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COMPARATIVE EVALUATION OF COMMERCIAL MOLECULAR GROUP B *STREPTOCOCCUS* ASSAYS TO DETERMINE TEMPORAL ASPECTS OF ASSAY PERFORMANCE

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ABSTRACT

Background: Several molecular platforms are commercially available for the detection of Group B *Streptococcus* (GBS) colonization of pregnant women. Although relatively equivalent with respect to analytical accuracy, the hands-on and result turnaround times differ between each system. These temporal parameters are important variables to consider when purchasing such systems, as tests that offer relatively hands-free usage and provide high sample throughput often help mitigate the effects of laboratory staffing shortages and increased workload burdens, respectively. For this study, we compared 4 commercial GBS detection assays with respect to total hands-on time (HoT) and result turnaround time (TAT).

Methods: A comparative analysis of the HoT and TAT for pre-analytical, analytical, and post-analytical tasks was performed using the ARIES® GBS, BD MAX GBS, *illumigene* Group B *Streptococcus*, and the Xpert GBS assays. Briefly, de-identified antepartum vaginal-rectal swab enrichment broth specimens were used to evaluate the assay set-up, run initiation, post-analytical, and total result turnaround times when testing 1, 6, and 12 samples by each platform according to their manufacturer's specifications. Because the *illumigene* system can only accept a maximum of 5 specimens at one time, 2 instruments were used and a total of 3 runs were performed in order to accommodate the testing of 12 specimens; the data from all 3 runs were combined.

Results: The total HoT for performance of single samples was less than 5 minutes (range, 1:55 – 3:32 [min:sec]) for all assays. For assay runs of 6 samples, HoT ranged from 4:45 (ARIES®) – 11:41 (illumigene), and for runs of 12 samples it ranged from 8:42 (ARIES®) – 19:07 (BD MAX). The result TATs varied slightly for each assay when 1, 6, and 12 samples were run, but the overall TATs compared between methods ranged from less than 1:12:00 (h:min:sec) for the *illumigene* platform to approximately 2 h for both the ARIES and BD MAX systems.

Conclusions: The amount of HoT and TAT required to perform GBS testing using the systems described herein is influenced by both the manual complexity of the tests and the quantity of specimens being tested during a single run. Assays that required minimal user manipulation (ARIES®, BD MAX, and Xpert) had shorter HoT, but these tests did not necessarily have shorter TAT. Despite having the longest HoT, the *illumigene* had relatively short TAT. Overall, temporal variables, including those evaluated, should factor into a laboratory's decision making process when purchasing diagnostic systems.

BACKGROUND

Determination of maternal *Streptococcus agalactiae* (Group B Streptococcus [GBS]) colonization status prior to labor and delivery is paramount for guiding appropriate intrapartum antimicrobial prophylaxis. Since the introduction of active GBS prevention, the incidence of early-onset (i.e., within 1 week following birth) neonatal GBS disease has declined by 80% (1). A key component of successful GBS disease prevention is detection of this opportunistic pathogen by analyzing maternal vaginal-rectal swabs obtained between weeks 35 and 37 of gestation (2, 3). Prior to the advent of GBS molecular diagnostics, the mainstay of surveillance depended upon isolating GBS from enrichment broths derived from vaginal-rectal swabs. Despite its relatively high sensitivity, selective culture is slow, requiring up to 48 hours for result reporting. Although this slow turnaround time is tolerable for routine surveillance, it is unsatisfactory when a result is needed much sooner.

Currently, there are several molecular GBS testing platforms that are commercially available as either *in vitro* diagnostic products or as research-use-only systems. These assays employ nucleic acid amplification or nucleic acid probes for detecting GBS-specific genetic determinants in either enrichment broths and/or directly from patient specimens. Depending upon the needs of the laboratory, systems are able to perform single tests if test volumes are relatively low or, for those with higher test volumes, multiple tests simultaneously. In addition, the hands-on time (HoT) of these assays vary from long to short, the latter accounted for by the almost total involvement of automation. Despite these differences, each assay platform is reported to have very high analytical sensitivities and specificities.

Among the factors to consider when purchasing GBS testing systems, the HoT required for tests is important since most laboratories are staffed by scientists who are required to carry out numerous tasks throughout their work-shifts. In laboratories that process large volumes of GBS specimens, test systems that offer relatively hands-free usage and provide high sample throughput often help mitigate the effects of laboratory staffing shortages and increased workload burdens, respectively. However, in small laboratories, systems that require a longer HoT may be acceptable especially if the test volume is low and the physical space required to accommodate large instrumentation is not readily available.

In our study, we were interested in determining both the HoT and the total turnaround time (TAT; HoT plus automation time [AuT]) for a variety of GBS testing systems that included both more and less labor-intensive systems. We evaluated the temporal aspects of testing with each of 4 systems (BD MAX™ GBS, Luminex ARIES® GBS, *illumigene*® Group B *Streptococcus*, and Xpert® GBS) using de-identified enrichment broths leftover from standard-of-care testing at the IU Health Pathology Laboratory in Indianapolis, IN. For specifics regarding the analytical parameters of this study, please see the companion poster, *Comparison of Four Commercial Molecular Assays for Detection of Group B Streptococcus in Antepartum Rectal-Vaginal Swab Specimens Following Broth Enrichment*.

Figure 1. Comparison of HoT for GBS test systems.

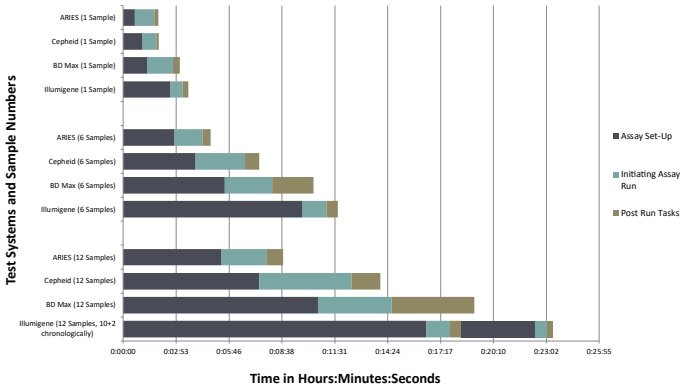
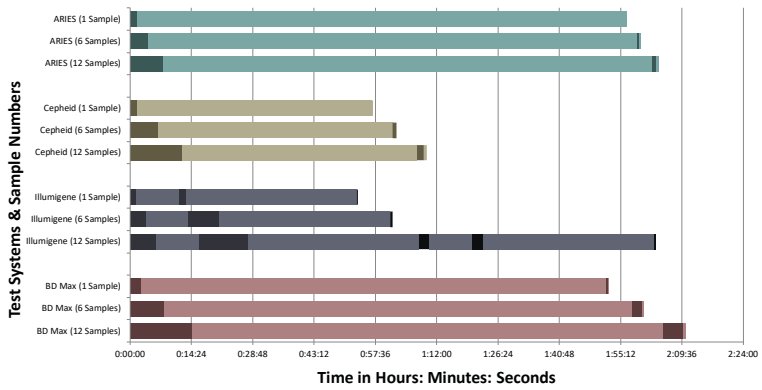


Figure 2. Comparison of total TAT for GBS test systems by sample number. Dark shading in each bar represents HoT whereas light shading indicates on-automation time.



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, vaginal-rectal 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.
- HoT and TAT analyses
  - Set-up, run initiation, analytical, and post-analytical times where measured.
  - HoT was evaluated for individual samples as well as when 6 and 12 samples were ran simultaneously.
    - Because the *illumigene*® system can only accommodate a maximum of 5 samples at once, 2 instruments were used and a total of 3 runs were performed in order to accommodate 12-sample testing. The data from these runs were combined.
  - The total TAT was calculated by adding the HoT and AuT.

RESULTS

- HoT and total TAT are presented in Figures 1 and 2, respectively.
- HoT for 1, 6, and 12 samples:
  - For single samples, the total HoT for all assays was less than 5 minutes (min) and ranged from 1:55 min (ARIES® GBS and Xpert® GBS) – 3:32 min (illumigene® GBS assay).
    - The BD MAX™ GBS assay had a HoT of 3:05 min.
  - For 6 samples ran simultaneously, HoT ranged from 4:45 min (ARIES® GBS) – 11:41 min (*illumigene*® GBS assay)
    - The Xpert® GBS and BD MAX™ GBS assays had HoT of 7:25 min and 10:22 min, respectively.
  - For 12 samples ran simultaneously, HoT ranged from 8:42 min (ARIES® GBS) – 19:07 min (BD MAX™ GBS).
    - The Xpert® GBS and *illumigene*® GBS assays had HoT of 14:00 min and 18:25 min, respectively.
- Total TAT for 1, 6, and 12 samples:
  - The ARIES® GBS assay had total TAT for 1, 6, and 12 samples of 1:56:43 hours (h), 1:59:49 h, and 2:04:06 h, respectively.
  - The BD MAX™ GBS assay had total TAT for 1, 6, and 12 samples of 1:52:16 h, 2:00:31 h, and 2:10:26 h, respectively.
  - The *illumigene*® GBS assay had total TAT for 1, 6, and 12 samples of 53:32 min, 1:01:41 h, and 2:03:23 h, respectively.
  - The Xpert® GBS assay had total TAT for 1, 6, and 12 samples of 56:59 min, 1:02:44 h, and 1:09:39 h, respectively.

CONCLUSIONS

- The ARIES® GBS assay required the least total HoT for testing 1, 6, and 12 samples while the *illumigene*® Group B *Streptococcus* assay required the most HoT when testing these 1, 6, and 12 samples.
- The fastest total TAT for 1 and 6 samples was seen with the *illumigene*® Group B *Streptococcus* assay and the fastest total TAT was recorded with the Xpert® GBS assay for 12 samples. The slowest total TAT was seen with the BD MAX™ GBS assay when 12 samples were tested.
- All systems tested produced actionable results much sooner than conventional selective bacterial culture making molecular GBS testing an ideal solution for GBS surveillance in modern clinical laboratories.
- **Please see the companion poster, *Comparison of Four Commercial Molecular Assays for Detection of Group B Streptococcus in Antepartum Rectal-Vaginal Swab Specimens Following Broth Enrichment*, for details regarding assay performance characteristics.**

REFERENCES

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