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COMPARISON OF FOUR COMMERCIAL MOLECULAR ASSAYS FOR DETECTION OF GROUP B *STREPTOCOCCUS* IN ANTEPARTUM RECTAL-VAGINAL SWAB SPECIMENS FOLLOWING BROTH ENRICHMENT

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ABSTRACT

Background: According to the CDC, the incidence of newborn group B streptococcal (GBS) disease has significantly declined since the widespread implementation of prenatal screening of expectant mothers for rectal and vaginal GBS colonization. Screening methods have evolved from largely time-consuming cultivation-dependent approaches to molecular methods that can rapidly provide results by either direct specimen testing or analysis of specimens following broth enrichment. To evaluate the performance of 4 commercially available GBS molecular tests for detection of GBS colonization, we performed a comparative analysis using antepartum rectal-vaginal specimens submitted to our laboratory for routine GBS screening.

Methods: A total of 299 de-identified antepartum rectal-vaginal swab enrichment broth specimens collected from females ranging in age from 16 – 42 years were enrolled in this study. For standard-of-care testing, rectal-vaginal swab specimens were incubated in Lim broth for approximately 18 h prior to analysis by the BD MAX GBS assay. Following analysis, remnant broth from each specimen was stored at 4°C until testing (within 48 h of BD MAX testing) by the ARIES® GBS, *illumigene*® Group B Streptococcus, and Xpert® GBS assays according to their manufacturers' specifications. Data were compared and discrepant results were arbitrated by nucleotide sequencing and culture.

Results: Nine of 299 (3%) specimens yielded discrepancies between methods. The BD MAX assay detected GBS in 150 / 299 (50.2%) specimens and produced 2 false-negative results. The ARIES®, *illumigene*®, and Xpert® assays each detected GBS in 151 (51%), 149 (49.6%), and 151 (51%) specimens, respectively. Five discrepant results (3 false-positives and 2 false-negatives) and one invalid result were obtained by the ARIES® method; upon retesting, a valid result was obtained. Seven discrepancies (4 false-positives and 3 false-negatives) resulted from *illumigene*® testing. The Xpert® assay resulted in 3 discrepancies (2 false-positives and 1 false-negative).

Conclusions: Prevention of newborn GBS disease requires vigilant screening and prophylaxis practices. Chief among the requirements for offering a robust screening program is the use of accurate GBS detection assays. Our comparison of 4 commercial molecular GBS testing platforms revealed that each produces highly accurate results that, for the most part, agree with the results obtained from comparator systems.

BACKGROUND

According to the Centers for Disease Control and Prevention (CDC), approximately 10 – 30% of pregnant women are colonized with Group B *Streptococcus* (GBS; *Streptococcus agalactiae*)(1). GBS is a Gram-positive, catalase-negative, facultative anaerobe that is known to harmlessly colonize the gastrointestinal and genitourinary tracts of humans. In susceptible hosts (e.g., neonates, pregnant women, and those with chronic medical conditions), GBS is a versatile opportunistic pathogen that is capable of causing a variety of diseases, including urinary tract, respiratory, wound, and central nervous infections (2). Human infections generally originate from endogenous sources or are linked to vertical transmission.

Historically, GBS has been a major cause of neonatal infections such as meningitis, pneumonia, and sepsis following vaginal birth from colonized mothers. Based upon the duration of time spanning from birth until the development of signs and symptoms, neonatal GBS diseases are classified as early-, late-, and very-late-onset. Early-onset GBS diseases appear within 1 to 7 days following birth, while late- and very-late-onset GBS infections manifest following 1 week to 3 months and >3 months from birth, respectively. In late- and very-late-onset infections, focal infections such as those of the bone and other sites are not uncommon.

CDC data indicate that since the nearly universal implementation of material prenatal GBS screening in the U.S., the incidence of neonatal GBS infections has been dramatically reduced (e.g., ~80% reduction in early-onset infections). Effective GBS surveillance strategies entail collection of vaginal-rectal specimens from pregnant women between 35 and 37 weeks of gestation (1,3). Swab specimens are subsequently analyzed for GBS by cultivation-based and/or molecular methods. To maximize the sensitivity of molecular assays, most commercially available platforms require broth enrichment prior to analysis. To date, several such tests are available and include both PCR and isothermal DNA amplification methods; however, data comparing these methods are lacking.

The aim of this study was to compare the performance characteristics of 4 commercially available GBS molecular diagnostic tests: the BD MAX GBS assay, the ARIES® GBS assay, the *illumigene* Group B *Streptococcus* assay, and the Xpert GBS assay. To do so, we tested remnant antepartum rectal-vaginal specimens submitted to our laboratory for routine GBS screening.

Table 1. Comparison of the BD MAX™ GBS and the ARIES® GBS assays

		BD MAX™ GBS		
		Positive	**Negative**	**Total**
ARIES® GBS	**Positive**	148	3	151
	Negative	2	146	148
	Total	150	149	299

Statistical test	**Result (95% CI)**
Positive percent agreement	98.70% (94.92% - 99.70%)
Negative percent agreement	98.00% (94.07% - 99.33%)
Positive likelihood ratio	72.53 (18.31 – 287.35)
Negative likelihood ratio	0.02 (0.01 – 0.06)

METHODS

- Clinical specimens
  - A total of 299 de-identified, remnant antepartum rectal-vaginal swab enrichment broths were enrolled in this study.
  - Patient age range: 16 – 42 years (median, 27 years).
- Standard-of-care testing
  - Upon receipt by the IU Health Division of Clinical Microbiology, rectal-vaginal swabs (Liquid Stuart medium; BD, Sparks, MD) were broken off into Lim broth (BD, Sparks, MD) and incubated for 18 – 24 h at 35°C in ambient air.
  - Following incubation, 15-µl aliquots of well-mixed Lim broth from each swab sample were tested by the BD MAX™ GBS assay (BD, Sparks, MD) using the BD MAX™ System according to the manufacturer's specifications.
- Comparator assay testing
  - Following standard-of-care testing, remnant Lim broths were stored at 4°C until testing by comparator methods.
    - All samples were tested within 48 h of BD MAX™ GBS testing.
  - Aliquots of remnant Lim broths were tested using the ARIES® GBS Assay (RUO; Luminex Corp., Austin, TX), *illumigene*® Group B *Streptococcus* Assay (Meridian, Cincinnati, OH), and the Xpert® GBS Assay (Cepheid, Sunnyvale, CA) in singlicate unless invalid results were obtained. In these cases, testing was repeated once more. All testing was performed according to the manufacturers' specifications.
- Discrepancy resolution
  - Discrepant molecular GBS test results were arbitrated by:
    - Bacterial culture
      - Ten-microliter aliquots of Lim broth were plated to sheep blood agar (TSA w/ 5% sheep blood; Remel, Lenexa, KS) and plates were incubated for 24 h at 35°C in 5% CO_2.
      - GBS isolates were identified by latex agglutination testing.
    - Nucleic acid sequencing
      - Total nucleic acids were extracted from Lim broth aliquots using the NucliSENS® easyMag® (bioMérieux, Durham, NC).
      - PCR targeting a distinct region of the GBS genome was subsequently performed and amplicons were sequenced by capillary electrophoresis on an Applied Biosystems 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were analyzed using BLAST.

Table 2. Comparison of the BD MAX™ GBS and the *illumigene*® Group B *Streptococcus* assays

		BD MAX™ GBS		
		Positive	**Negative**	**Total**
***Illumigene*® GBS**	**Positive**	147	4	151
	Negative	3	145	148
	Total	150	149	299

Statistical test	**Result (95% CI)**
Positive percent agreement	98.00% (94.11% - 99.34%)
Negative percent agreement	97.32% (93.23% - 99.00%)
Positive likelihood ratio	48.03 (15.66 – 147.25)
Negative likelihood ratio	0.03 (0.01 – 0.07)

RESULTS

- The BD MAX™ GBS (non-reference standard) detected GBS in 150/299 (50.2%) specimens.
- The ARIES® GBS Assay detected GBS in 151/299 (51%) specimens and produced 5 discrepant results (3 false-positives and 2 false-negatives) when compared to the gold standard. Also, 1 invalid result was obtained.
  - False-positives: In 2/3 (67%) samples, GBS was detected by either culture and/or sequencing.
  - False-negatives: GBS was detected in all samples by either culture and/or sequencing.
  - Invalid: Resolved by repeat testing.
- The *illumigene*® Group B Streptococcus Assay detected GBS in 149/299 (49.6%) specimens and resulted in 7 discrepant results (4 false-positives, 3 false-negatives, and 1 true-positive) when compared to the gold standard.
  - False-positives: In 2/3 (67%) samples, GBS was detected by sequencing.
  - False-negatives: GBS was detected in all samples by either culture and/or sequencing.
- The Xpert® GBS Assay detected GBS in 151/299 (51%) specimens and resulted in 3 discrepant results (2 false-positives and 1 false-negative) when compared to the gold standard.
  - False-positives: In 1/2 (50%) sample, GBS was detected by sequencing only.
  - False-negative: GBS was detected by sequencing only.
- Discrepancy resolution by culture and sequencing detected GBS in 2 samples that the gold standard declared negative.
- Comparative data are given in Tables 1 - 3.

Table 3. Comparison of the BD MAX®™ GBS and the Xpert® GBS assays.

		BD MAX™ GBS		
		Positive	**Negative**	**Total**
Xpert® GBS	**Positive**	149	2	151
	Negative	1	147	148
	Total	150	149	299

Statistical test	**Result (95% CI)**
Positive predictive value	99.3% (95.5% - 99.9%)
Negative predictive value	98.7% (94.9% - 99.7%)
Positive likelihood ratio	146.0 (20.7 – 1,030.0)
Negative likelihood ratio	0.01 (0.00 – 0.05)

CONCLUSIONS

- Highly accurate detection of GBS is paramount to its surveillance. Testing vaginal-rectal swabs following broth enrichment increases molecular test sensitivity.
- Each comparator method evaluated agreed with the gold standard 98% of the time or greater.
- Discrepancy resolution by culture and sequencing detected GBS in 2 samples that the gold standard declared negative, validating the importance of arbitration.
- **Please see the companion poster, *Comparative Evaluation of Commercial Molecular Group B* Streptococcus *Assays to Determine Temporal Aspects of Assay Performance*, for a comparison of time and motion study data from this evaluation.**

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