artus® GBS QS-RGQ Kit

Performance Characteristics

artus GBS QS-RGQ Kit, Version 1, REF 4576366

Version management

This document is the *artus* GBS QS-RGQ Kit Performance Characteristics, Version 1, R1.

Check availability of new electronic labeling revisions at www.qiagen.com/p/artus-GBS-QS-RGQ-Kit-CE before test execution.

Limit of detection

The limit of detection (LoD) was assessed for the *artus* GBS QS-RGQ Kit using two serotypes of group B Streptococcus (GBS): GBS Serotype Ia (ATCC® BAA-1177), and Serotype III (ATCC 12403). Each strain was serially diluted in Lim broth plus matrix (Lim cultures inoculated with vaginal/rectal swabs and found to be negative for GBS) using 2-fold dilutions and run in replicates of 20 on the QIAsymphony RGQ. The LoD for each strain was determined by plotting the detection rate against the bacterial input titer and performing probit analysis (Figures 1 and 2).

The limit of detection of the *artus* GBS QS-RGQ Kit is 74.07 CFU/ml for GBS Serotype Ia and 24.29 CFU/ml for Serotype III. This means that there is a 95% probability that 74.07 CFU/ml of GBS Serotype Ia and 24.29 CFU/ml of GBS Serotype III will be detected.

May 2014

Sample & Assay Technologies

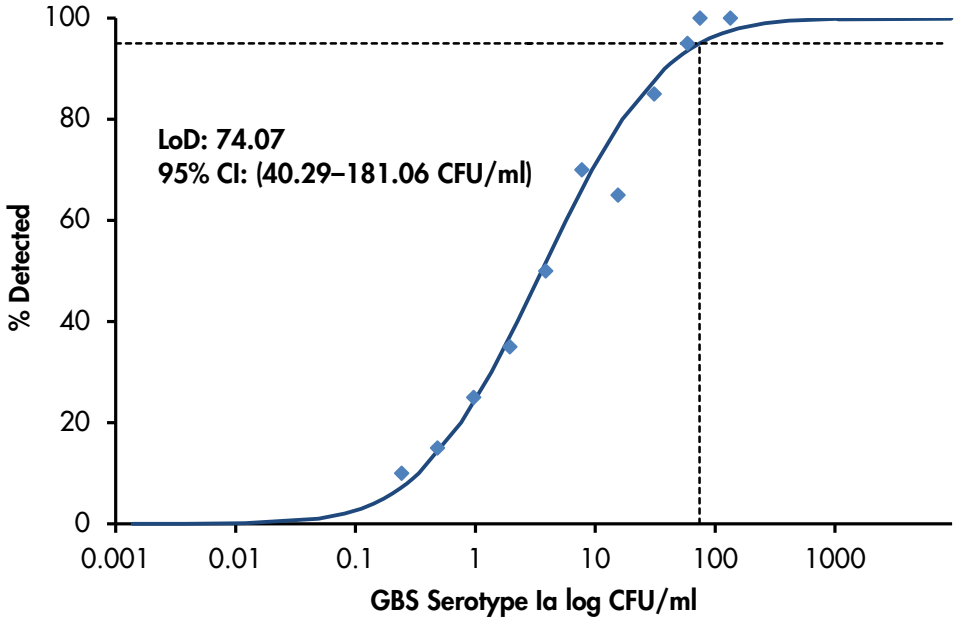

Figure 1. Probit analysis: GBS Serotype Ia. Limit of detection of the *artus* GBS QS-RGQ Kit.

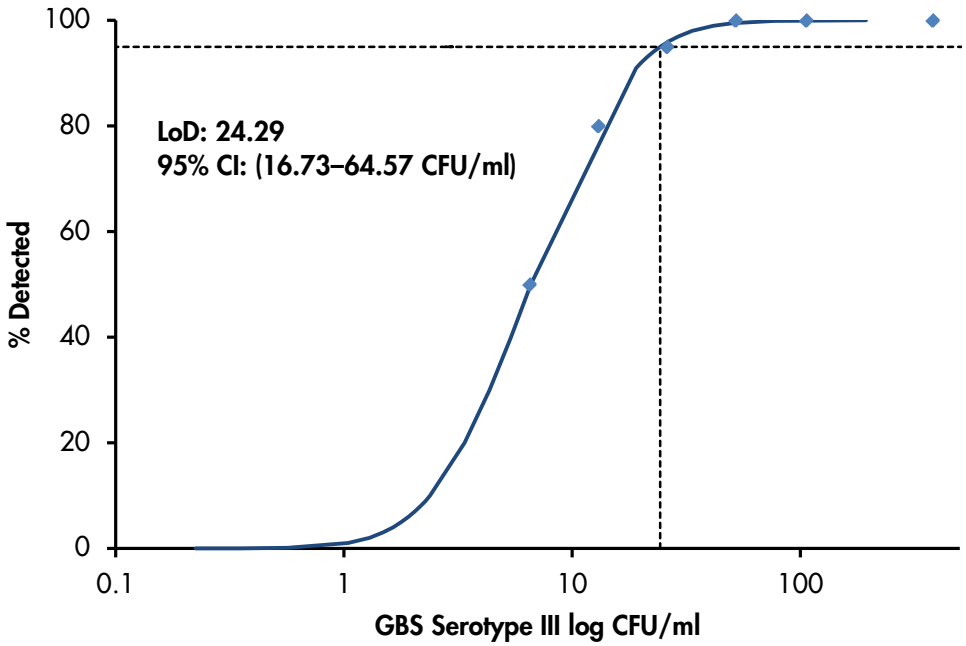

Figure 2. Probit analysis: GBS Serotype III. Limit of detection of the *artus* GBS QS-RGQ Kit.

Analytical reactivity

The analytical reactivity of the *artus* GBS QS-RGQ Kit was assessed to determine whether the kit could detect an array of GBS serotypes and clinical isolates. A total of 21 GBS isolates representing various serotypes (Table 1) were diluted in Lim broth plus matrix to approximately 2–3x LoD and tested with the *artus* GBS QS-RGQ Kit. GBS target was detected in all strains tested.

Table 1. GBS strains tested in analytical reactivity studies

Serotype	Strain ID	Hemolysis	GBS target detected	Internal control
Ib	ATCC*: BAA 1174	Hemolytic	+	+
V	ATCC: BAA 23	Hemolytic	+	+
VI	CDC†: ABC 20028*757*	Hemolytic	+	+
VII	CDC: ABC20013332	Hemolytic	+	+
VIII	CDC: ABC20026068	Hemolytic	+	+
IV	ATCC: 49446	Hemolytic	+	+
Ia	ATCC: BAA1138	Hemolytic	+	+
V	ATCC: 700046	Hemolytic	+	+
III	ATCC: BAA22	Hemolytic	+	+
V	ATCC: 700048	Hemolytic	+	+
V	ATCC: 49447	Hemolytic	+	+
Ib	BEI‡: MNZ929	Hemolytic	+	+
II	BEI: MNZ933	Hemolytic	+	+
III	BEI: MNZ938	Hemolytic	+	+

* American Type Culture Collection.

† Centers for Disease Control and Prevention.

‡ BEI Resources.

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Table 1. Continued

Serotype	Strain ID	Hemolysis	GBS target detected	Internal control
V	CDC*: ABC020033655	Hemolytic	+	+
II	ATCC†: BAA1175	Hemolytic	+	+
Ib	ATCC: 55193	Hemolytic	+	+
Ic	ATCC: 27591	Hemolytic	+	+
Unknown	York Hosp 130	Non-Hemolytic	+	+
Unknown	York Hosp 144	Non-Hemolytic	+	+
Unknown	Evanston 2	Non-Hemolytic	+	+

* Centers for Disease Control and Prevention.

† American Type Culture Collection.

Cross-reactivity and microbial interference

A panel of microorganisms that may be present in patient specimens was tested to determine whether these microorganisms interfered with the detection of GBS or were cross-reactive with the *artus* GBS QS-RGQ Kit. To assess microbial interference, GBS Serotype III was diluted in Lim broth plus matrix to a concentration of approximately 2–3x LoD. Potential interfering organisms were diluted in Lim broth plus matrix. Bacteria and fungi were diluted to approximate working concentrations of ≥1.0 x 10^6 CFU/ml and viruses were diluted to approximate concentrations of ≥1.0 x 10^5 $TCID_{50}$/ml or PFU/ml. Bacterial and viral nucleic acids were diluted to a final concentration of ≥1 x 10^6 genome copies/ml. Human genomic DNA was diluted to a final concentration of ≥1 x 10^6 genome copies/ml. Each potentially interfering organism was tested with the *artus* GBS QS-RGQ Kit in the presence of GBS Serotype III. To assess cross reactivity, each organism from the panel was tested in the absence of GBS, at the same concentrations described above.

None of the potential interfering organisms cross-reacted or interfered with the detection of GBS Serotype III by the *artus* GBS QS-RGQ Kit.

Table 2. Panel of organisms tested for interference and cross-reactivity

		Interference		Cross-reactivity	
Organism tested	**Source ID**	**GBS target detected**	**Internal control**	**GBS target detected**	**Internal control**
Acinetobacter baumannii	ATCC* 19606	+	+	–	+
Adenovirus 40	ZMC† 0810084CF	+	+	–	+
Aeromonas hydrophila	ATCC 7966	+	+	–	+
Bacillus cereus	ATCC 11778	+	+	–	+
Bacteroides fragilis	ZMC 0601533	+	+	–	+
Campylobacter coli	ATCC 33559	+	+	–	+
Candida albicans	ATCC 10231	+	+	–	+
C. glabrata	ZMC Z007	+	+	–	+
C. guilliermondii	ZMC Z008	+	+	–	+
C. krusei	ZMC Z009	+	+	–	+
C. parapsilosis	ZMC Z011	+	+	–	+

* American Type Culture Collection.

† Zeptometrix Corporation.

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Table 2. Continued

		Interference		Cross-reactivity	
Organism tested	**Source ID**	**GBS target detected**	**Internal control**	**GBS target detected**	**Internal control**
Candida tropicalis	ZMC* Z012	+	+	–	+
Chlamydia trachomatis	ATCC† VR885	+	+	–	+
Citrobacter amalonaticus	ATCC 25405	+	+	–	+
C. freundii	ATCC 8090	+	+	–	+
C. koseri	ATCC 27028	+	+	–	+
Coxsackie virus	ZMC 0810075CF	+	+	–	+
Cytomegalovirus	ZMC 0810003CF	+	+	–	+
Echovirus	ZMC 0810023CF	+	+	–	+
Enterobacter aerogenes	ATCC 13048	+	+	–	+
E. cloacae	ATCC 13047	+	+	–	+
Enterococcus casseliflavus	ATCC 25788	+	+	–	+
E. faecalis	ATCC 35550	+	+	–	+

* Zeptometrix Corporation.

† American Type Culture Collection.

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Table 2. Continued

		Interference		Cross-reactivity	
Organism tested	**Source ID**	**GBS target detected**	**Internal control**	**GBS target detected**	**Internal control**
Enterococcus faecium	ATCC* BAA-2319	+	+	–	+
Escherichia coli	ATCC 23511	+	+	–	+
Enterovirus Type 71	ZMC† 0810047CF	+	+	–	+
Herpes simplex virus 1	ATCC VR-1493	+	+	–	+
Herpes simplex virus 2	ATCC VR-734	+	+	–	+
HIV	ZMC 0801032CF	+	+	–	+
HPV 11	ATCC 45151D	+	+	–	+
HPV 16	ATCC 45113D	+	+	–	+
HPV 18	ATCC 45152	+	+	–	+
Klebsiella pneumoniae	ATCC 13883	+	+	–	+
Lactobacillus acidophilus	ATCC 4356	+	+	–	+
Lactococcus lactis	ATCC 11454	+	+	–	+

* American Type Culture Collection.

† Zeptometrix Corporation.

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Table 2. Continued

		Interference		Cross-reactivity	
Organism tested	**Source ID**	**GBS target detected**	**Internal control**	**GBS target detected**	**Internal control**
Listeria monocytogenes	ATCC* 19115	+	+	–	+
Mobiluncus mulieris BV 64-5	ATCC 35240D-5	+	+	–	+
Moraxella catarrhalis	ATCC 8176	+	+	–	+
Mycoplasma hominis	ATCC 23114D	+	+	–	+
Neisseria gonorrhoeae	ATCC 19424	+	+	–	+
N. meningitides	ATCC 13077	+	+	–	+
Proteus mirabilis	ATCC 25933	+	+	–	+
P. penneri	ATCC 35198	+	+	–	+
Pseudomonas aeruginosa	ATCC 35554	+	+	–	+
Rotavirus	ZMC 0810041CF	+	+	–	+
Serratia marcescens	ATCC 13880	+	+	–	+
Streptococcus mutans Z072	ZMC Z072	+	+	–	+

* American Type Culture Collection.

† Zeptometrix Corporation.

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Table 2. Continued

		Interference		Cross-reactivity	
Organism tested	**Source ID**	**GBS target detected**	**Internal control**	**GBS target detected**	**Internal control**
Staphylococcus aureus	ATCC* 29213	+	+	–	+
S. epidermidis	ATCC 51625	+	+	–	+
Streptococcus dysgalactiae	ATCC 43078	+	+	–	+
S. mitis	ZMC† Clinical isolate	+	+	–	+
S.pneumoniae	ATCC 33400	+	+	–	+
S.pyogenes	ATCC 49399	+	+	–	+
Toxoplasma gondii	ZMC 0810007CF	+	+	–	+
Trichomonas vaginalis	ATCC 30238	+	+	–	+
Ureaplasma urealyticum	ATCC 27618	+	+	–	+
Vibrio parahaemolyticus	ATCC 17802	+	+	–	+
Yersinia enterolitica	ATCC 23715	+	+	–	+
Human gDNA	Promega G304A	+	+	–	+

* American Type Culture Collection.

† Zeptometrix Corporation.

Precision

The precision of the *artus* GBS QS-RGQ Kit was assessed using a 7-member panel consisting of 2 serotypes of GBS: Serotype Ia (ATCC BAA-1177) and Serotype III (ATCC 12403). Panel members were formulated in Lim broth plus matrix with a single serotype present (Ia or III) at 3 concentrations; positive (approximately 2–3x LoD), low positive (1x LoD), and high negative (<1x LoD). A seventh panel member (negative) was prepared using Lim broth plus matrix only. The data obtained were used to determine the mean C_T, standard deviation (SD), and the coefficient of variation (%CV) for each target and the internal control.

For the within laboratory repeatability study, the seven-member panel was tested in replicates of 3, once a day for a total of 12 days. The testing was conducted by 2 alternating operators using one instrument system (QIAsymphony RGQ) and one lot of the *artus* GBS QS-RGQ Kit.

Table 3. Summary of within laboratory repeatability for the *artus* GBS QS-RGQ Kit

		Internal control			GBS		
Serotype	**Panel member**	**Mean C_T**	**SD**	**%CV**	**Mean C_T**	**SD**	**%CV**
Ia	**Positive**	29.87	0.32	1.08	31.19	0.53	1.71
	LoD	29.77	0.30	1.00	32.85	1.04	3.15
	High negative	29.78	0.31	1.05	35.54	0.88	2.47
III	**Positive**	29.72	0.30	1.01	32.16	0.50	1.55
	LoD	29.82	0.27	0.90	33.35	0.82	2.45
	High negative	29.76	0.29	0.96	35.93	0.62	1.72
Negative		30.09	0.25	0.84	n.a.*	n.a.	n.a.

* n.a.: not applicable.

Lot-to-lot reproducibility was assessed using 3 different lots of the *artus* GBS QS-RGQ Kit. A single run was performed for each of the 3 lots (for a total of 3 runs) using a single instrument system (QIAsymphony RGQ), by a single operator. For each run, each panel member was tested in replicates of 6 (Table 4).

Table 4. Summary of lot-to-lot reproducibility for the *artus* GBS QS-RGQ Kit

			Internal control			GBS		
Strain	**Panel member**	**Lot**	**Mean C_T**	**SD**	**%CV**	**Mean C_T**	**SD**	**%CV**
Ia	Positive	1	29.50	0.42	1.41	29.88	0.27	0.90
		2	29.46	0.17	0.59	29.87	0.13	0.43
		3	30.00	0.50	1.65	30.10	0.32	1.07
		Overall	**29.65**	**0.44**	**1.49**	**29.95**	**0.26**	**0.87**
	LoD	1	29.44	0.20	0.69	31.30	0.46	1.46
		2	29.39	0.19	0.64	31.19	0.35	1.11
		3	30.15	0.38	1.28	31.73	0.39	1.24
		Overall	**29.66**	**0.44**	**1.48**	**31.41**	**0.44**	**1.42**
	High negative	1	29.65	0.13	0.44	n.a.*	n.a.	n.a.
		2	29.46	0.33	1.13	34.00	0.51	1.50
		3	29.90	0.35	1.16	36.16	n.a.	n.a.
		Overall	**29.67**	**0.33**	**1.11**	**34.72**	**1.30**	**3.74**
III	Positive	1	29.61	0.31	1.06	31.77	0.92	2.89
		2	29.49	0.35	1.17	32.03	0.45	1.41
		3	30.05	0.31	1.04	31.91	0.41	1.28
		Overall	**29.72**	**0.39**	**1.33**	**31.90**	**0.61**	**1.90**

* n.a.: not applicable.

Table continued next page

Table 4. Continued

Strain	Panel member	Lot	Internal control Mean C_T	Internal control SD	Internal control %CV	GBS Mean C_T	GBS SD	GBS %CV
III	LoD	1	29.49	0.12	0.42	33.28	0.49	1.47
		2	29.54	0.26	0.87	32.56	0.44	1.37
		3	29.96	0.36	1.20	33.62	0.69	2.04
		Overall	**29.66**	**0.33**	**1.12**	**33.15**	**0.69**	**2.08**
	High negative	1	29.46	0.25	0.83	35.72	0.12	0.34
		2	29.40	0.32	1.10	36.69	0.60	1.64
		3	29.99	0.39	1.29	36.05	n.a.	n.a.
		Overall	**29.62**	**0.41**	**1.38**	**36.32**	**0.64**	**1.76**
Negative		1	29.79	0.27	0.89	36.01	n.a.	n.a.
		2	29.69	0.18	0.59	n.a.*	n.a.	n.a.
		3	30.20	0.22	0.73	n.a.	n.a.	n.a.
		Overall	**29.90**	**0.31**	**1.04**	**36.01**	**n.a.**	**n.a.**

* n.a.: not applicable.

To measure instrument reproducibility, the 7-member panel was run on 3 different QIAsymphony RGQ instrument systems. Panel members were tested in replicates of 6 by a single operator with one lot of the *artus* GBS QS-RGQ Kit (see Table 5).

Table 5. Summary of instrument-to-instrument reproducibility for the *artus* GBS QS-RGQ Kit

Strain	Panel member	Instrument	Internal control			GBS		
			Mean C_T	SD	%CV	Mean C_T	SD	%CV
Ia	Positive	1	29.63	0.24	0.80	30.11	0.20	0.66
		2	29.89	0.20	0.68	29.96	0.19	0.64
		3	29.63	0.20	0.68	30.03	0.22	0.72
		Overall	**29.72**	**0.24**	**0.80**	**30.04**	**0.20**	**0.67**
	LoD	1	29.49	0.19	0.65	31.81	0.72	2.26
		2	29.87	0.17	0.58	31.66	0.37	1.18
		3	29.57	0.35	1.19	30.90	0.17	0.56
		Overall	**29.64**	**0.29**	**0.99**	**31.46**	**0.61**	**1.93**
	High negative	1	29.66	0.36	1.22	36.37	0.01	0.02
		2	30.00	0.30	1.00	35.88	n.a.*	n.a.
		3	29.55	0.29	0.98	34.68	1.74	5.02
		Overall	**29.74**	**0.36**	**1.21**	**35.59**	**1.22**	**3.43**
III	Positive	1	29.83	0.20	0.67	32.55	0.31	0.95
		2	29.93	0.37	1.23	32.77	0.55	1.67
		3	29.47	0.22	0.74	31.99	0.26	0.82
		Overall	**29.74**	**0.33**	**1.10**	**32.44**	**0.50**	**1.54**

* n.a.: not applicable.

Table continued next page

Table 5. Continued

Strain	Panel member	Instrument	Internal control Mean C_T	Internal control SD	Internal control %CV	GBS Mean C_T	GBS SD	GBS %CV
III	LoD	1	29.56	0.22	0.76	33.41	0.72	2.16
		2	30.10	0.32	1.05	34.17	1.24	3.63
		3	29.63	0.24	0.81	33.16	0.57	1.73
		Overall	**29.76**	**0.35**	**1.17**	**33.58**	**0.95**	**2.82**
	High negative	1	29.51	0.26	0.88	36.22	0.82	2.26
		2	30.10	0.24	0.81	36.12	1.44	3.99
		3	29.56	0.27	0.92	35.65	0.77	2.16
		Overall	**29.72**	**0.37**	**1.23**	**36.04**	**1.12**	**3.10**
Negative		1	30.07	0.16	0.53	n.a.*	n.a.	n.a.
		2	30.95	0.48	1.54	n.a.	n.a.	n.a.
		3	30.15	0.40	1.31	n.a.	n.a.	n.a.
		Overall	**30.39**	**0.54**	**1.77**	**n.a.**	**n.a.**	**n.a.**

* n.a.: not applicable.

To measure site-to-site reproducibility, the 7-member panel was run by 2 users at each of 3 sites. Each of the 2 users performed 5 runs on alternating testing days. Panel members were tested in replicates of 3 that were randomized and blinded to the user. A single QIAsymphony RGQ instrument system and one lot of the *artus* GBS QS-RGQ Kit were used at each site to conduct the study (Table 6).

Table 6. Summary of site-to-site reproducibility for the *artus* GBS QS-RGQ Kit

Strain	Panel member	Site	Internal control Mean C_T	Internal control SD	Internal control %CV	GBS Mean C_T	GBS SD	GBS %CV
Ia	Positive	1	29.51	0.31	1.05	30.25	0.34	1.14
		2	29.15	0.35	1.19	30.21	0.78	2.59
		3	29.47	0.27	0.90	30.30	0.28	0.94
		Overall	**29.38**	**0.34**	**1.17**	**30.25**	**0.51**	**1.70**
	LoD	1	29.49	0.39	1.33	31.63	0.47	1.49
		2	29.21	0.41	1.39	31.40	0.93	2.96
		3	29.43	0.32	1.07	31.40	0.39	1.26
		Overall	**29.38**	**0.39**	**1.32**	**31.48**	**0.65**	**2.05**
	High negative	1	29.47	0.35	1.17	35.32	0.95	2.70
		2	29.24	0.67	2.28	34.66	0.77	2.23
		3	29.51	0.37	1.26	35.39	1.41	4.00
		Overall	**29.40**	**0.49**	**1.68**	**35.13**	**1.07**	**3.05**
III	Positive	1	29.37	0.33	1.14	31.45	0.33	1.06
		2	29.14	0.47	1.60	31.35	0.90	2.89
		3	29.55	0.34	1.14	31.92	0.45	1.40
		Overall	**29.36**	**0.41**	**1.41%**	**31.57**	**0.65**	**2.07**

Table continued next page

Table 6. Continued

Strain	Panel member	Site	Internal control Mean C_T	Internal control SD	Internal control %CV	GBS Mean C_T	GBS SD	GBS %CV
III	LoD	1	29.48	0.33	1.14	31.67	0.52	1.65
		2	29.19	0.41	1.42	31.79	0.74	2.32
		3	29.40	0.33	1.13	32.50	0.54	1.67
		Overall	**29.36**	**0.38**	**1.29**	**31.99**	**0.70**	**2.20**
	High negative	1	29.38	0.35	1.20	35.29	0.82	2.33
		2	29.06	0.29	0.99	35.59	1.19	3.34
		3	29.46	0.25	0.84	35.89	0.99	2.75
		Overall	**29.30**	**0.34**	**1.17**	**35.57**	**1.03**	**2.88**
Negative		1	29.81	0.32	1.08	36.70	0.23	0.62
		2	29.44	0.33	1.12	n.a.*	n.a.	n.a.
		3	29.73	0.39	1.30	36.77	n.a.	n.a.
		Overall	**29.66**	**0.38**	**1.28**	**36.72**	**0.17**	**0.45**

* n.a.: not applicable.

Carryover

Less than 1% carryover (cross-contamination) between samples for the entire workflow was proven by the correct detection of >99% of high positive and negative samples in alternating positions. Contrived positive GBS and negative GBS samples were prepared in Lim broth plus matrix. High positive samples were formulated at a concentration of ≥2 × 10^6 CFU/ml. These samples were processed with the complete *artus* GBS QS-RGQ workflow.

Interfering substances

A panel of 34 substances that may be present in patient specimens was tested to determine whether these substances interfered with the performance of the *artus* GBS QS-RGQ Kit. GBS Serotype III was used for this study and diluted to approximately 2–3x LoD in Lim broth plus matrix. GBS was individually spiked with each potentially inhibitory substance and tested with the *artus* GBS QS-RGQ Kit. The samples and brands are shown in Table 7. None of the substances showed an inhibitory effect on the signals of the internal control and GBS.

Table 7. Substances tested for potential interference

Substance	Potential interfering substance	Concentration tested*
Aleve®	Naproxen sodium	0.5mg/ml
Anti-hemorrhoidal cream/gel	Phenylephedrine HCL	0.25%
Amniotic fluid	Amniotic fluid	10% v/v
Astringent	Witch Hazel	50%
Barium sulfate	Barium sulfate	12 mg/ml
Condoms	Nonoxynol-9	7% v/v
Ethanol	Ethanol	0.05% v/v
ex-lax®	Sennosides	0.6% v/v
Feces	Feces	100%
Gaviscon®	Aluminum hydroxide	42 µg/ml
Germicidal soap	Triclosan	0.015% v/v
Human hemoglobin	Hemoglobin	0.025mg/ml
Imodium®	Loperamide HCL	0.8 µg/ml
Isopropyl alcohol	Isopropyl alcohol	0.05% v/v
Leukocytes	Leukocytes	2.5% v/v
Methicillin	Methicillin	0.5mg/ml

* Represents physiologically relevant concentrations of substances

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Table 7. Continued

Substance	Potential interfering substance	Concentration tested*
Mineral oil	Mineral oil	1.5% v/v
Moist towelettes	Benzalkonium chloride	0.30%
Monistat Derm	Miconazole nitrate cream	2%
Mucus	Mucin	1% v/v
Palmitic acid	Palmitic acid	0.025mg/ml
Pepto Bismol®	Bismuth subsalicylate	1% v/v
Personal deodorant spray	Personal deodorant spray	100%
Preparation H®	Hydrocortisone	1%
Prilosec®	Omeprazole magnesium	8 µg/ml
Sodium hypochlorite	Sodium hypochlorite	0.05% v/v
Stearic acid	Stearic acid	0.025mg/ml
Tagamet®	Cimetidine	80 µg /ml
Tums®	Calcium carbonate	200 µg/ml
Urine	Urine	10% v/v
Vancomycin	Vancomycin	0.5mg/ml
Vaseline®	Petroleum jelly	100%
Whole blood	Whole blood EDTA	5% v/v
Zinc oxide	Zinc oxide	20% w/w

* Represents physiologically relevant concentrations of substances

Diagnostic performance evaluation

The performance of the *artus* GBS QS-RGQ Kit was evaluated using clinical samples from 4 geographically diverse locations within the United States in 2014. A total of 369 Lim broth cultures were evaluated and results from the *artus* GBS QS-RGQ Kit were compared to results obtained from reference culture and bi-directional sequencing. Clinical samples were considered positive if the C_T values were ≤39.7. With the clinical samples tested, the *artus* GBS QS-RGQ Kit showed an overall diagnostic sensitivity of 95% (with a positive predictive value of 87%) and specificity of 95% (with a negative predictive value of 98%) for GBS in comparison with enriched culture and bi-directional sequencing (Tables 8 and 9).

Table 8. GBS clinical agreement study results

		Culture + bi-directional sequencing		
		+	–	Total
artus GBS QS-RGQ Kit	+	90	13	103
	–	5	261	266
	Total	95	274	369

Table 9. GBS clinical agreement study results

		95% CI
Sensitivity	95%	88–98%
Specificity	95%	92–97%
Positive predictive value	87%	80–92%
Negative predictive value	98%	96–99%
Prevalence	26%	22–30%
Agreement	95%	N/A

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