Diagnostic Accuracy of Loop-Mediated Isothermal Amplification Assay for Group B Streptococcus Detection in Recto-Vaginal Swab : Comparison with Polymerase Chain Reaction Test and Conventional Culture

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Background

- *Streptococcus agalactiae*, also known as group B streptococcus (GBS), is the leading cause of newborn infection
  - CDC recommends
    - All pregnant women at 35-37 weeks of gestation : screening for GBS
- Until recently, the standard method for GBS screening : microbiological culture
  - Limitation
    - A long turnaround time and Low sensitivity (only 54-87%)
    - Vaginal GBS colonization can be intermittent during pregnancy
  - Therefore, it is recognized that a rapid, sensitive, and specific GBS test may have benefit during the intrapartum period or following the rupture of membranes.
- Several commercial GBS molecular tests using polymerase chain reaction (PCR) for intrapartum screening.
  - require not readily available equipment and reagents
  - more expensive than those needed for cultures
- The loop-mediated isothermal amplification (LAMP) method can amplify target nucleotide sequences at isothermal conditions (usually 60°C-65°C) within 90 minutes using 4 or 6 primers.
  - A rapid, practical, and relatively straightforward method

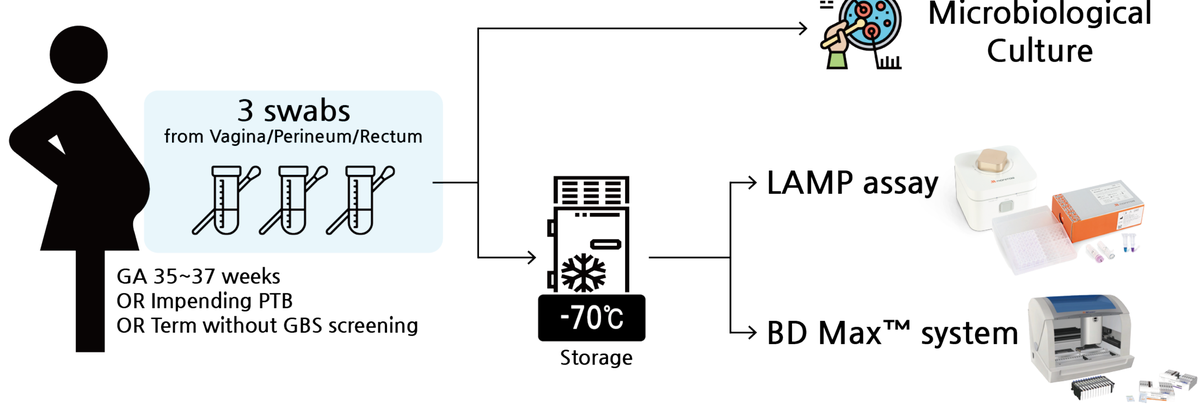
Objective

- To evaluate the diagnostic performance of LAMP assay (Isopollp® easy GBS Detection Kit) for detection of GBS in maternal recto-vaginal swabs and to compare it with PCR testing (BD MAX™ System) and microbiological culture.

Materials and Methods

▶ Study Design and Participants

- between June 2018 and November 2021



▶ Microbiological Culture of GBS

- incubation in BBL™ Lim broth enrichment media
  - 35 to 37°C in ambient air or 5% CO_2 for 18-24 hours
- Sub-culture onto 5% sheep blood agar plate
- Vitek2 & MALDI-TOF MS → to identify a colony
- 16S rRNA sequencing → to confirm the exact identification of bacteria.

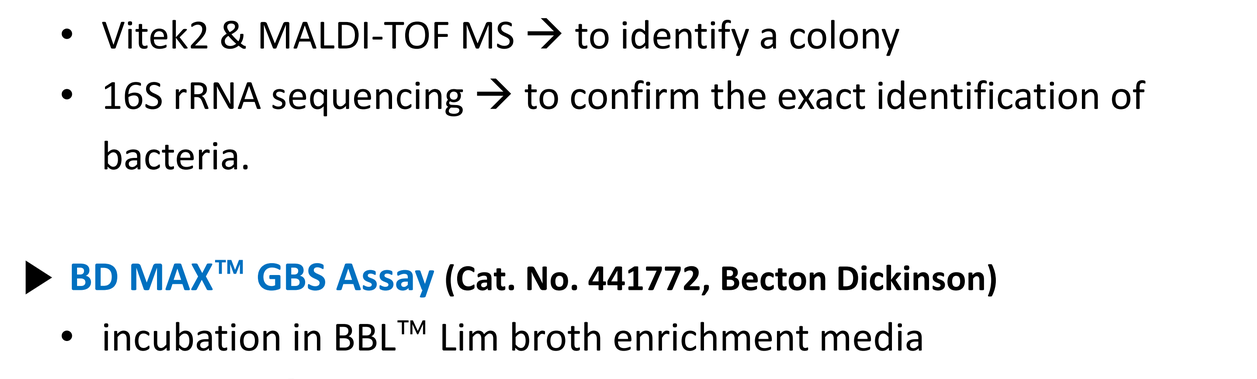
▶ BD MAX™ GBS Assay (Cat. No. 441772, Becton Dickinson)

- incubation in BBL™ Lim broth enrichment media
  - 35 to 37°C in ambient air for 18-24 hours
- 15μL enrichment broth : used for BD MAX
- BD MAX system automatically extracts the nucleic acid using a combination of heat, lytic enzymes, and magnetic capture beads.
- Target gene : ***cfb* gene** sequence of the GBS chromosome

▶ LAMP Assay Using MmaxSure™ EZ GBS Detection Kit

(Cat. No. 52313, Mmonitor, Daegu, South Korea)

- Incubation in BBL™ Lim broth enrichment media (35~37°C for 18-24 hrs)
- Target gene : ***sip* gene**



▶ Statistical Analysis

- BD MAX™ GBS assay : as the reference for the clinical validation
- Sensitivity, specificity, and diagnostic accuracy with a 95% CI
- The results of the microbiological culture compared to LAMP assay
- Statistical analysis was performed using SAS version 9.4.

Results

▶ Basal Characteristics

- Total 527 patients
  - Mean maternal age: 32.4 ± 4.2 years
  - Median gestational age: 36.5 (22.6-39.4) at sampling
    38.6 (24.4-41.1) at delivery
- GBS (+): 23 (4.4%) on microbiological culture
  115 (21.8%) by PCR (BD MAX™ GBS Assay, LAMP assay)

▶ LAMP assay vs. BD MAX™ GBS Assay

- The LAMP assay showed 100% diagnostic accuracy compared to the BD MAX™ System

Table 1. Diagnostic performance of Loop-Mediated Isothermal Amplification (LAMP) assays with reference to BD MAX™.

Group B Streptococcus (GBS) Diagnosis		BD MAX™ GBS Assay		Total
		Positive	Negative	
LAMP assay	Positive	115	0	115
	Negative	0	412	412
Total		115	412	527
Sensitivity (%, 95% CI)*		100 (96.8-100.0)		
Specificity (%, 95% CI)*			100 (99.1-100.0)	
Diagnostic accuracy (%, 95% CI)*				100 (99.3-100.0)

*Wald's with continuity correction.

▶ LAMP assay vs. Microbiological culture

- The LAMP assay showed acceptable sensitivity and specificity considering microbiological culture as the reference.

Table 2. Diagnostic performance of LAMP assay with reference to microbiological culture.

GBS Diagnosis		Microbiological culture		Total
		Positive	Negative	
LAMP assay	Positive	20	95	115
	Negative	3	409	412
Total		23	504	527
Sensitivity (%, 95% CI)*		87.0 (71.0-100.0)		
Specificity (%, 95% CI)*			81.2 (77.6-84.7)	
Diagnostic accuracy (%, 95% CI)*				81.4 (78.0-84.8)

Discussion

- The prevalence of GBS colonization in Korean pregnant women reported a colonization rate increasing during the last three decades.
- Until now, the microbiological culture remains the gold standard screening method for GBS colonization to reduce EOD in neonates.
  - However, it takes more than 48hours.
  - There was a considerable number of pregnant women who delivered their babies without antepartum GBS screening.

  ➔ Rapid and accurate methods for GBS screening is required. We thought our approach may help solving this problem.
- GBS BD MAX™ System is a PCR test including ≥ 18hrs incubation.
  - Provide results in about 2.5 hours after 18hrs incubation
  - Limitation : Requires a PCR machine & NA purification system
- The LAMP assay using MmaxSure™ EZ GBS Detection Kit
  - Good sensitivity and specificity for detection of GBS
  - Moreover, it required shorter turnaround time (60-80 min. after 18 hrs incubation) and simple equipment (heat block for 63°C and simple nucleic purification solutions).

Conclusion

This test could be used in the identification of intrapartum GBS prophylaxis candidates who presented in labor without antepartum GBS result.

※ This study is undergoing minor revisions in *diagnostics* after submission of this abstract.