

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

K133936

B. Purpose for Submission:

To obtain substantial equivalence for artus[®] *C. difficile* QS-RGQ MDx kit

C. Measurand:

C. difficile Toxin A gene (*tcdA*) and Toxin B gene (*tcdB* and *tcdBv*)

D. Type of Test:

Real-time PCR with DNA amplification

E. Applicant:

Qiagen GmbH

F. Proprietary and Established Names:

artus[®] *C. difficile* QS-RGQ MDx kit

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3130

2. Classification:

Class II

3. Product codes:

OZN – *C. difficile* toxin gene amplification assay

OOI – Real time nucleic acid amplification system

4. Panel:

83-Microbiology

H. Intended Use:

1. Intended use(s):

The *artus C. difficile* QS-RGQ MDx Kit is an *in vitro* polymerase chain reaction (PCR) assay for use on the QIASymphony RGQ MDx system for the qualitative detection of toxigenic *Clostridium difficile* toxin A and toxin B genes in human liquid or soft stool specimens from patients suspected of having *Clostridium difficile* associated disease. The test is intended to be used directly on patient samples.

The *artus C. difficile* QS-RGQ MDx Kit is intended to be used to aid in diagnosis of *Clostridium difficile* infection.

2. Indication(s) for use:

The *artus C. difficile* QS-RGQ MDx Kit is an *in vitro* polymerase chain reaction (PCR) assay for use on the QIASymphony RGQ MDx system for the qualitative detection of toxigenic *Clostridium difficile* toxin A and toxin B genes in human liquid or soft stool specimens from patients suspected of having *Clostridium difficile* associated disease. The test is intended to be used directly on patient samples.

The *artus C. difficile* QS-RGQ MDx Kit is intended to be used to aid in diagnosis of *Clostridium difficile* infection.

3. Special conditions for use statement:

For Prescription Use

4. Special instrument requirements:

QIA symphony RGQ MDx System

I. Device Description:

The contents of the *artus C. difficile* QS-RGQ MDx Kit are sufficient for 72 tests in one to three batches of 24 reactions on the QIASymphony RGQ MDx system. Kit components are dispensed in 2 mL tubes with color-coded caps. Each component is provided in 3 separate aliquots sufficient to perform up to 24 reactions each, and the components are provided in a single kit carton. The list of materials included in the kit is provided in Table 1.

Table 1: Materials provided with the *artus C. difficile* QS-RGQ MDx Kit

Color of tube	Component	Number of tubes provided in kit	Volume per tube
Blue	<i>C. difficile</i> Master A	3	330 µL
Violet	<i>C. difficile</i> Master B	3	600 µL
Green	<i>C. difficile</i> Internal Control	3	540 µL
Red	<i>C. difficile</i> Positive Control	3	330 µL
White	<i>C. difficile</i> Negative Control	3	330 µL

C. difficile Master A: A mixture containing thermostable DNA polymerase, buffer, dNTPs, and proprietary PCR enhancers and stabilizers.

C. difficile Master B: A buffered solution containing *C. difficile* and IC-specific primers and probes. It contains primer sets for the amplification of conserved regions of *C. difficile* *tcdA*, *tcdB*, and *tcdBv* genes. In addition, the mix contains primers for the amplification of a conserved region of the *Geobacillus stearothermophilus* (GeoB) genome contained in an intact, chemically inactivated organism (NATrol). The mix also contains specific fluorophore labeled probes to allow for the detection and differentiation of the amplification products.

C. difficile Internal Control: Consists of intact inactivated *Geobacillus stearothermophilus* in a soy peptone buffer.

C. difficile Positive Control: Consists of plasmids that encode the *tcdA* and *tcdB* genes in a buffered solution.

C. difficile Negative Control: Consists of *Bacteroides Thetaiotaomicron* DNA in 10 mM Tris / 1 mM EDTA, pH 8.0 buffer.

J. Substantial Equivalence Information:

1. Predicate device name:

Quidel Molecular Direct *C. difficile* assay

2. Predicate 510(k) number:

K123998

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Intended for the qualitative detection of toxigenic <i>C. difficile</i> toxin A and toxin B genes in stool specimens from patients suspected of having CDAD	Similar
Technology	Real-time PCR; DNA amplification	Same

Differences		
Item	Device	Predicate
Procedure	Assay uses the QIA Symphony SP/AS for automated sample preparation and assay setup	Assay uses proprietary sample preparation buffer and manual assay setup
Instrument System	Assay uses the Rotor-Gene Q MDx	Assay can be performed using either the: <ul style="list-style-type: none"> • Life Technologies QuantStudio Dx • Applied Biosystems 7500 fast Dx • Cepheid Smart Cycler II
Controls	All controls required to run the assay (Internal Control, Positive Control, Negative Control) are included in the kit.	Assay includes a process control and recommends the use of external controls

K. Standard/Guidance Document Referenced:

Draft Guidance for Industry and Food and Drug Administration Staff –“ Establishing the Performance Characteristics of In Vitro diagnostic Devices for the Detection of *Clostridium difficile*”, issued November 29, 2010.

L. Test Principle:

The *artus C. difficile* QS-RGQ MDx Kit assay uses PCR to generate an amplified product from the *tcdA* and *tcdB/tcdBv* genes of toxigenic *C. difficile* DNA in clinical specimens. Samples are extracted and prepared using the QIA Symphony SP instrument with the QIA Symphony DSP Virus/Pathogen Mini Kit, followed by assay setup on the QIA Symphony AS. Amplification and detection are carried out using the *artus C. difficile* QS-RGQ MDx Kit with the Rotor-Gene Q MDx (RGQ MDx) and Rotor-Gene AssayManager software. The presence of a toxigenic *C. difficile* target sequence is indicated by the fluorescent signal generated through the use of fluorescently labeled oligonucleotide probes. The probes do not generate a signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the RGQ MDx is inversely proportional to the toxigenic *C. difficile* DNA target concentration present in the original

specimen. A bacterial species unrelated to toxigenic *C. difficile* is introduced into each specimen during sample preparation to serve as an internal control. The internal control bacteria are lysed simultaneously with toxigenic *C. difficile* in the specimen, and amplified in the same reaction as the *C. difficile* targets using PCR, and serve to demonstrate that the entire assay process has proceeded correctly for each specimen.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of the *artus C. difficile* QS-RGQ Kit was assessed using a 7-member panel consisting of 2 *C. difficile* strains: NAP-1/BI/027 strain, toxinotype III A+B+ (ATCC BAA-1870) and 1470 strain, toxinotype VIII A-B+ (ATCC 43598). Panel members were initially diluted in TE buffer then tested in Buffer ATL containing negative stool matrix with a single strain present (NAP-1/BI/027 or 1470) 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 TE buffer only and also tested in Buffer ATL containing negative stool matrix. 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 three, once a day for a total of twelve days (for a total of 262 data points for the 12 runs). The testing was conducted by two operators using one instrument (QS/AS RGQ MDx) and one reagent kit lot (Table 2).

Table 2: Within Laboratory Repeatability Study Results

Panel Member	Internal Control			<i>tcdA</i>			<i>tcdB</i>		
	MEAN	STDEV	%CV	MEAN	STDEV	%CV	MEAN	STDEV	%CV
NAP1 Positive	29.78	0.49	1.64%	30.84	0.47	1.52%	33.58	0.64	1.91%
NAP1 Low Positive	30.07	0.55	1.82%	32.19	0.99	3.08%	34.92	0.68	1.94%
NAP1 High Negative	29.99	0.72	2.41%	34.21	0.91	2.66%	36.18	0.23	0.64%
1470 Positive	29.85	0.52	1.73%	30.95	0.55	1.78%	33.72	0.45	1.33%
1470 Low Positive	29.89	0.41	1.39%	32.14	0.65	2.03%	35.13	0.63	1.80%
1470 High Negative	29.92	0.53	1.76%	34.14	0.66	1.92%	36.39	0.03	0.07%
Negative	30.00	0.44	1.46%	N/A	N/A	N/A	N/A	N/A	N/A

To measure site-to-site reproducibility, the 7-member panel was run by 2 users at each of 3 sites (IMDx and 2 external 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 QIA Symphony RGQ MDx system and one lot of the *artus C. difficile* QS-RGQ MDx Kit were used at each site to conduct the study (Table 3). The reproducibility study results are acceptable.

Table 3: Site-to-Site Reproducibility Study Results

	Panel Member	Site	Mean Ct	SD	%CV	Mean Ct	SD	%CV	Mean Ct	SD	%CV
NAP-1/BI/027	Positive	1	30.61	0.42	1.38	31.85	0.73	2.3	34.32	0.69	2.02
		2	30.58	0.51	1.65	32.16	0.8	2.5	34.43	0.71	2.05
		3	30.55	0.32	1.05	31.97	0.85	2.65	34.87	0.78	2.25
		overall	30.58	0.42	1.37	32	0.8	2.49	34.53	0.76	2.19
	Low Positive	1	30.63	0.4	1.3	33.38	0.71	2.14	35.58	0.55	1.55
		2	30.73	0.6	1.94	33.27	0.81	2.45	35.01	0.53	1.52
		3	30.86	0.62	2	33.07	0.84	2.53	35.45	0.69	1.96
		overall	30.74	0.55	1.78	33.24	0.79	2.38	35.36	0.62	1.76
	High Negative	1	30.71	0.35	1.15	34.21	0.54	1.58	35.88	N/A	N/A
		2	30.59	0.33	1.09	34.21	0.29	0.86	35.92	N/A	N/A
		3	30.64	0.47	1.53	34.17	0.57	1.68	N/A	N/A	N/A
		overall	30.65	0.39	1.27	34.19	0.45	1.32	35.91	0.16	0.43
1470	Positive	1	30.67	0.49	1.58	32.22	0.86	2.67	34.74	0.61	1.76
		2	30.69	0.41	1.32	31.74	0.95	2.99	34.66	0.74	2.14
		3	30.86	0.33	1.06	31.81	0.78	2.45	34.95	0.7	2.01
		overall	30.74	0.42	1.35	31.92	0.88	2.77	34.78	0.69	1.99
	Low Positive	1	30.74	0.42	1.37	33.17	0.94	2.83	35.61	0.49	1.38
		2	30.51	0.42	1.38	33.06	0.89	2.7	35.62	0.6	1.7
		3	30.7	0.33	1.08	33.41	1.08	3.22	35.77	0.46	1.29
		overall	30.65	0.4	1.31	33.2	0.96	2.9	35.65	0.53	1.48
	High Negative	1	30.76	0.46	1.5	34.64	0.14	0.4	36.41	N/A	N/A
		2	30.55	0.45	1.46	34.15	0.28	0.83	35.82	N/A	N/A
		3	30.44	0.42	1.38	34.63	0.27	0.77	N/A	N/A	N/A
		overall	30.58	0.46	1.49	34.49	0.31	0.91	36.12	0.42	1.16
Negative	1	30.68	0.37	1.2	N/A	N/A	N/A	N/A	N/A	N/A	
	2	30.66	0.47	1.55	N/A	N/A	N/A	N/A	N/A	N/A	
	3	30.67	0.42	1.37	33.58	N/A	N/A	N/A	N/A	N/A	
	overall	30.67	0.42	1.36	33.58	N/A	N/A	N/A	N/A	N/A	

b. *Linearity/assay reportable range:*

N/A

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

For controls: See # I - Device Description above

Traceability for the QIASymphony Instrument, Firmware, and Software is maintained between requirements, specifications, testing, and risk mitigations. Product requirements that are defined at the highest system level (QS-RGQ) are translated into product requirements for the SP and AS subsystem, and translated further into specifications for hardware, firmware, software, and disposables.

d. *Detection limit:*

The limit of detection (LOD) was assessed for the *artus C. difficile* QS-RGQ MDx Kit using 3 toxigenic *Clostridium difficile* strains: NAP-1/BI/027 strain, toxinotype III A+B+ (ATCC® BAA-1870); 1470 strain, toxinotype VIII A-B+ (ATCC 43598); and VPI 10463 strain, toxinotype 0 A+B+ (ATCC 43255). The LOD is defined as the toxigenic *C. difficile* bacterial titer (CFU/mL) detected with a probability of 95% or greater and was determined by probit analysis. The results, representative of the analytical sensitivity of the *artus C. difficile* QS-RGQ MDx Kit, are summarized in Table 4.

Table 4: Limit of Detection

Strain	LoD (95%CI)
<i>C. difficile</i> NAP1 ATCC BAA-1870, strain:4118	7.9 CFU/mL (6.1- 15.0)
<i>C. difficile</i> 1470 ATCC 43598, strain:1470	11.2 CFU/mL (8.7- 16.8)
<i>C. difficile</i> 10463 ATCC 43255, strain:10463	2.8 CFU/mL (2.1- 4.2)

e. *Analytical specificity:*

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 *tcdA* or *tcdB* targets or were cross-reactive with the *artus C. difficile* QS-RGQ MDx Kit. Organisms were tested at a target concentration of approximately 1×10^6 CFU/ml for bacteria and fungi or $\geq 1 \times 10^5$ units/ml for viruses separately in the presence of 2–3x LOD of each

of three *C. difficile* strains: NAP-1/BI/027 strain, 1470 strain, and VPI 10463 strain. None of the potential interfering organisms cross-reacted or interfered with the detection of any of the 3 *C. difficile* strains by the *artus C. difficile* QS-RGQ MDx Kit (Table 5). Cross-reactivity for *Clostridium botulinum* was analyzed *in silico* and predicted no cross reactivity or microbial interference for the *artus C. difficile* QS-RGQ MDx Kit.

Table 5: Organisms Tested in Cross Reactivity and Microbial Interference

Organism Tested	Source ID
<i>Abiotrophia defectiva</i>	ATCC 49176
<i>Acinetobacter baumannii</i>	ATCC 19606
<i>Aeromonas hydrophila</i>	ATCC 7966
<i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	ATCC 15554
<i>Bacillus cereus</i>	ATCC 13472
<i>Bacteroides fragilis</i>	ZMC 0601533
<i>Campylobacter coli</i>	ATCC 43479
<i>Campylobacter coli</i>	ATCC 33559
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	ATCC 33292
<i>Candida albicans</i>	ATCC 10231
<i>Citrobacter freundii</i>	ATCC 8090
<i>Clostridium bifermentans</i>	ATCC 638
<i>Clostridium butyricum</i>	ATCC 19398
<i>Clostridium haemolyticum</i>	ATCC 9650
<i>Clostridium novyi</i>	ATCC 19402
<i>Clostridium orbiscindens</i>	ATCC 49531
<i>Clostridium perfringens</i>	ATCC 13124
<i>Clostridium scindens</i>	ATCC 35704
<i>Clostridium septicum</i>	ATCC 12464
<i>Clostridium sordellii</i>	ATCC 9714
<i>Clostridium difficile</i> (non-toxigenic)	ATCC 43593
<i>Clostridium difficile</i> (non-toxigenic)	ATCC 43601
<i>Clostridium sporogenes</i>	ATCC 15579
<i>Edwardsiella tarda</i>	ATCC 15947
<i>Enterobacter aerogenes</i>	ATCC 13048
<i>Enterobacter cloacae</i>	ATCC 13047
<i>Enterococcus faecalis</i> (vanB)	ATCC 51299
<i>Escherichia coli</i>	ATCC 23511
<i>Escherichia coli</i> O157:H7	ATCC 700927
<i>Helicobacter pylori</i> DNA	ATCC 43504D-5
<i>Klebsiella oxytoca</i>	ATCC 33496
<i>Lactobacillus acidophilus</i>	ATCC 4356
<i>Listeria monocytogenes</i>	ZMC 0801534
<i>Peptostreptococcus anaerobius</i>	ATCC 27337
<i>Plesiomonas shigelloides</i>	ATCC 14029

Organism Tested	Source ID
<i>Plesiomonas shigelloides</i>	ATCC 14029
<i>Porphyromonas asaccharolytica</i>	ATCC 25260
<i>Prevotella melaninogenica</i>	ATCC 25845
<i>Proteus mirabilis</i>	ATCC 25933
<i>Providencia alcalifaciens</i>	ATCC 9886
<i>Pseudomonas aeruginosa</i>	ATCC 35554
<i>Salmonella choleraesuis (Typhimurium)</i>	ATCC 14028
<i>Salmonella enterica</i> subsp. <i>arizonae</i>	ATCC 13314
<i>Salmonella enterica</i> subsp. <i>enterica</i>	ATCC 7001
<i>Serratia liquefaciens</i>	ATCC 27592
<i>Serratia marcescens</i>	ATCC 13880
<i>Shigella boydii</i>	ATCC 9207
<i>Shigella dysenteriae</i>	ATCC 11835
<i>Shigella sonnei</i>	ATCC 29930
<i>Staphylococcus aureus</i>	ATCC 43300
<i>Staphylococcus epidermidis</i>	ATCC 14990
<i>Streptococcus agalactiae</i>	ATCC 27541
<i>Vibrio parahaemolyticus</i>	ATCC 17802
Adenovirus	ZMC 0810110 CF
Rotavirus	ZMC 0810041 CF
Norovirus	ZMC 0810086 CF
Enterovirus	ZMC 0810047 CF
Echovirus	ZMC 0810023 CF
Coxsackie virus	ZMC 0810075 CF
Cytomegalovirus	ZMC 0810003 CF
Human Genomic DNA	Promega G3041

Analytical Reactivity

The analytical reactivity of the *artus C. difficile* QS-RGQ MDx Kit was assessed to determine whether the kit could detect a broad range of toxigenic *C. difficile* strains representing temporal and geographical diversity. A total of 27 strains and characterized clinical isolates were diluted in TE buffer to 2–3x LOD of the reference strain and tested with the *artus C. difficile* QS-RGQ MDx Kit. *C. difficile* target was detected in all strains tested (Table 6).

Table 6: Strains Tested in Analytical Reactivity

Name	Strain	Toxinotype	Origin
ATCC 17857	870	O	unknown
ATCC 17858	1253	N/A	unknown
ATCC 43594	W1194	N/A	Human feces; Belgium

Name	Strain	Toxinotype	Origin
ATCC 43596	545	N/A	Human feces; Belgium
ATCC 43599	2022	N/A	Human feces; Belgium
ATCC 43600	2149	N/A	Human feces; Belgium
ATCC 51695	BDMS 18AN	N/A	Becton Dickinson Microbiology Systems, Johns Hopkins Univ. Hosp. Lab
ATCC 700792	14797-2	N/A	Human feces; Michigan, USA
ATCC 9689	90556- M6S	O	unknown
ATCC BAA- 1382	630	X	Switzerland
ATCC BAA- 1805	N/A	III	unknown
ATCC BAA- 1871	4111	O	Human; New Jersey, USA
ATCC BAA- 1872	4206	O	Human; Maine, USA
ATCC BAA- 1873	5283	O	Human; New York, USA
ATCC BAA- 1874	4205	O	Human; Oregon, USA
ATCC BAA- 1875	5325	V	Human; Georgia, USA
ATCC BAA- 2155	LBM 0801058	N/A	Human; New Mexico, USA
ATCC BAA- 2156	LBM 0801040	N/A	Human; Cambridge UK
CCUG 20309	8864	X	Birmingham, UK
Illinois VA Hospital isolate 278	N/A	II	Illinois, USA
Illinois VA Hospital isolate 464	N/A	IV	Illinois, USA
Illinois VA Hospital isolate 4092	N/A	VIII	Illinois, USA
Illinois VA Hospital isolate 5572	N/A	VIII	Illinois, USA

Name	Strain	Toxinotype	Origin
Illinois VA Hospital isolate 3430	N/A	IX	Illinois, USA
Illinois VA Hospital isolate 1753	N/A	XII	Illinois, USA
Illinois VA Hospital isolate 5090	N/A	XXI	Illinois, USA
Illinois VA Hospital isolate 3130	N/A	XXII	Illinois, USA

Interfering Substances

A panel of 23 substances that may be present in patient specimens was tested to determine whether these substances interfered with the performance of the *artus C. difficile* QS-RGQ MDx Kit. Three toxigenic *C. difficile* strains: NAP-1/BI/027 strain, 1470 strain, and VPI 10463 strain, were diluted to approximately 2–3x LOD and spiked with each potentially inhibitory substance. None of the substances showed an inhibitory effect on the detection of *C. difficile* by the *artus C. difficile* QS-RGQ MDx Kit (Table 7).

Table 7: Potentially Interfering Substances Tested

Type	Substance	Potential Interferent	Concentration Tested*
Anti-fungal	Miconazole nitrate cream	Miconazole nitrate	2% w/v
Cream/Suppositories	Preparation H	Hydrocortizone	2% w/v
	Zinc oxide	Zinc oxide	40% w/v
	Vaseline	Petroleum jelly	100%
Anti-hemorrhoid creams	Hemorrhoid gel	Phenylephrine hydrochloride	2% w/v
Condoms	Condoms	Nonoxynol-9	7%
Moist Towelettes	Moist Towelettes	Benzalkonium Chloride	0.12% w/v
Antacids	Gaviscon	Aluminum hydroxide, Magnesium carbonate	0.1 mg/mL
	Tums	Ca carbonate	0.5 mg/mL
	Tagamet	Cimetidine	0.5 mg/mL
	Prilosec (delayed release)	Omeprazole magnesium	0.5 mg/mL
Enemas	Mineral Oil	Mineral Oil	2% v/v

Type	Substance	Potential Interferent	Concentration Tested*
Anti-Diarrheal Medication	Imodium	Loperamide HCl	0.00667 mg/mL
	Pepto Bismol	Bismuth Subsalicylate	0.87 mg/mL
Laxative	ExLax	Sennosides	0.1 mg/mL
Antibiotics	Vancomycin HCl	Vancomycin	12.5 mg/mL
	Metronidazole	Metronidazole	14 mg/mL
Anti-inflammatory	Naproxen Sodium (Aleve)	Naproxen Sodium	14 mg/mL
Blood	Whole blood	Glucose, hormones, enzymes, iron, etc.,	5% v/v
Fecal Components	Mucus	Mucin	3 mg/mL
	Palmitic acid	Palmitic acid	2 mg/mL
	Stearic acid	Stearic acid	4 mg/mL
MRI Contrast Agents	Barium sulfate	Barium sulfate	5 mg/mL

*Represents physiologically relevant concentrations of substances

f. Assay cut-off:

In order to determine the CT cutoff values for *tcdA* and *tcdB* targets in *C. difficile* positive samples, the following studies were performed: (1) Serial dilutions of contrived *C. difficile* specimens and- (2) Testing of in-house *C. difficile* positive and negative clinical specimens.

Based on the studies conducted to establish the cutoff values for the artus *C. difficile* QS-RGQ MDx Kit, the following parameters were established (Table 8).

Table 8: CT threshold and cutoff parameters

Target	CT	CT cutoff
<i>tcdA</i> (Cycling Orange)	0.03	< 38.3
<i>tcdB</i> (Cycling Green)	0.03	<36.5
Internal Control	0.03	<35.9

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. *Clinical Sensitivity:*

The performance of the *artus C. difficile* QS-RGQ MDx Kit was evaluated at 3 external testing sites using patient samples from patients suspected of *C. difficile* infection. Patient samples were collected from 5 geographically diverse locations within the United States. The *artus C. difficile* QS-RGQ MDx Kit was compared to direct and/or enriched culture. For direct culture, the stool specimen was swabbed to a CCFA plate and streaked for isolation. For enriched culture, the same swab was used to inoculate enrichment broth (CCMB-TAL). At 36-48 hours, the enrichment broth was subcultured to a CCFA plate which was streaked for isolation. Suspicious colonies were identified and the cytotoxin assay was performed from a cell free supernatant. The tables below present the data from these studies.

Assay vs. Enriched Culture Comparison

A total of 741 specimens were tested by both the *artus C. difficile* QS-RGQ MDx Kit and enriched toxigenic culture. The overall assessment of sensitivity and specificity versus enriched toxigenic culture is shown below (Table 9).

Table 9: Clinical Performance of *artus C. difficile* QS-RGQ MDx Kit vs. Enriched Toxigenic Culture

Combined Sites – Combined Ages				
		Enriched Toxigenic Culture		Total
		Positive	Negative	
<i>artus C. difficile</i> QS-RGQ MDx Kit	Positive	114	17*	131
	Negative	13**	597	610
	Total	127	614	741
	95% CI (%)			
	Sensitivity	90%	83-94	
	Specificity	97%	96-98	
	PPV	87%	80-92	
	NPV	98%	96-99	
	Prevalence	17%	15-20	

*17 discordant specimens (*artus C. difficile* QS-RGQ MDx Positive, Enriched Toxigenic Culture Negative) reported were analyzed by alternative PCR followed by bi-directional sequencing and the result was that 12 out of 17 were positive for toxigenic *C. difficile*, agreeing with the *artus C. difficile* QS-RGQ MDx result.

** 12 discordant specimens (*artus C. difficile* QS-RGQ MDx Negative, Enriched Toxigenic Culture Positive) reported were analyzed by alternative PCR followed by bi-directional sequencing and the result

was that 10 out of 12 were negative for toxigenic *C. difficile*, agreeing with the *artus C. difficile* QS-RGQ MDx result. The remaining 1 discordant specimen was unavailable for testing.

Assay vs. Direct Toxigenic Culture Comparison

A direct toxigenic culture result was available for 699 specimens. The overall assessment of sensitivity and specificity versus direct toxigenic culture is shown below (Table 10).

Table 10: Clinical Performance of *artus C. difficile* QS-RGQ MDx Kit vs. Direct Toxigenic Culture

Combined Sites – Combined Ages				
		Direct Toxigenic Culture		Total
		Positive	Negative	
<i>artus C. difficile</i> QS-RGQ MDx Kit	Positive	84	21*	105
	Negative	1**	593	594
	Total	85	614	699
	95% CI (%)			
	Sensitivity	99%	94-100	
	Specificity	97%	95-98	
	PPV	89%	71-87	
	NPV	100%	99-100	
	Prevalence	12%	10-15	

*19 discordant specimens (*artus C. difficile* QS-RGQ MDx Positive, Direct Toxigenic Culture Negative) reported were analyzed by alternative PCR followed by bi-directional sequencing and the result was that 14 out of 19 were positive for toxigenic *C. difficile*, agreeing with the *artus C. difficile* QS-RGQ MDx result. The remaining 2 specimens were unavailable for testing.

**The 1 discordant specimen (*artus C. difficile* QS-RGQ MDx Negative, Direct Toxigenic Culture Positive) was unavailable for testing.

b. Clinical specificity:

See 3 a. above for specificity results.

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

The prevalence for *C. difficile* observed during a multi-center clinical trial

was estimated as 18.2% (135/741) when tested using the *artus* C. difficile QS-RGQ MDx Kit. Of the patient population included in the study, the majority of patients were senior adults (≥ 60 years) and the prevalence of *C. difficile* in this age group was found to be 19% (75/395). The next largest age group was adults (age >21 to ≤ 59 years) and the prevalence was found to be 16% (52/324). The remaining patient population included one infant (<2 years), 6 children (≥ 2 to <12 years), 9 adolescents (≥ 12 to <18 years), and 6 transitional adolescents (≥ 18 to ≤ 21 years).

N. Instrument Name:

QIASymphony RGQ MDx system

O. System Descriptions:

1. Modes of Operation:

The QIASymphony RGQ MDx system consists of 3 subsystems: the QIASymphony SP (QSSP) for sample preparation, the QIASymphony AS (QSAS) for assay set-up and the Rotor-Gene Q MDx (RGQ MDx) for target amplification and detection.

2. Software:

The QIASymphony SP and QIASymphony AS are used in combination with the QIASymphony software (version 4.0) and the RGQ MDx is used in combination with the Rotor-Gene AssayManager analysis software (version 1.0). As part of the QIASymphony RGQ MDx system, cyclor control, data analysis and reporting with the Rotor-Gene MDx instrument are done by the Rotor-Gene AssayManager 1.0 software using plug-ins, which contain specific analysis algorithms and configurations.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No _____

3. Specimen Identification:

For specimen identification, switch on the QIASymphony SP/AS, and wait until the "Sample Preparation" screen appears and the initialization procedure has finished. Then log in to the instrument and load the sample drawer with the positive and negative controls. Next load the "Sample" drawer with the samples and using the "Integrated run" setup on the QIASymphony touchscreen, enter the required information for each batch of samples to be processed. Then define the assay to run, define the QIASymphony AS batch, load the "Sample" drawer with the internal control mixture, define the internal control positions and start the run.

4. Specimen Sampling and Handling:

The QIASymphony SP instrument is designed to perform automated purification of nucleic acids in combination with QIASymphony DSP Kits. An assay is developed for use with a specific QIASymphony DSP Kit based on the application and sample type. The QIASymphony AS instrument is designed to perform automated assay setup and is physically connected to the QIASymphony SP. The eluate transfer between QIASymphony SP and QIASymphony AS is done automatically. After the assay setup is complete, the user needs to remove the assay rack from the QIASymphony AS. The Rotor-Gene strip tubes must be sealed with lids, transferred to the Rotor-Gene Q MDx and secured with a locking ring. The Rotor-Gene Q MDx instrument is a real-time nucleic acid amplification and detection system.

5. Calibration:

N/A. The QS-RGQ MDx system does not require calibration.

6. Quality Control:

A bacterial species unrelated to toxigenic *C. difficile* is introduced into each specimen during sample preparation to serve as an internal control. The internal control bacteria are lysed simultaneously with toxigenic *C. difficile* in the specimen, and amplified in the same reaction as the *C. difficile* targets using PCR, and serve to demonstrate that the entire assay process has proceeded correctly for each specimen. Kit also contains a positive and negative control for use in the assay run.

~~P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:~~

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.