Evaluation of the Simplexa and Illumigene Assays for Detection of Group B *Streptococcus*

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Background

Since the 1970's Group B *Streptococcus* (GBS) has been the leading cause of early-onset disease (EOD). GBS can be a dangerous and/or deadly pathogen to the neonatal population. GBS has the potential to cause meningitis, bacteremia, and sepsis as well as other less clinically significant illnesses.

As many as 30% of women are unknowingly colonized with GBS. To help reduce the risk of EOD, a prenatal screen is recommended for pregnant women between week 35 and 37. Specimens for GBS screening are collected on swabs from the vagina and rectums and placed in an enrichment broth for 18-24 hours before testing. Incubating these specimens can increase the detection rate by as much as 50%.

The emergence of molecular testing has reduced the turn-around-times and increased the level of GBS detection. Antibiotics are started sooner reducing the number EOD deaths caused by GBS.

With the availability of a new molecular assay, the Simplexa GBS assay manufactured by Diasorin (Cypress, CA), our laboratory evaluated the assay performance, assay hands-on time and turnaround time.

Materials & Methods

Specimens. A total of one hundred specimens were submitted to the Microbiology Laboratory at Vanderbilt University Medical Center, for routine Group B *Streptococcus* testing. These samples were received in or placed in Lim Broth upon arrival. The Lim Broth tubes were incubated at 37° C with loose lids for 18 – 24 hours. At the end of the incubation, specimens were placed in a refrigerator (2-8° C) until the time of testing.

Discrepant Results. Any specimens not correlating were plated to chromID Strepto B agar by bioMerieux. The plates were held for 48 hours and observed for Group B *Streptococcus*.

Limits of detection. A 1:10 serial dilution was performed. Each sample was then run on the Simplexa and illumigene assays.

Time requirements. Hands-on-time was calculated for each assay. Times for each step assays were recorded. Assay run time was also included.

Results

Only one sample of the 100 samples tested was discrepant between the two methods. The specimen was undetected by the *illumigene* assay, but was detected by the Simplexa assay. A resolution was sought by culturing the specimen on chromID Strepto B agar and incubating for 48 hours. The culture was reviewed with no Group B Streptococcus present. The high outlying CT value for this specimen could be indicative of a nonviable organism.

The limit of detection was compared on five specimens. Results showed a comparable level of detection between both assays. All specimens were within one dilution factor of each other.

Hands-on time was 25 minutes for the Simplexa assay with an overall turn around time of 85 minutes. In comparison 52 minutes hands on time for the *illumigene* assay with an overall turn around time of 102 minutes. The Simplexa assay reduces the need to batch specimens decreasing the overall turn around time.

Table 1. Method Comparison. Using chi-square a p-value of 0.88 was calculated indicating no significant difference in sample reproducibility between the assays.

Method	Positive Samples	Negative Samples	Discrepant Samples
illumigene	32	68	1
Simplexa	33	67	1

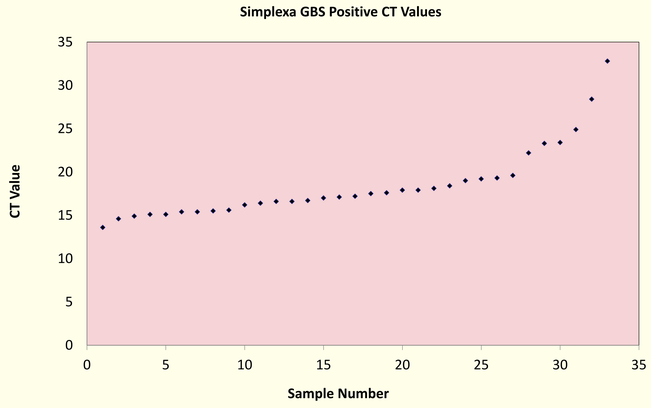

Table 2. Limit of Detection

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Simplexa	1:10,000	1:100,000	1:10,000	1:100,000	1:10,000
illumigene	1:1,000	1:10,000	1:10,000	1:100,000	1:100,000

Table 3. Assay Comparison of Hands-on Time (HOT) and Turn Around Time (TAT). Using chi-square a p-value of 0.052 was calculated showing a significant difference in both hands-on time and the total-turn-around time between the assays.

Simplexa	HOT (min)	TAT (min)	illumigene	HOT (min)	TAT (min)
1 Load Sample well	10	10	1 Prepare Diluent Tube	10	10
2 Load Instrument	5	5	2 Specimen Transfer	4	4
3 Run Time	0	60	3 Vortex (10 seconds)	3	3
4 Analyze and Report Results	10	10	4 Heat Treatment (10 minutes)	0	10
			5 Vortex (10 seconds)	3	3
			6 Specimen Transfer	4	4
			7 Vortex (10 seconds)	3	3
			8 Load Reaction Vessels	10	10
			9 Load Instrument	5	5
			10 Run Time	0	40
			11 Analyze and Report Results	10	10
Total Time	**25**	**85**	**Total Time**	**52**	**102**

Summary

- No significant difference was found in the reproducibility of the Simplexa GBS assay and the illumigene assay.
- The Simplexa GBS assay has similar sensitivity for detection of GBS as the illumigene assay.
- There are fewer steps in the Simplexa assay as compared to the illumigene assay. This reduces the risks of cross-contamination and opportunities for technologists errors.
- The Simplexa assay has significant reductions in both hands-on time and total-turn-around time compared to the illumigene assay. The reduction in hands-on time allows technologists more time to complete other tasks and a higher volume of samples to be tested.