JCM
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Antepartum Screening for Group B *Streptococcus* by Three FDA-Cleared Molecular Tests and Effect of Shortened Enrichment Culture on Molecular Detection Rates

Brianne A. Couturier,[a] Trent Weight,[b] Haley Elmer,[a] Robert Schlaberg[a,c]

ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah, USA[a]; ARUP Laboratories, Salt Lake City, Utah, USA[b]; Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah, USA[c]

Neonatal *Streptococcus agalactiae* infections cause significant morbidity and mortality, and antenatal screening is recommended. We compared three U.S. Food and Drug Administration (FDA)-cleared nucleic acid amplification tests (NAATs) to culture using 314 vaginal/rectal swabs after 18 to 24 h (recommended period) and 4 to 8 h (shortened period) of broth enrichment. Agreement of the NAATs with each other was high (97.1% to 98.4%), but culture was less sensitive than all NAATs (67% to 73%). A shortened period of broth culture enrichment resulted in 1 false-negative result in 68 (1.5%). The NAATs performed comparably and were more sensitive than culture.

Neonatal *Streptococcus agalactiae* (group B *Streptococcus* [GBS]) infection is a leading cause of sepsis and death in this age group. In the United States, antenatal screening of pregnant women between weeks 35 and 37 of gestation for rectal and vaginal colonization with GBS is recommended by the Centers for Disease Control and Prevention (CDC) and endorsed by the American Society for Microbiology, American College of Obstetricians and Gynecologists, American Academy of Pediatrics, and American Academy of Family Physicians (1–5). Available detection methods include culture, with or without the aid of chromogenic media, and nucleic acid amplification tests (NAATs) (6–9). Several U.S. Food and Drug Administration (FDA)-cleared NAATs are commercially available for testing directly from specimens or after broth culture enrichment (10, 11). Broth enrichment cultures are incubated for 18 to 24 h prior to testing by culture or NAATs (1). NAATs provide shorter turnaround times and higher sensitivity than culture-based identification (1). However, culture is often used as the reference method for determining the performance of different NAATs and is required if antimicrobial susceptibility testing is indicated. The sensitivity and specificity of culture vary by protocol and are operator dependent, making it difficult to compare NAATs tested in different studies (6, 8, 9, 12, 13). Thus, we performed a direct study comparing three FDA-cleared NAATs, BD Max GBS (MAX; Becton, Dickinson), illumigene GBS (Illumigene; Meridian Bioscience), and BD GeneOhm StrepB (GeneOhm; Becton, Dickinson), to each other and to culture-based detection of GBS.

The MAX assay is intended for testing of vaginal/rectal swab specimens from antepartum women after Lim broth enrichment for ≥18 h (11). The illumigene assay has been cleared by the FDA for use with vaginal/rectal swab specimens from antepartum women after broth (Lim, TransVag, carrot) enrichment for 18 to 24 h (10). The GeneOhm assay is intended for direct testing of vaginal/rectal specimens from prepartum or intrapartum women (14) but was used off-label in this study for testing of Lim broth after culture enrichment based on a laboratory validation. For evaluation of samples with initially discordant results, samples were retested with all three assays and the Smart GBS assay (Cepheid; Lim broth protocol) (15). As far as can be determined given the commercial nature of these NAATs, different molecular targets are used for all 4 tests (10, 11, 14). To aid interpretation of discordant results, threshold cycle (C_T) data were retrieved (GeneOhm, Smart GBS) or estimated (MAX) if applicable. Given its endpoint detection design, no C_T data were available for the illumigene assay. Vaginal/rectal swabs ($n = 314$) collected at the University of Utah Hospital and Clinics from pregnant women between weeks 35 and 37 (±3 days) of gestation (University of Utah IRB number 56504) were included for this study. After Lim broth culture enrichment for 4 to 8 h at 37°C, 50 μl of Lim broth was used for off-label GeneOhm testing (16), placed in the lysis tube, subjected to a vortex procedure (5 min), heated to 95°C (2 min), and chilled on a cold block (15 min), and 2 μl was added to 23 μl of diluent for amplification on a SmartCycler (Cepheid) using the manufacturer's instructions (a shortened period of broth culture enrichment). An aliquot (~1 ml) of the Lim broth was further incubated for a total of 18 to 24 h for testing with the MAX, illumigene, and GeneOhm tests as well as for culture-based detection of group B *Streptococcus*. After 18 to 24 h of total incubation, 10 μl was removed for culture on Columbia blood agar plates per CDC guidelines (1). Presumptive GBS colonies were confirmed by latex agglutination (Hardy Diagnostics, Santa Maria, CA) or matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Billerica, MA). The remaining Lim broth was stored at 4°C for a maximum of 7 days prior to NAAT analysis in accordance with package insert instructions. All three NAATs were performed on the same day. Samples with invalid or indeterminate results were retested with the relevant assay, and the first valid result was used

Received 16 April 2014 **Returned for modification** 9 May 2014
Accepted 3 July 2014
Published ahead of print 9 July 2014
Editor: K. C. Carroll
Address correspondence to Robert Schlaberg, robert.schlaberg@path.utah.edu.

doi:10.1128/JCM.01081-14

TABLE 1 Culture detects GBS in fewer samples than any of three FDA-cleared molecular tests[a]

Test	Result	No. of culture results before (after) analysis of discordant results		
		Positive	Negative	Total
GeneOhm	Positive	48 (48)	24 (21)	72 (69)
	Negative	0 (0)	241 (244)	241 (244)
MAX	Positive	48 (48)	24 (20)	72 (68)
	Negative	0 (0)	241 (245)	241 (245)
illumigene	Positive	48 (48)	19 (20)	67 (68)
	Negative	0 (0)	246 (245)	246 (245)
Total		48	265	313

[a] Data represent detection of GBS by culture compared to detection by three FDA-cleared molecular tests for 313 antepartum vaginal/anal swabs before and after (in parentheses) analysis of discordant results. The first valid result is reported. For culture versus MAX, agreement = 93.3%, κ = 0.79. For culture versus illumigene, agreement = 93.6%, κ = 0.79. For culture versus GeneOhm, agreement = 93.0%, κ = 0.78.

for test comparisons. Samples with discordant results were retested simultaneously with the three study NAATs and the Smart GBS assay on the SmartCycler within 7 days of the initial test. A composite standard was determined for each sample using a majority rule for the 6 NAAT results (initial and repeat results obtained with the MAX, illumigene, and GeneOhm tests). In the event of a tie, the composite standard was interpreted as "indeterminate." Results of the Smart GBS test were not used for analysis of discordant results.

Culture was positive for 48 (15.2%) samples, and the NAATs were initially positive for 72 (22.9%; MAX), 66 (21.0%; illumigene), and 72 (22.9%; GeneOhm) (Table 1). Twenty-one samples initially had nonvalid results ("indeterminate" by MAX, $n = 4$, 1.3%; "invalid" by illumigene, $n = 1$, 0.3%; "unresolved" by GeneOhm, $n = 16$, 5.1%). Upon repeat testing, all but one sample (GeneOhm) produced valid results (Table 2). Culture was less sensitive than the NAATs, with a relative sensitivity of 70.6% compared to that of the composite standard. No samples had culture-positive and NAAT-negative results (Table 1).

Agreement between NAATs was generally high, ranging from 98.4% for GeneOhm versus illumigene (κ = 0.95) and 98.1% for illumigene versus MAX (κ = 0.94) to 97.1% for GeneOhm versus MAX (κ = 0.92) (Table 3). Overall, nine (2.9%) samples had discordant results (Table 2). Five were positive by MAX only, three were positive by GeneOhm only, and one was positive by GeneOhm and MAX but negative by illumigene (Table 2). After resolution testing, 68 (21.6%; MAX), 67 (21.3%; illumigene), and 68 (21.6%; GeneOhm) had positive results (Table 1). Based on initial results, sensitivity and specificity compared to those of the composite standard were 100% and 98.4% (MAX), 98.5% and 100% (illumigene), and 100% and 98.4% (GeneOhm). Results were persistently discordant for only two samples (Table 2). Sample 077 had one positive and one negative result with each of the 3 NAATs and a negative result by Smart GBS. Sample 024 was reproducibly positive by GeneOhm and MAX but negative by illumigene. In addition, this sample also had a positive result with Smart GBS; thus, the illumigene result likely represents a false negative. C_T values for both samples are shown in Table 2 and suggest a small amount of target organism. Inconsistent results may thus be due to a concentration of GBS that was close to the detection limit for the NAATs. With one exception (sample 443), all of the remaining samples with initially discordant results had late C_T values, suggesting small amounts of target organism or false-positive initial results. Sample 443 was initially positive by GeneOhm only (C_T of 21 per automatic software interpretation), but no amplification curve could be detected visually. Retesting with the GeneOhm assay produced a negative result. Samples 226 and 228 initially had positive MAX results but were suspected to be false positives due to late C_T values and proximity to positive samples with early C_T values on the same run. Thus, they were retested with the MAX assay only (Table 2).

In conclusion, with a relative sensitivity of 70.6%, culture-based identification of GBS was less sensitive than identification by all three FDA-cleared NAATs after 18 to 24 h of Lim broth enrichment culture. Reported relative sensitivities of culture-based detection and NAATs differ significantly and depend on the specific methods used, but results obtained in this study are consistent with previous reports (1, 6, 7, 16–18). In addition, agreement between the three NAATs was high. Only two (0.6%) samples had consistently discordant results, one likely illumigene false-negative sample (024) and one low-positive sample (077) (Table 2).

TABLE 2 Samples with discrepant results based on three FDA-cleared NAATs[a]

ID	Initial result (C_T)			Repeat result (C_T)			Composite result	Smart GBS result
	GeneOhm	MAX	illumigene	GeneOhm	MAX	illumigene		
432[b]	Inv.	Neg.	Neg.	Inv.	n/d	n/d	Neg.	n/d
010	Pos. (40)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	n/d
168	Pos. (40)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
443	Pos. (21[c])	Neg.	Neg.	Neg.	n/d	n/d	Neg.	n/d
077	Neg.	Pos. (>35)	Neg.	Pos. (40)	Neg.	Pos.	Ind.	Neg.
095	Neg.	Pos. (>35)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
226	Neg.	Pos. (>35)	Neg.	n/d	Neg.	n/d	Neg.	n/d
228	Neg.	Pos. (>35)	Neg.	n/d	Neg.	n/d	Neg.	n/d
426	Neg.	Pos. (>35)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
024	Pos. (33)	Pos. (>35)	Neg.	Pos. (33)	Pos. (>35)	Neg.	Pos.	Pos. (40)

[a] All initial tests and repeat tests were performed on the same day. Threshold cycle values are indicated in parentheses where applicable. ID, identification number; Pos., positive; Neg., negative; Inv., invalid; n/d, not done; Ind., indeterminate.
[b] Excluded from analysis as a valid result could not be obtained for the GeneOhm test.
[c] No amplification curve was detected.

TABLE 3 High degree of agreement for three FDA-cleared tests for detection of GBS after 18 to 24 h of broth enrichment culture[a]

Test and result	No. of samples with indicated result						
	MAX		illumigene		Composite		
	Positive	Negative	Positive	Negative	Positive	Negative	Indeterminate
GeneOhm							
Positive	68	4	67	5	68	0	1
Negative	5	236	0	241	0	244	0
illumigene							
Positive	67	0			67	0	1
Negative	6	240			1	244	0
MAX							
Positive					68	0	1
Negative					0	244	0

[a] Data represent comparisons of the GeneOhm, MAX, and illumigene tests for 313 antepartum vaginal/anal swabs based on the first valid result. One sample consistently produced invalid results with GeneOhm and was omitted from this table. For GeneOhm versus MAX, agreement = 97.1%, $\kappa = 0.92$. For GeneOhm versus illumigene, agreement = 98.4%, $\kappa = 0.95$. For illumigene versus MAX, agreement = 98.1%, $\kappa = 0.94$. For GeneOhm versus the composite standard, agreement = 99.6%, $\kappa = 0.99$. For illumigene versus the composite standard, agreement = 99.4%, $\kappa = 0.98$. For MAX versus the composite standard, agreement = 99.6%, $\kappa = 0.99$.

While broth enrichment for 18 to 24 h increases the sensitivity of GBS screening by culture, it also introduces an increase in the turnaround time. Rapid results may be desirable in cases of incomplete prenatal care or premature labor. However, the effect of a shortened period of broth culture enrichment on test sensitivity is not well understood. We compared the effect of a shortened period of culture enrichment to the recommended overnight period (18 to 24 h) of broth culture enrichment using the GeneOhm assay as an example of NAAT. Results for MAX and illumigene after overnight enrichment culture were used for analysis of discordant results. Cultures with shortened periods of enrichment were incubated for 4 to 8 h or for additional 4-hour increments until visually cloudy (≤8 h in 90% of samples). Of the 313 specimens, 68 (21.7%) were positive at 4 to 8 h whereas 72 (23.0%) were positive at 18 to 24 h. One sample (410) was negative at 4 to 8 h but positive after overnight incubation by all 3 NAATs. Four additional samples (443, 168, 091, and 010) had negative results upon repeat testing by GeneOhm and were likely false positives at the later time point. Another sample (240) was positive at 4 h but negative at 18 to 24 h of incubation. However, no amplification curve was present, and all NAATs at 18 to 24 h were negative, indicating that this might represent a false-positive result at the earlier time point. Thus, after repeat testing, results agreed for 311 of 313 samples (99.4%) and only one false-negative result was obtained after a shortened period of Lim broth culture enrichment. These data suggest that with highly sensitive NAATs, overnight enrichment culture may only provide a small increase in sensitivity compared to a shortened incubation period of 4 to 8 h. A previous study showed increased sensitivity of broth enrichment culture with incubation for at least 6 h compared to shorter incubation times (19). Taken together, these results confirm optimal sensitivity after 18 to 24 h of broth culture enrichment prior to NAAT-based detection and provide evidence for a relative loss of sensitivity in situations where a shortened incubation period may be required or desired. However, given the effect of organism concentrations in original specimens, larger studies will be required to conclusively determine the effect of a shortened period of broth culture enrichment on the sensitivity of NAATs.

ACKNOWLEDGMENTS

This study was funded in part by Meridian Bioscience and Becton, Dickinson, which provided instrumentation and reagent support, and by the ARUP Institute for Clinical and Experimental Pathology.

We thank the Molecular Amplified Detection laboratory at ARUP Laboratories for assistance with specimen preparation.

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