Detection of Group B *Streptococcus* Using the Simplexa™ GBS Direct Kit

R. Martin, A.T. Tran Ha, L. Plascencia, R. Hazelo, Y. Parocua, H. Gregson, P. Naranatt, M. Tabb.

DiaSorin Molecular LLC, Cypress, CA, USA

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Correspondence: Regina Martin
Regina.Martin@diasorin.com

Revised Abstract

Introduction: Group B *Streptococcus* (GBS) is a gram positive bacteria commonly found in the gastrointestinal and urogenital tracts of healthy adults. Approximately 10-30% of all pregnant women are colonized with GBS and transmission to the newborn remains the leading cause of early-onset neonatal sepsis.

The Simplexa™ GBS Direct kit is currently in development to detect GBS from antepartum vaginal/rectal swab samples enriched in Lim broth. This test utilizes real-time PCR amplification without any nucleic acid extraction.

The analytical performance characteristics, including analytical sensitivity/limit of detection (LoD), analytical reactivity, analytical specificity/cross-reactivity, and interfering substances, were determined. Clinical agreement studies compared Simplexa™ GBS Direct kit performance to reference culture according to CDC guidelines. Positive and negative agreement were also determined for Simplexa™ GBS Direct kit versus an FDA cleared Nucleic Acid Amplification Test (NAAT) method.

Methods: The analytical sensitivity/limit of detection was determined for two GBS strains, ATCC 12403 and BAA-1138. Analytical reactivity was performed by testing sixteen (16) GBS strains. In order to evaluate analytical specificity, a panel of 123 bacteria, viruses, fungi, and parasites were tested for cross-reactivity. The potential inhibitory effect of 31 endogenous and exogenous substances on detection of ATCC BAA-1138 was evaluated.

The performance of Simplexa™ GBS Direct kit was compared to reference culture and an FDA cleared molecular NAAT method.

Results: The LoD for the Simplexa™ GBS Direct kit was 46,000 CFU/mL for ATCC 12403 strain and 33,700 CFU/mL for ATCC BAA-1138. Simplexa™ GBS Direct kit was able to detect all 16 strains, which include serotypes I-IX and non-hemolytic strains, at <92,000 CFU/mL tested for analytical reactivity. The kit did not have any cross-reactivity to the 123 microorganisms tested. Simplexa™ GBS Direct kit was able to detect ATCC BAA-1138 in the presence of each potentially interfering substance tested.

In comparison to reference culture, Simplexa™ GBS Direct kit had a positive agreement of 100% and a negative agreement of 88.1%. The performance of Simplexa™ GBS Direct kit compared to an FDA cleared NAAT method demonstrated 99.0% positive agreement and 90.4% negative agreement.

Materials and Methods

Real-time PCR Amplification and Detection: The Simplexa™ GBS Direct kit (MOL3550) contains all-in-one reagent vials for real-time PCR. No sample extraction or preparation is required. Vaginal/rectal swabs were enriched in Lim broth for 18-24 hours according to CDC guidelines. For each reaction on the Direct Amplification Disc (DAD), 50 µL of enriched sample was loaded into the sample port and 50 µL of GBS Direct Reaction Mix was loaded into the reaction port. All testing was performed using the

Materials and Methods (continued)

LIAISON® MDX system.

Limit of Detection: Twenty (20) replicates of each GBS strain ATCC 12403 and BAA-1138 spiked into negative Lim broth matrix were tested with the Simplexa™ GBS Direct kit.

Method Comparison: A panel of clinical specimens from two geographically diverse sites was evaluated using the Simplexa™ GBS Direct kit and reference culture method as well as a commercially available FDA-cleared molecular Nucleic Acid Amplification Test (NAAT) method. Positive and negative agreements were determined.

Analytical Reactivity: Sixteen (16) GBS strains were individually spiked into negative Lim broth matrix at <2X LoD and tested.

Cross-reactivity: The test panel consisted of 10^6 CFU/mL for bacteria and fungi, 10^6 cells/mL for parasites or 10^5 $TCID_{50}$/mL for viruses and was formulated in negative Lim broth matrix.

Potentially Interfering Substances: Each individual substance was spiked into a baseline sample consisting of GBS ATCC BAA-1138 at 1.5X LoD in negative Lim broth matrix.

Results

Limit of Detection was 46,000 CFU/mL or less for GBS strains tested in Table 1.

Table 1. Limit of Detection

GBS Bacterial Strain	Serotype	LoD Concentration (CFU/mL)
BAA-1138	Ia	33,700
12403	III	46,000

Method Comparison – Reference Culture: Positive Agreement was 100%. Negative Agreement was 88.1% (Table 2).

Table 2. Simplexa™ GBS Direct kit vs. Reference Culture

Simplexa GBS Direct	Reference Culture		
	Detected	Not Detected	Total
Detected	94	14[a,b]	108
Not Detected	0	104	104
Total	94	118	212
Positive Agreement	100.0% (94/94) 95% CI: 96.2% to 100.0%	Negative Agreement	88.1% (104/118) 95% CI: 80.9% to 93.4%

[a] 4 samples were GBS positive in an alternate FDA cleared NAAT method
[b] 8 of 11 samples sent for DNA analysis (Agilent Bioanalyzer DNA 1000) had GBS peaks present.

Results (continued)

Method Comparison – FDA cleared NAAT: Positive Agreement was 99.0%. Negative Agreement was 90.4% (Table 3).

Table 3. Simplexa™ GBS Direct kit vs. FDA Cleared NAAT

Simplexa GBS Direct	FDA Cleared NAAT		
	Detected	Not Detected	Total
Detected	97	11[a,b]	108
Not Detected	1[c]	103	104
Total	98	114	212
Positive Agreement	99.0% (97/98) 95% CI: 94.4% to 100.0%	Negative Agreement	90.4% (103/114) 95% CI: 83.4% to 95.1%

[a] 1 sample was GBS positive on an alternate FDA cleared NAAT method
[b] 8 of 11 samples sent for DNA analysis (Agilent Bioanalyzer DNA 1000) had GBS peaks present
[c] Sample was GBS negative on an alternate FDA cleared NAAT method

Analytical Reactivity: All GBS strains tested were detected at 87,000 CFU/mL or lower (Table 4).

Table 4. GBS Strains Tested

ATCC GBS strain	Serotype	Concentration tested (CFU/mL)
49446	4	23,100
51487	1b	184
27591	1c	37,700
BAA-1176	3	55,300
13813	Non-hemolytic	62,000
BAA-2674	3	70,700
BAA-2671	6	87,000
BAA-2672	5	10,700
BAA-2669	8	11,900
12973	2	12,000
BAA-2673	4	12,200
BAA-2666	Non-hemolytic	14,000
BAA-2675	2	17,400
BAA-2668	9	26,000
BAA-611	5	62,000
BAA-2670	7	62,700

Results (continued)

No cross-reactivity was observed with the organisms tested in Table 5.

Table 5. Cross Reactivity Pathogens (representative of the 123 organisms tested).

Abiotrophia defectiva	*Haemophilus influenzae*	*Staphylococcus intermedius*
Acinetobacter baumannii	*Hafnia alvei*	*Staphylococcus saprophyticus*
Aeromonas hydrophila	*Helicobacter pylori*	*Staphylococcus simulans*
Alcaligenes faecalis	*Klebsiella oxytoca*	*Stenotrophomonas maltophilia*
Anaerococcus prevotii	*Klebsiella pneumoniae*	*Streptococcus anginosus*
Bacillus cereus	*Lactobacillus acidophilus*	*Streptococcus canis*
Bacteroides fragilis	*Lactobacillus jensenii*	*Streptococcus dysgalactiae*
Bacteroides stercoris	*Lactobacillus plantarum*	*Streptococcus equi*
Bifidobacterium adolescentis	*Lactobacillus reuteri*	*Streptococcus intermedius*
Burkholderia cepacia	*Lactococcus lactis*	*Streptococcus mitis*
Campylobacter coli	*Legionella pneumophila*	*Streptococcus mutans*
Campylobacter jejuni	*Listeria monocytogenes*	*Streptococcus pneumoniae*
Candida albicans	*Micrococcus luteus*	*Streptococcus pyogenes*
Candida glabrata	*Moraxella catarrhalis*	*Streptococcus salivarius*
Candida parapsilosis	*Morganella morgani*	*Streptococcus sanguinis*
Chlamydia trachomatis	*Mycoplasma hominis*	*Streptococcus uberis*
Citrobacter freundii	*Mycoplasma pneumoniae*	*Trichomonas vaginalis*
Citrobacter koseri	*Neisseria gonorrhoeae*	*Ureaplasma urealyticum*
Clostridium bifermentans	*Neisseria meningitidis*	*Veillonella parvula*
Clostridium butyricum	*Pasteurella aerogenes*	*Vibrio cholerae*
Clostridium difficile	*Peptoniphilus asaccharolyticus*	*Vibrio parahaemolyticus*
Clostridium histolyticum	*Peptostreptococcus anaerobius*	*Yersinia enterocolitica*
Clostridium novyi	*Plesiomonas shigelloides*	*G. lamblia-S. cerevisiae*
Clostridium perfringens	*Porphyromonas asaccharolytica*	*Toxoplasma gondii*
Clostridium sepiticum	*Prevotella melaninogenica*	*Trichomonas vaginalis*
Clostridium sordellii	*Prevotella oralis*	Adenovirus type 40
Clostridium sporogenes	*Proteus mirabilis* Z050	BKV
Clostridium tetani	*Proteus Vulgaris*	Cytomegalovirus
Corynebacterium genitalium	*Providencia stuartii*	Enterovirus 71
Cryptococcus neoformans	*Pseudomonas aeruginosa*	Epstein Barr virus
Enterobacter aerogenes	*Pseudomonas fluorescens*	HHV-6
Enterobacter cloacae	*Pseudomonas putida*	HHV-7 SB
Enterococcus dispar	*Salmonella enterica* subsp. Arizonae	HHV-8
Enterococcus faecalis	*Serratia marcescens*	HIV-1 IIIB
Enterococcus faecium	*Shigella boydii*	HSV-1
Enterococcus gallinarum	*Shigella dysenteriae*	HSV-2
Escherichia coli	*Shigella flexneri*	Human papilloma virus
Escherichia fergusonii	*Shigella sonnei*	JCV
Escherichia hermannii	*Staphylococcus aureus*	Norovirus
Fusobacterium varium	*Staphylococcus epidermidis*	Rotavirus
Gardnerella vaginalis	*Staphylococcus haemolyticus*	VVZ

Results (continued)

No interference was detected with the substances tested in Table 6.

Table 6. Potentially Interfering Substances Tested

calcuim carbonate	bismuth subsalicylate
calcuim carbonate/ magnesium hydroxide	sennosides
aluminum hydroxide/ magnesium hydroxide	clotrimazole cream
mineral oil enema	naproxen sodium
K-Y Yours+ Mine- HIS	nonoxynol-9
K-Y Yours+ Mine- HER	white blood cells
Summers Eve spray	miconazole
Norforms Feminine Deodorant	phenylephrine HCl
esomeprazole magnesium	seminal fluid
Loperamide HCl	barium sulfate
whole blood	metronidazole
Mesalazine	fluconazol
benzalkonium chloride	hydrocortisone
ethanol	stool
palmitic acid	meconium
amniotic fluid	

Conclusions

- The Simplexa™ GBS Direct kit and LIAISON® MDX system from DiaSorin Molecular LLC provide sample-to-answer detection of GBS from unextracted Lim broth enriched vaginal/rectal samples in approximately one hour, with a simple workflow.
- The Simplexa™ GBS Direct kit demonstrated good clinical agreement, was able to detect all serotypes/strains tested, can tolerate various potentially interfering substances, and does not show cross-reactivity to other organisms found in vaginal/rectal swabs.

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