enriched broth not only inhibits the growth of Gram-negative bacteria but also indicates GBS positivity by changing its color after incubation; the change of sample color to yellow suggests GBS growth, while the sample color remaining purple or changing to an intermediate color indicates GBS absence. Each cultured broth was categorized based on the color change (Fig. 1) and then subcultured at 37 °C for 18—24 h in 5% sheep blood agar supplemented with colistin and aztreonam (CA agar plate; Kyokuto Pharmaceutical Industrial). GBS was identified by standard culture-based testing according to guidelines,[1,2] i.e., if GBS-like colonies were present, they were identified using a latex agglutination test kit (Prolex "Iwaki" Streptococcus® for Group B, Iwaki, Tokyo, Japan) according to the manufacturer's protocol. Culture-based testing was conducted consecutively at each facility using the same procedure.

NAAT-based assay

Non-yellow samples were analyzed using real-time polymerase chain reaction (PCR; StepOnePlus, Thermo Fisher Scientific, Waltham, MA, USA) targeting the GBS-specific *cfb* gene, which encodes the CAMP factor, according to previous studies.[7—9] Briefly, DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The forward and reverse sequences of the primers and appropriate probe for *cfb* were 5′-TTT CAC CAG CTG TAT TAG AAG TA-3′, 5′-GTT CCC TGA ACA TTA TCT TTG AT-3′ and 5′-(FAM)-CCC AGC AAA TGG CTC AAA AGC-(BHQ1)-3′, respectively. Amplification was performed with one cycle at 25 °C for 10 min, followed by one cycle at 95 °C for 20 s for initial denaturation, followed by 40 cycles at 95 °C for 1 s and 60 °C for 20 s for amplification. The standard curve was calculated using 10-fold serial dilutions of DNA from *S. agalactiae* ATCC 12386. The detection limit of the PCR assay for *cfb* was 1000 CFU/assay. If the threshold cycle (Ct) value was more than 33, amplification of the targeted gene was confirmed by agarose gel electrophoresis. The NAAT-based assay was conducted at Fukushima Medical University.

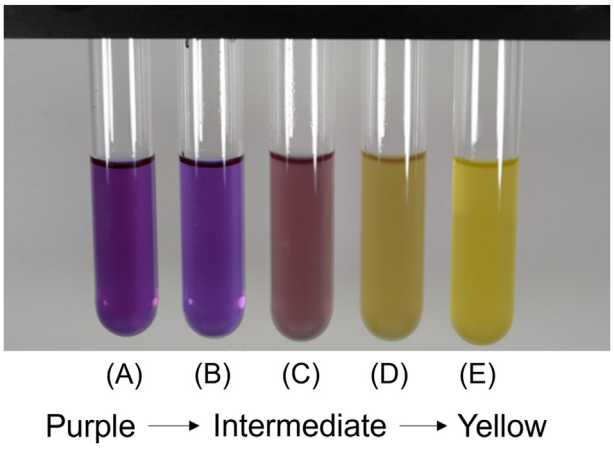

Figure 1. Change in the color of the Kyokuto enrichment broth after 18—24 h of incubation. Initially, the Kyokuto enrichment broth has a purple color. Group B *Streptococcus* (GBS) is present when the broth turns yellow after 18—24 h of aerobic incubation at 37 °C (D, E). In contrast, GBS is considered absent when the color remains purple (A, B) or changes to an intermediate color (C).

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the color of Kyokuto enrichment broth by referring to the results of GBS detection using culture-based testing. Additionally, 95% confidence intervals (CIs) were calculated by using the Clopper–Pearson exact method using R (version 4.3.0).

Results

Of the 1839 vaginal–rectal swab specimens inoculated into the Kyokuto enrichment broth, 77.1% (1417/1839) broths turned yellow, whereas 22.9% (422/1839) broths remained purple or changed to an intermediate color after 18–24 h of incubation (Table 1). Of the 1417 yellowed broths, GBS, including nonhemolytic strains, was detected via subculture in 389 broths. Meanwhile, GBS was not detected in all of 422 non-yellow broths by both culture-based testing and NAAT-based assay using real-time PCR. The sensitivity and specificity of the color change of the Kyokuto enrichment broth to detect GBS were 100% (389/389, 95% CI = 99.1%–100%) and 29.1% (422/1450, 95% CI = 26.8%–31.5%), respectively, whereas the PPV and NPV were 27.5% (389/1417, 95% CI = 25.1%–29.9%) and 100% (422/422, 95% CI = 99.1%–100%), respectively.

Discussion

Based on the results of the current study, it was concluded that GBS was absent in the specimens that did not turn the Kyokuto enrichment broth yellow, which accounted for 22.9% (422/1839) of the total specimens. This conclusion was achieved by simply observing the broth's color and without performing further examinations. Adequate accuracy and efficiency, which are important for GBS screening during pregnancy checkups, may be yielded using Kyokuto enrichment broth. Using Kyokuto enrichment broth can eliminate the subculture procedures that were once necessary to ensure the GBS absence, which would reduce both the workload of personnel and the costs for GBS screening.

Another modified enrichment broth, Granada-type broth,[4] that indicates the presence of GBS by a change in color is the commercially available. The broth is reported to show red-orange pigment specific for GBS growth after 18–24 h of incubation and eliminates additional subculturing. However, the Granada-type broth can detect only hemolytic strains, therefore, negative sample broths that does not change color, should further be tested by either subculture or NAAT to detect nonhemolytic GBS strains.[4] In this study, nonhemolytic GBS strains were detected in 2.8% (11/389) of broths, all of which turned yellow. This shows that the Kyokuto enrichment broth can indicate even the growth of nonhemolytic GBS strains. If cost is disregarded, even more efficient GBS screening can be achieved by combining the Kyokuto enrichment broth and Granada-type broth.

NAAT-based assays have been conducted more frequently for GBS screening because of the publication of the CDC guidelines in 2010. In the CDC survey of 10 states participating in the Active Bacterial Core Surveillance, 82% of laboratories used an enrichment broth before conducting NAAT assays for GBS screening.[10] Meanwhile, the Kyokuto enrichment broth, which is similar to the conventional Lim broth, is applicable for use in NAAT.[8,9] Therefore, the Kyokuto enrichment broth is more efficient because it can exclude GBS-negative samples that do not need any further testing using NAAT-based assays. Furthermore, the Kyokuto enrichment broth may be used at any facility without using any complicated techniques, and it may reduce hospital costs without compromising the detection accuracy.

In conclusion, the adoption of the Kyokuto enrichment broth for GBS screening in pregnant women is recommended because it has the potential to reduce both medical costs and workload without compromising the detection accuracy. This study was based on the evaluation conducted across multiple facilities; however, it should be noted that the results were limited to Japan. Considering the variability in bacterial epidemiology across different regions and countries, further studies are warranted to substantiate our findings. As some countries or regions in Asia have not yet adopted universal GBS screening using a selective enrichment broth for pregnant women, we hope that our study contributes to the widespread adoption of the GBS screening program.

Table 1 Number of yellow and non-yellow samples in which group B *Streptococcus* was detected.

Color of cultured broth		Group B *Streptococcus*		Total
		Positive	Negative	
Yellow		389	1028	1417
Non-yellow	**Intermediate**	0	135	422
	Purple	0	287	
Total		389	1450	1839

Ethical approval

The study protocol was approved by the Ethics Committee of Fukushima Medical University, Fukushima, Japan (approval no. 2019-209).

Funding

This study was funded by Kyokuto Pharmaceutical Industrial, Tokyo, Japan. The sponsor had no control over the interpretation, writing, or publication of this work.

CRediT authorship contribution statement

Daiki Tanno: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Kyoichi Saito:** Supervision, Validation, Writing – review & editing, Visualization. **Yasuaki Tomii:** Investigation. **Yukari Nakatsuka:** Investigation. **Kohei Uechi:** Investigation. **Kazutaka Ohashi:** Investigation.

Yukio Yamadera: Investigation. **Atsuko Hata:** Supervision, Investigation. **Masahiro Toyokawa:** Investigation, Supervision. **Hiroki Shimura:** Funding acquisition, Project administration, Supervision, Visualization, Writing — review & editing.

Declaration of competing interest

All authors report no conflicts of interest relevant to this article.

Acknowledgment

We thank all the staff of Fukushima Medical University Hospital, Aiiku Hospital, and Kitano Hospital for their cooperation in this study.

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