QUIDEL®
CORPORATION

DEC 1 3 2012

DECISION Quidel AmpliVue™ C. difficile Assay 510(k) SUMMARY

A. 510(k) Number:

K123355

B. Date of Preparation:

December 10, 2012

C. Purpose for Submission:

To determine substantial equivalence of the AmpliVue™ C. difficile Assay for the qualitative detection of toxigenic *Clostridium difficile* directly from unformed stool samples.

D. Measurand:

A fragment from the conserved 5' end of the Toxin A (*tcdA*) gene, located within the PaLoc of toxigenic *Clostridium difficile* strains.

E. Type of Test:

Qualitative, in vitro diagnostic nucleic acid amplification test; based on the isothermal, helicase-dependent amplification (HDA) scheme combined with a manual read of assay results.

F. Applicant:

QUIDEL Corp.

G. Proprietary and Established Names:

AmpliVue™ C. difficile Assay

H. Regulatory Information:

1. Regulation section:

   21 CFR 866.3130 - C. difficile Nucleic Acid Amplification Test Assay

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2. Classification:

   II

3. Product code:

   OZN - Amplification assay for the detection of *Clostridium difficile* toxin genes from stool specimens of symptomatic patients

4. Panel:

   Microbiology (83)

I. Intended Use:

1. Intended use(s):

   The AmpliVue™ C. difficile Assay is an *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridium difficile* Toxin A gene (*tcdA*) in unformed stool specimens of patients suspected of having *Clostridium difficile*-associated disease (CDAD). The AmpliVue™ C. difficile Assay is intended for use as an aid in diagnosis of CDAD. The assay utilizes helicase-dependent amplification (HDA) for the amplification of a highly conserved fragment of the Toxin A gene sequence and a self-contained disposable amplicon detection device that allows for manual evaluation of assay results.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   None, detection is by manual read.

J. Device Description:

The AmpliVue™ C. difficile Assay combines simple specimen processing, an isothermal amplification technology named helicase-dependent amplification (HDA), and a self- contained disposable amplicon detection device for the detection of *Clostridium difficile* directly from CDAD-suspected stool specimens.

A swab is used to transfer a small amount of specimen into a dilution tube. The diluted sample is then transferred into a sample lysis buffer tube, and the cells are lysed by simple heat treatment. After heat treatment, an aliquot of the lysed sample is added to a reaction tube containing a lyophilized mix of HDA reagents, including primers specific for the amplification of a fragment from the conserved 5' end of the *tcdA* gene that are located within the PaLoc of toxigenic *C. difficile* strains. The assay also includes a process control to monitor specimen processing and to confirm the integrity of the assay reagents and cassette detection, as well as to assess for HDA-inhibitors that may be present within the stool specimen. Competitive amplification of the process control DNA also takes place unless inhibitory substances are present or the specimen processing fails. The HDA reaction is asymmetric so that an excess of single stranded DNA is formed from a biotinylated primer present within the reaction mix. The capture probes for each amplicon bind to the corresponding biotinylated single-stranded DNA forming dual labeled amplicons.

After completion of the HDA reaction, the reaction tube is transferred to a single-use, disposable cassette for detection. The self-contained cassette is comprised of two individual components: an amplicon cartridge that holds the running buffer and the single 0.2-ml thin wall reaction tube containing the amplified product. The detection chamber houses the amplicon cartridge and a vertical-flow DNA detection strip embedded into the cassette. The DNA detection strip is coated with an anti-FITC antibody and an anti-DNP antibody that serve as the *C. difficile* test (T2) line and the control (C) line in the assay, respectively. A razor blade and a plastic pin located at the bottom of the detection chamber opens the HDA reaction tube and the running buffer bulb when the handle of the detection chamber is closed.

The mixture flows through a fiberglass paper connected to the DNA detection strip that contains a fiberglass pad pre-loaded with streptavidin-conjugated color particles for color visualization. The dual-labeled probe-amplicon hybrid is then detected by the lateral flow strip within the cassette. The bottom line captures the FITC-labeled probe-amplicon and the top line captures the DNP-labeled probe-amplicon. The biotin label attracts the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines visible to the naked eye. Detection of *C. difficile* DNA is reported when the T2 and C lines are visible through the detection window of the cassette. No detection of *C. difficile* DNA is reported when only the C line is displayed. The assay is regarded as unresolved when neither line is displayed. The whole procedure takes approximately 75 minutes (Note: The T1 line present on the self-contained cassette is for a triplex assay and is not applicable/used for the AmpliVue C. difficile Assay).

The Quidel AmpliVue C. difficile Assay contains sufficient reagents to process 16 specimens or quality control samples. The kit contains the following:

Component	Quantity	Storage
Detection Cassettes: Each cassette comes preloaded with all required probes and signal amplification solutions necessary to generate a test result.	16/kit	2° to 30°C
Dilution Buffer: Ready for use.	16 tubes/kit 1.8 mL	2° to 8°C
Lysis Buffer: Ready for use.	16 tubes/kit 1 mL	2° to 8°C
Reaction Tubes: Ready for use.	16 tubes/kit	2° to 8°C
Amplicon Cartridge: Each cartridge comes preloaded with all solutions required amplification.	16/kit	2° to 30°C
Flocked Swabs: Ready for use.	16 swabs/kit	2° to 30°C

K. Substantial Equivalence Information:

1. Predicate device name(s):

   Great Basin Scientific Portrait Toxigenic *C. Difficile* Assay

2. Predicate 510(k) number(s):

   Great Basin Scientific Portrait Toxigenic C. Difficile Assay - K113358

3. Comparison with predicate:

Similarities		
Item	Device	Predicate (Great Basin Scientific Portrait Toxigenic C. Difficile Assay)
Intended Use	The AmpliVue™ C. difficile Assay is an in vitro diagnostic test for the direct, qualitative detection of the Clostridium difficile Toxin A gene (tcdA) in unformed stool specimens of patients suspected of having Clostridium difficile-	Portrait Toxigenic C. difficile Assay, a prescription device under 21 CFR Part 801.109 that is indicated for the detection of toxigenic Clostridium difficile in human fecal samples collected from patients suspected of having

Similarities		
Item	Device	Predicate (Great Basin Scientific Portrait Toxigenic C. Difficile Assay)
	associated disease (CDAD). The AmpliVue™ C. difficile Assay is intended for use as an aid in diagnosis of CDAD. The assay utilizes helicase-dependent amplification (HDA) for the amplification of a highly conserved fragment of the Toxin A gene sequence and a self-contained disposable amplicon detection device that allows for manual evaluation of assay results.	Clostridium difficile infection (CDI). The test utilizes automated blocked primer enabled helicase-dependent amplification (bpHDA) to detect toxin gene sequences associated with toxin producing C. difficile. The Portrait Toxigenic C. difficile Assay is intended as an aid in the diagnosis of CDI.
Specimen	Unformed stool	Same
Assay time	75-90 min	same
Test Cartridge	Disposable, single-use, multi-chambered fluidic cartridge.	Same
Technological principle	Nucleic acid amplification	Same
Assay technique	Isothermal, helicase-dependent nucleic acid amplification	Same

Differences		
Item	Device	Predicate
Analyte	Toxin A gene (*tcdA*)	Toxin B gene (*tcdB*)
Detection technique/instrument	The HDA reaction generates a biotinylated amplicon for both the target (tcdA) and the internal control. Capture	Amplification primers are biotin-labeled primers and hybridized to probes immobilized on a silicon chip.

Differences		
Item	Device	Predicate
	probes specifically bind to the corresponding biotinylated single-stranded DNA forming dual labeled amplicons. The reaction tube containing this mixture is transferred to a single-use, disposable cassette for detection. The mixture flows through a fiberglass paper connected to the DNA detection strip that contains a fiberglass pad pre-loaded with streptavidin-conjugated color particles for color visualization. The dual-labeled probe-amplicon hybrid is then detected by the lateral flow strip within the cassette. The bottom line captures the FITC-labeled probe-amplicon and the top line captures the DNP-labeled probe-amplicon. The biotin label attracts the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines visible to the naked eye.	Incubation with anti-biotin antibody conjugated to the HRP with TMB allows visualization by Portrait Analyzer
Detection method	Manual	Automated

L. Standard/Guidance Document Referenced (if applicable):

"Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of *Clostridium difficile*." DRAFT Guidance - November 29, 2010

M. Test Principle:

The AmpliVue™ C. difficile Assay combines simple specimen processing, an isothermal amplification technology named helicase-dependent amplification (HDA), and a self- contained disposable amplicon detection device for the detection of *Clostridium difficile* directly from CDAD-suspected stool specimens.

A swab is used to transfer a small amount of specimen into a dilution tube. The diluted sample is then transferred into a sample lysis tube and the cells are lysed by simple heat treatment. After heat treatment an aliquot of the lysed sample is added to a reaction tube containing lyophilized mix of HDA reagents including primers specific for the amplification of a fragment from the conserved 5' end of the *tcdA* gene, located within the PaLoc of toxigenic *C. difficile* strains. The assay also includes a process control to monitor sample processing and to confirm the integrity of the assay reagents and cassette detection as well as assay for HDA-inhibitors that may be present within the diarrheal sample. After completion of the HDA reaction, the reaction tube is transferred to a disposable lateral flow detection cassette for rapid detection with test result displayed as test and/or control lines in the window of the detection cassette. The whole procedure takes approximately 75 minutes, depending on the number of specimens processed.

N. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision: For the Precision/Within Laboratory Repeatability study, a blinded four-member panel consisting of *C. difficile* positive and negative sample was tested by two operators, twice a day using a single assay lot of AmpliVue C. difficile Assay for thirteen (13) days.

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Category	Operator #1		Operator #2		Overall Percent Agreement		95% Confidence Interval
	#expected results/# tested	*% Agreement*	*#expected results/# tested*	*% Agreement*			
C. difficile High Negative (T2-/C+; 20-80%)	**0/24**	**0%**	**0/24**	**0%**	**0/48**	**0%**	**0% - 8%**
C. difficile Low Positive (T2+/C+, ≥95%)	**24/24**	**100%**	**24/24**	**100%**	**48/48**	**100%**	**91.2% - 100%**
C. difficile Moderate Positive (T2+/C+, ≥95%)	**24/24**	**100%**	**24/24**	**100%**	**48/48**	**100%**	**91.2% - 100%**
Negative (T2-/C+, 100%)	**24/24**	**100%**	**24/24**	**100%**	**48/48**	**100%**	**91.2% - 100%**
C. difficile Positive Control	**24/24**	**100%**	**24/24**	**100%**	**48/48**	**100%**	**91.2% - 100%**
Assay Negative Control	**24/24**	**100%**	**24/24**	**100%**	**48/48**	**100%**	**91.2% - 100%**

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Reproducibility: In order to confirm the reproducibility of the AmpliVue™ C. difficile Assay a blinded and randomized study panel containing *Clostridium difficile* negative and positive samples were tested at three (3) test sites, two of which were clinical sites. Each site tested a reproducibility panel and assay controls for five (5) days in triplicate. The testing was done by two operators at each site. Each operator ran the panel once a day using one lot of AmpliVue™ C. difficile Assay.

Category	SITE						Overall Percent Agreement		95% Confidence Interval
	Site #1		Site #2		Site #3				
	#expected results/# tested	*% Agreement*	*#expected results/# tested*	*% Agreement*	*#expected results/# tested*	*% Agreement*			
C. difficile High Negative	25/30	83%	23/30	77%	24/30	80%	72/90	80%	71% - 87%
C. difficile Low Positive	30/30	100%	29/29	100%	30/30	100%	89/89*	100%	95% - 100%
C. difficile Moderate Positive	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95% - 100%
Negative	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95% - 100%
C. difficile Positive Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95% - 100%
Assay Negative Control	30/30	100%	30/30	100%	30/30	100%	90/90	100%	95% - 100%
Lot#	CLIN-005								

* **Note: One (1) sample was invalid.**

b. Linearity/assay reportable range:

N/A

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c. *Controls:*

The AmpliVue C. difficile assay incorporates a process control which is included in the lysis buffer tube and is used to monitor sample processing and evaluates the presence of inhibitory substances. The process control confirms the integrity of assay reagents and cassette detection. Additional controls are performed in accordance with end user laboratory guidelines and requirements.

d. *Detection limit:*

The analytical sensitivity (limit of detection or LoD) of the Quidel AmpliVue C. difficile Assay was determined using quantified (CFU/mL) cultures of two *C. difficile* strains (ATCC 43255 {toxinotype 0} and CCUG 8864 {toxinotype X}) serially diluted in a negative fecal matrix. Analytical sensitivity (LoD) is defined as the lowest concentration at which 95% of all replicates tested positive.

Strain	Toxinotype	LoD (CFU/ Assay)
ATCC 43255	0	4.2
CCUG 8864	X	0.7

The final assay LoD is defined as the higher of the two strain concentrations where 95% positivity was observed. The final assay LoD is 4.2 CFU/assay.

e. *Analytical Reactivity (Inclusivity)*

Twenty-four toxigenic strains for *C. difficile* were tested at 2 to 3x LoD in negative specimen matrix using three (3) AmpliVue C. difficile assay lots. Strains were reported to originate from at least five states and four countries (USA, Belgium, France and Sweden). Seven (7) toxinotypes were represented: 0, IIIb, IIIc, IV, V, VIII and XXIII. The analytical reactivity (inclusivity) testing conducted demonstrated that the AmpliVue C. difficile assay can detect a broad range of toxigenic *Clostridium difficile* strains.

f. Analytical specificity:

Cross Reactivity

The analytical specificity of the Quidel AmpliVue C. difficile Assay was evaluated by testing a panel consisting of sixty-six (66) bacterial, viral and yeast microorganisms and human DNA representing common enteric pathogens, flora or nucleic acid commonly present in the intestine. Microorganisms or nucleic acid was mixed with pooled negative matrix and tested directly or in the presence of 2 to 3x LoD level of *C. difficile* for cross reactivity and microbial interference, respectively. Bacteria were tested at concentrations greater than 1.0E+06 CFU/mL and viruses at greater than 1.0E+05 PFU/mL. In addition, *in silico* analysis showed that the AmpliVue C. difficile Assay had no predicted cross-reactivity for *C. botulinum*. The results of this study demonstrate that the AmpliVue C. difficile Assay does not cross react with medically relevant levels of viruses or bacteria found in stool specimens.

Interfering Substances

Two toxigenic strains of C. difficile (ATCC 43255, toxinotype 0; CCUG 8864, toxinotype X) were evaluated against a test panel consisting of thirty-four substances found in stool specimens. Substances were introduced into the assay dilution tubes at concentrations which were medically relevant. Each of the strains was tested for each substance. None of the substances tested were found to interfere with the AmpliVue C. difficile Assay.

g. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the AmpliVue C. difficile Assay was evaluated at four geographically diverse locations within the United States between January 2012 and October 2012. Eight hundred and forty (840) samples were tested by both the AmpliVue C. difficile Assay and the Tissue Culture Cytotoxicity Assay. One specimen (0.1%) was indeterminate in the cytotoxin assay due to toxicity in the antitoxin well. Four specimens (0.5%) were invalid in the AmpliVue C. difficile Assay when initially tested. Three (3) of these specimens yielded valid results (all were negative) when retested according to the AmpliVue C. difficile Assay's instructions for use. One (1) specimen remained invalid upon repeat testing. The data below is based on the initial result for the eight hundred and thirty-five (835) specimens.

	Tissue Culture Cytotoxin						95% CI	
AmpliVue C. difficile		POS	NEG	Total	Sensitivity	93.6%	87.3%	96.9%
	POS	102	43*	145	Specificity	94.1%	92.1%	95.6%
	NEG	7**	683	690	ppv	70.3%	62.5%	77.2%
	Total	109	726	835	npv	99.0%	97.9%	99.5%

* Of these forty three (43) discordant specimens (AmpliVue Positive/Tissue Culture Cytotoxin Negative) reported, thirty-seven (37) were positive for *C. difficile* by a FDA-cleared molecular device, and six (6) were negative.

** Of these seven (7) discordant specimens (AmpliVue Negative/Tissue Culture Cytotoxin positive) reported, two (2) were positive for *C. difficile* by a FDA-cleared molecular device, and five (5) were negative.

The distribution of positive specimens is parsed by gender and age below (incidence based on results from the AmpliVue C. difficile Test):

Age	Male			Female			Total		
	n	AmpliVue+	%	n	AmpliVue+	%	n	AmpliVue+	%
<2	14	6	42.9%	12	2	16.7%	26	8	30.8%
≥2 and <12	45	9	20.0%	27	6	22.2%	72	15	20.8%
≥12 and <18	21	2	9.5%	24	3	12.5%	45	5	11.1%
≥18 and ≤21	14	3	21.4%	5	0	0.0%	19	3	15.8%
>21 and ≤59	166	25	15.1%	167	26	15.6%	333	51	15.3%
≥60	134	25	18.7%	206	38	18.4%	340	63	18.5%
Total	**394**	**70**	**17.8%**	**441**	**75**	**17.0%**	**835**	**145**	**17.4%**

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

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:

N/A

O. Proposed Labeling:

The labeling is per the requirements of 21 CFR Part 809.10.

P. Conclusion:

Quidel has submitted this 510(k) in accordance with the requirements of SMDA 1990 and 21 CFR 807.92. This summary of 510(k) safety and effectiveness information provides the necessary detail for a determination of substantial equivalence for the Quidel AmpliVue™ C. difficile Assay.

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Decision Summary, k123355

This 510(k) was reviewed under OIR's Pilot Triage Program. This program represents an internal workload management tool intended to reduce internal FDA review resources for 510(k) applications that are of good quality upon receipt by FDA.

The information in the 510(k) is complete and supports a substantial equivalence (SE) determination. Please refer to the applicant's 510(k) summary for a summary of the information that supports this SE determination.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-002

Quidel Corporation
Mr. Ron H. Lollar
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San Diego, California 92121

DEC 13 2012

Re: K123355

Trade/Device Name:	AmpliVue™ C. difficile Assay
Regulation Number:	21 CFR 866.3130
Regulation Name:	C. Difficile Nucleic Acid Amplification Test Assay
Regulatory Class:	Class II
Product Code:	OZN
Dated:	October 26, 2012
Received:	November 6, 2012

Dear Mr. Lollar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostics and Radiological Health at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

510(k) Number k123355:

Device Name: AmpliVue™ C. difficile Assay

Indication for Use:

The AmpliVue™ C. difficile assay is an *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridium difficile* Toxin A gene *(tcdA)* in unformed stool specimens of patients suspected of having *Clostridium* difficile-associated disease (CDAD). The AmpliVue™ C. difficile assay is intended for use as an aid in diagnosis of CDAD. The assay utilizes helicase-dependent amplification (HDA) for the amplification of a highly conserved fragment of the Toxin A gene sequence and a self-contained disposable amplification detection device that allows for manual evaluation of assay results.

Prescription Use		Over-The-Counter Use
X	AND/OR	
(Part 21 CFR 801 Subpart D)		(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED

Concurrence of CDRH, Office of Device Evaluation (ODE)

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) k 123355