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

Running Title: Detection of GBS by NAAT

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Neonatal *Streptococcus agalactiae* infections cause significant morbidity and mortality and antenatal screening is recommended. We compared three FDA-cleared nucleic acid amplification tests (NAAT) to culture using 314 vaginal/rectal swabs after 18-24 hour (recommended) and 4-8 hour (shortened) broth enrichment. Agreement of NAATs to each other was high (97.1-98.4%) but culture was less sensitive than all NAATs (67-73%). Shortened broth enrichment resulted in 1/68 (1.5%) false-negative result. NAATs performed comparably and were more sensitive than culture.
Neonatal *Streptococcus agalactiae*, or group B *Streptococcus* (GBS), infection is a leading cause of sepsis and death in this age group. In the U.S., antenatal screening of pregnant women between 35-37 weeks 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 (NAAT) (6-9). Several U.S. Food and Drug Administration (FDA)-cleared NAATs are commercially available for testing directly from specimens or after broth enrichment culture (10, 11). Broth enrichment cultures are incubated for 18-24 hours prior to testing by culture or NAATs (1). NAATs provide faster turn-around-time and higher sensitivity compared with culture-based identification (1). However, culture is often used as reference method when determining the performance of different NAATs and is required if antimicrobial susceptibility testing is indicated. Sensitivity and specificity of culture varies by protocol and is operator-dependent making it difficult to compare NAATs tested in different studies (6, 8, 9, 12, 13). Thus, we performed a direct comparison study of three FDA-cleared NAATs, BD MAX GBS (MAX, Becton Dickinson), illumigene GBS (illumigene, Meridian Bioscience), and BD GeneOhm StrepB assays (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 hours (11). The illumigene assay is FDA-cleared for vaginal/rectal swab specimens from antepartum women after broth (LIM, TransVag, Carrot) enrichment for 18-24 hours (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 enrichment culture based on a laboratory validation. For evaluation of samples with initially discordant results, samples were re-tested with all three assays and the Smart GBS (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 cycles (Ct) were retrieved (GeneOhm, Smart GBS) or estimated (MAX) if applicable. Given its endpoint detection design, no Ct’s were available for the illumigene assay. Vaginal/rectal swabs (n=314) collected at the University of Utah Hospital and Clinics from pregnant women between 35 and 37 weeks (+/-3 days) gestation (University of Utah IRB #56504) were included for this study. After LIM broth enrichment culture for 4-8 hours at 37°C, 50µL of LIM broth was used for off-label GeneOhm testing (16), placed in the lysis tube, vortexed (5 minutes), heated to 95°C (2 minutes), chilled on a cold block (15 minutes), and 2µL added to 23µL of diluent for amplification on a SmartCycler (Cepheid) using the manufacturer’s instructions (shortened broth enrichment culture). An aliquot (~1ml) of the LIM broth was further incubated for a total of 18-24 hours for testing with the MAX, illumigene, and GeneOhm tests as well as for culture-based detection of group B Streptococcus. After 18-24 hours of total incubation, 10 µL 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 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 NAAT were performed on the same day. Samples with invalid or indeterminate results were re-tested with the relevant assay and the first valid result was used for test comparison.
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 case of a tie, the composite standard was interpreted as ‘indeterminate’. Results of the Smart GBS test were not used for discordant analysis.

Culture was positive for 48 samples (15.2%), and NAAT were initially positive for 72 (22.9%, MAX), 66 (21.0%, illumigene), and 72 (22.9%, GeneOhm, Table 1). Twenty-one samples initially had non-valid 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 NAATs with a relative sensitivity of 70.6% compared to 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), 98.1% for illumigene versus MAX (κ =0.94) to 97.1% for GeneOhm versus MAX (κ =0.92, Table 3). Overall, nine samples (2.9%) 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 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 and the illumigene result thus likely represents a false-negative. Ct’s for both samples are shown in Table 2 and suggest a low amount of target organism. Inconsistent results may thus be due to a concentration of GBS close to the detection limit for the NAATs. With one exception (sample 443), all of the remaining samples with initially discordant results had late Ct’s suggesting low amounts of target organism or false-positive initial results. Sample 443 was initially positive by GeneOhm only (Ct 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 Ct’s and proximity to positive samples with early Ct’s on the same run. Thus, they were only retested with the MAX assay (Table 2).

In conclusion, with a relative sensitivity of 70.6%, culture-based identification of GBS was less sensitive than all three FDA-cleared NAATs after 18-24 hours of LIM broth enrichment culture. Reported relative sensitivities of culture-based detection and NAATs vary 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 samples (0.6%) had consistently discordant results, one likely illumigene false negative (024) and one low-positive (077) sample (Table 2).

While 18-24 hour broth enrichment culture increases the sensitivity of GBS screening, it also introduces a delay in turn-around-time. Rapid results may be desirable in cases of incomplete prenatal care or premature labor. However, the effect of shortened broth enrichment
culture on test sensitivity is not well understood. We compared the effect of shortened
enrichment culture to the recommended overnight (18-24 hour) broth enrichment culture using
the GeneOhm assay as an example NAAT. Results for MAX and illumigene after overnight
enrichment culture were used for discordant analysis. Shortened enrichment cultures were
incubated for 4 to 8 hours or additional 4-hour increments until visually cloudy (≤ 8 hours in
90% of samples). Of the 313 specimens, 68 (21.7%) were positive at 4-8 hours while 72 (23.0%)
were positive at 18-24 hours. One sample (410) was negative at 4-8 hours but positive after
overnight incubation by all 3 NAATs. Four additional samples (443, 168, 091, 010) had negative
results upon repeat testing by GeneOhm and were likely false positive at the later time point.
Another sample (240) was positive at 4 hours but negative at 18-24 hour incubation. However,
no amplification curve was present and all NAATs at 18-24 hours were negative, indicating that
this may have been 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 shortened LIM broth enrichment culture. These data suggest that with highly sensitive
NAATs, overnight enrichment culture may only provide a small increase in sensitivity compared
to shortened incubation for 4-8 hours. A previous study showed increased sensitivity of broth
enrichment culture when incubated for at least 6 hours compared to shorter incubation times
(19). Taken together, these results confirm optimal sensitivity after 18-24 hours of broth
enrichment culture prior to NAAT-based detection and provide evidence for the relative loss of
sensitivity in situations where shortened incubation 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 shortened broth enrichment culture on sensitivity of
NAATs.
ACKNOWLEDGEMENTS

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REFERENCES


10. Meridian Bioscience. 2012. DNA amplification assay for the detection of Group B *Streptococcus* in vaginal/rectal antepartum specimens. Meridian Bioscience, Cincinnati, OH.


Table 1. Culture detects GBS in fewer samples than any of three FDA-cleared molecular tests. Detection of GBS by culture compared to three FDA-cleared molecular tests for antepartum vaginal/anal swabs before and after (in parenthesis) discordant analysis. First valid result is reported.

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td><strong>GeneOhm</strong></td>
<td>Positive</td>
<td>48(48)</td>
<td>24(21)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0(0)</td>
<td>241(244)</td>
</tr>
<tr>
<td><strong>MAX</strong></td>
<td>Positive</td>
<td>48(48)</td>
<td>24(20)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0(0)</td>
<td>241(245)</td>
</tr>
<tr>
<td><strong>illumigene</strong></td>
<td>Positive</td>
<td>48(48)</td>
<td>19(20)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>0(0)</td>
<td>246(245)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>48</td>
<td>265</td>
</tr>
</tbody>
</table>

Culture vs. MAX: Agreement = 93.3%; κ = 0.79

Culture vs. illumigene: Agreement = 93.6%; κ = 0.79

Culture vs. GeneOhm: Agreement = 93.0%; κ = 0.78
Table 2: Samples with discrepant results based on three FDA-cleared NAAT. All initial testing and repeat testing was performed on the same day. Threshold cycles are indicated in parentheses where applicable.

<table>
<thead>
<tr>
<th>ID</th>
<th>Initial Result</th>
<th>Repeat Result</th>
<th>Composite</th>
<th>Smart GBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GeneOhm MAX</td>
<td>illumigene</td>
<td>GeneOhm MAX</td>
<td>illumigene</td>
</tr>
<tr>
<td>432</td>
<td>Inv. Neg.</td>
<td>Neg. Inv. n/d</td>
<td>Neg. n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>443</td>
<td>Pos. (21*) Neg.</td>
<td>Neg. Neg. n/d</td>
<td>Neg. n/d n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>226</td>
<td>Neg. Pos. (&gt;35)</td>
<td>Neg. n/d Neg.</td>
<td>Neg. n/d n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>228</td>
<td>Neg. Pos. (&gt;35)</td>
<td>Neg. n/d Neg.</td>
<td>Neg. n/d n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

* No amplification curve was detected.

** Excluded from analysis as a valid result could not be obtained for the GeneOhm test.

Pos. – positive; Neg. – negative; Inv. – invalid; n/d - not done; Ind. – indeterminate
Table 3. High degree of agreement for three FDA-cleared tests for detection of GBS after 18-24 hours of broth enrichment culture. Comparison of the GeneOhm, MAX, and illumigene tests for 313 antepartum vaginal/anal swabs based on first valid result. One sample consistently produced invalid results with GeneOhm and was omitted from this table.

<table>
<thead>
<tr>
<th>GeneOhm</th>
<th>MAX</th>
<th>illumigene</th>
<th>Composite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>68</td>
<td>67</td>
<td>68</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>236</td>
<td>0</td>
</tr>
<tr>
<td>illumigene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>67</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>240</td>
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</tr>
<tr>
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<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

GeneOhm vs. MAX: Agreement = 97.1%; $\kappa = 0.92$

GeneOhm vs. illumigene: Agreement = 98.4%; $\kappa = 0.95$

illumigene vs. MAX: Agreement = 98.1%; $\kappa = 0.94$

GeneOhm vs. Composite: Agreement = 99.6%; $\kappa = 0.99$

illumigene vs. Composite: Agreement = 99.4%; $\kappa = 0.98$

MAX vs. Composite: Agreement = 99.6%; $\kappa = 0.99$