A Multi-center Clinical Evaluation of the ARIES® GBS Assay, a Sample to Answer Real-Time PCR Assay for the Detection of Group B *Streptococcus* in Antepartum Women

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Abstract

Group B *Streptococcus* (GBS) is a leading cause of serious neonatal infection. Published guidelines recommend universal screening for vaginal and rectal GBS colonization of pregnant women at 35-37 weeks gestation.[1] Enriched culture is the current gold standard for prenatal GBS screening; however, nucleic acid amplification tests (NAATs) are increasingly being adopted in microbiology laboratories to decrease turnaround time and improve detection accuracy. The Luminex ARIES® Group B *Streptococcus* (GBS) Assay is a qualitative real-time PCR assay for detection of GBS in Lim Broth-enriched vaginal-rectal swab cultures. The assay cassette is processed on ARIES® Systems, multiplex test systems that automate nucleic acid preparation from a clinical sample, perform real-time PCR detection and report results in about two hours. In this study, we determined the clinical performance of the ARIES® GBS Assay for vaginal-rectal swab samples inoculated into Lim Broth.

The ARIES® GBS Assay was evaluated using 688 prospective remnant specimens from three U.S. clinical sites. Specimens were de-identified Lim Broth cultures of vaginal-rectal swabs. Reference culture was performed according to CDC guidelines: Lim Broth was incubated for 18 to 24 hours at 35-37°C, subcultured to 5% sheep blood agar (SBA) and incubated 18-24 hours at 35-37°C with 5% CO_2. SBA plates with no GBS growth at 24 hours were incubated for an additional 24 hours before being categorized as negative. Suspected GBS colonies were Gram stained, tested for catalase activity, and confirmed as GBS by latex agglutination. The ARIES® GBS Assay was run according to manufacturer's instructions on enriched Lim Broth specimens that were stored at 2-8°C for ≤24 hours or frozen at -70°C. For discordant analysis, a frozen aliquot was tested by bidirectional sequencing.

When compared to the reference method, clinical sensitivity for the ARIES® GBS Assay was 96.1% (95% confidence interval [CI], 91.3-98.3%) and clinical specificity was 91.4% (95% CI, 88.8-93.5%). Of the 688 specimens, 53 (7.7%) had ARIES® GBS Assay results discordant with the reference method. Bidirectional sequencing showed the ARIES® GBS Assay result to be correct for 47 of the 53. The final sensitivity and specificity for the ARIES® GBS Assay was 98.3% (95% CI, 95.0-99.6%) and 99.4% (95% CI, 98.3-99.9%), respectively.

Results from this clinical evaluation demonstrate that the ARIES® GBS Assay is a sensitive and specific assay for the detection of Group B *Streptococcus* in Lim Broth-enriched vaginal-rectal swab specimens from pregnant women.

Materials and Methods

Specimen Collection and Processing

A total of 688 clinical specimens (vaginal-rectal swabs in non-nutritive liquid transport media) were collected from pregnant women between 35 and 37 weeks gestation who presented at care sites supported by one of the three clinical testing sites located in the United States between May and August 2016. Upon receipt at the laboratory, vaginal-rectal swab specimens were inoculated into Lim Broth and incubated at 35-37°C for ≥18 to ≤24 hours. Following enrichment, an aliquot of the Lim Broth was used for each testing lab's standard-of-care test. Leftover enriched Lim Broth was then blinded and prepared into four aliquots. The first aliquot was used for reference culture testing and the second for ARIES® GBS Assay testing. The remaining two aliquots were kept for potential discrepant analysis by bidirectional sequencing or re-testing.

Reference Culture Testing

The clinical performance of the ARIES® GBS Assay was compared to the culture-based method outlined in MMWR "Prevention of Perinatal Group B Streptococcal Disease, Revised Guidelines from the CDC, 2010".[1] Briefly, enriched Lim Broth specimens were cultured on 5% sheep blood agar (SBA) plates for 24 hours at 35-37°C with 5% CO_2. Following this 24-hour incubation, SBA plates were examined for colonies with morphology and color suggestive of GBS (both hemolytic and non-hemolytic). If no suspicious growth was observed following the initial 24-hour incubation period, the SBA plates were re-incubated for an additional 24 hours. Suspected GBS isolated colonies were Gram stained and tested for catalase production. Colonies of Gram-positive cocci that were catalase negative were confirmed as GBS by latex agglutination.

ARIES® GBS Assay Testing

The ARIES® GBS Assay was performed at each of the clinical sites according to manufacturer's instructions on enriched Lim Broth specimens that were either stored refrigerated (2-8°C) or frozen (-70°C). Fresh specimens were tested with the ARIES® GBS Assay within 24 hours following completion of Lim Broth enrichment. Frozen specimens were tested with the ARIES® GBS Assay within 7 days of completion of Lim Broth enrichment.

Result

TABLE 1: General Demographic Details of the Clinical Study Population (N=688)

Age (yrs)	Site 1 [n (%)]	Site 2 [n (%)]	Site 3 [n (%)]	All Subjects
≤20	10 (9.0%)	15 (7.9%)	56 (14.4%)	81 (11.8%)
21 - 30	67 (60.4%)	120 (63.5%)	225 (58.0%)	412 (59.9%)
31 - 40	33 (29.7%)	54 (28.6%)	102 (26.3%)	189 (27.5%)
>40	1 (0.9%)	0 (0.0%)	5 (1.3%)	6 (0.9%)
Total	**111 (100%)**	**189 (100%)**	**388 (100%)**	**688 (100%)**

TABLE 2: Expected GBS Values by Age Groups and by Sites (N=688)

Age (yrs)	Site 1 (N=111)		Site 2 (N=189)		Site 3 (N=388)	
	Total Positive	Expected Value	Total Positive	Expected Value	Total Positive	Expected Value
≤20	0	0.0%	5	2.6%	15	3.9%
21 - 30	14	12.6%	32	16.9%	59	15.2%
31 - 40	5	4.5%	16	8.4%	23	5.9%
>40	1	0.9%	0	0.0%	2	0.5%
Overall	**20**	**18.0%**	**53**	**28.0%**	**99**	**25.5%**

Note: Expected GBS Values are based on the the prevalence of GBS observed with the ARIES® GBS Assay.

TABLE 3: ARIES® GBS Assay Performance Compared with Reference Culture

ARIES® GBS Assay	Reference Culture Method		
	Positive	Negative	TOTAL
Positive	124	48[2]	172
Negative	5[1]	510	515
TOTAL	129	558	687[3]
		95% CI	
Sensitivity	96.1%	91.3% - 98.3%	
Specificity	91.4%	88.8% - 93.5%	

[1] Two (2) ARIES® GBS Assay negative specimens that were positive by the reference method (i.e., False Negative) were confirmed as negative by bidirectional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® GBS Assay.

[2] Forty-five (45) ARIES® GBS Assay positive specimens that were negative by the reference method (i.e., False Positive) were confirmed as positive by bidirectional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® GBS Assay.

[3] One (1) specimen (01027) generated an invalid result by the ARIES® GBS Assay after allowable re-run. This specimen was negative by the reference method and was excluded from the device performance calculations.

Discussion

- The ARIES® GBS Assay clinical sensitivity was reported to be 96.1% (124/129) with a lower bound 95% confidence interval of 91.3%. Clinical specificity was 91.4% (510/558) with a lower bound 95% confidence interval of 88.8%.
- Five (5) specimens were identified as positive by reference culture method but negative by the ARIES® GBS Assay (i.e., False Negative). Of these, two (2) specimens were confirmed as negative by bidirectional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® GBS Assay. Sensitivity after discordant resolution was 98.3% (95% CI, 95.0-99.6%).
- Forty-eight (48) specimens tested negative by reference culture, but positive by ARIES® GBS Assay. These results may be attributed to low titer organisms or the presence of non-hemolytic colonies that were not visibly detected by culture. Another explanation could be that GBS isolates present in those specimens became non-viable during transport to the testing laboratory, leading to negative culture results and positive identification by molecular methods. All of these 48 ARIES ® False Positive specimens (based on reference culture) were further assessed by bidirectional sequencing using analytically validated primers that targeted genomic regions distinct from the ARIES® GBS Assay. Forty-five (45) of these 48 specimens (93.8%) were positive for GBS by bidirectional sequencing. Specificity after discordant resolution was 99.4% (95% CI, 98.3-99.9%). These results are consistent with published data suggesting that culture is less sensitive than NAATs for the detection of GBS colonization in antepartum women.[2]

Conclusion

Results generated from this investigative study indicate that the diagnostic accuracy of the ARIES® GBS Assay is acceptable for the safe and effective detection of Group B *Streptococcus* in vaginal-rectal swab specimens from pregnant women, following Lim Broth enrichment.

References

(1) Centers for Disease Control and Prevention (2010), Prevention of Perinatal Group B Streptococcal Disease, Revised Guidelines from the CDC, 2010. Morbidity and Mortality Weekly Report 59(RR10); 1-32.

(2) Couturier BA, WeightT, Helmer H and Schlaberg R (2014), Antepartum Screening for Group B *Streptococcus* by Three FDA-Cleared Molecular Tests and Effect of Shortened Enrichment Culture on Molecular Detection Rates. J Clin Microbiol 52: 3429–3432.

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