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QUIDEL | AmpliVue® C. difficile ASSAY

For the qualitative detection and identification of toxigenic *Clostridium difficile* nucleic acids extracted from stool samples

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

Prescription Use only

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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*-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 visual evaluation of assay results.

SUMMARY AND EXPLANATION

C. difficile is a major cause of antibiotic-associated diarrhea and colitis, accounting for up to 25% of all cases.[1] It is thought that the exposure to antibiotics disrupts the flora of the intestine, allowing an opportunistic colonization by *C. difficile* (which is present in the gut flora of up to 3% of healthy adults). The virulence of *C. difficile* is believed to be mediated by the production of two toxins (Toxin A and Toxin B). Both toxin genes (*tcdA* and *tcdB,* respectively) are located within a 19.6 Kb pathogenicity locus (PaLoc), along with 3 other gene products. Recently, the incidence and severity of *C. difficile*-associated disease corresponding to short-term hospital stays has been on the rise.[1,2]

PRINCIPLE OF THE PROCEDURE

The AmpliVue C. difficile Assay combines simple sample processing, an isothermal amplification technology named helicase-dependent amplification (HDA), and a self-contained disposable amplicon detection device for the detection of *C. difficile* directly from CDAD-suspected stool specimens.[3,4] The assay targets a conserved fragment of the *C. difficile* DNA, which is intact in all known A+B+ and A–B+ toxinotypes of *C. difficile*.

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 lyophilized mix of HDA reagents including primers specific for the amplification of a fragment from the conserved region of the *C. difficile* DNA. 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 to assess for HDA-inhibitors that may be present within the diarrheal sample. Competitive amplification of the process control DNA also takes place unless amplification inhibitory substances are present or the sample 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 Cassette for rapid detection with the test result displayed as test and/or control lines in the window of the Cassette. The dual-labeled probe-amplicon hybrid is then detected by the lateral flow strip within the Cassette. The bottom line captures the test amplicon and the top line captures the control amplicon. The biotin label binds the streptavidin-conjugated color particles for visualization and the test result is shown as colored lines visible to the naked eye.

The self-contained Cassette is comprised of two individual components: an Amplicon Cartridge that holds the running buffer and a single 0.2 mL thin wall Reaction Tube containing the amplified product; and the detection chamber which houses the Amplicon Cartridge and a vertical-flow DNA detection strip embedded into the Cassette. The DNA detection strip is coated with different anti-hapten antibodies that serve as the *C. difficile* test (T) line and the control (C) line in the assay. 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. Detection of *C. difficile* DNA is reported whenever the T2 (Test line 2) is visible through the

detection window of the Cassette. The presence of the C line is not required for positive results. No detection of *C. difficile* DNA is reported when only the C line is displayed. The assay is regarded as invalid when neither line is displayed.

MATERIALS PROVIDED

Cat. #M201
16 Tests per Kit

Component	Quantity	Storage
Detection Cassettes Part 1185001	16/kit	2°C to 30°C
Dilution Buffer Part M5015	16 tubes/kit 1.8 mL	2°C to 8°C
Lysis Buffer Part M5016	16 tubes/kit 1 mL	2°C to 8°C
Reaction Tubes Part M5017	16 tubes/kit	2°C to 8°C
Amplicon Cartridge Part 1215400	16/kit	2°C to 30°C
Flocked Swabs Part M5013	16 swabs/kit	2°C to 30°C

OPTIONAL MATERIALS

- External controls for toxigenic *C. difficile* (e.g. Quidel Molecular C. difficile Control Set #M108, which contains positive and negative controls, serves as an external processing and extraction control)
- Thermometer

MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- Micropipettor
- Stopwatch or timer
- Scissors or a blade
- Heat block capable of 95°C $\pm$ 2°C temperature
- Heat block with heated lid capable of 64°C $\pm$ 2°C temperature

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Performance characteristics of this test have been established with the specimen types listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Only open the tubes when adding aliquots into tubes or removing aliquots from tubes. Keep the tubes closed at any other time to avoid contamination.
- To avoid contamination of the environment with *C. difficile* amplicons, do not open the reaction tubes post-amplification.
- Avoid microbial and deoxyribonuclease (DNAse) contamination of reagents when removing aliquots from tubes. The use of sterile DNAse-free disposable filter-blocked or positive displacement Pipettor tips is recommended.
- Use a new Pipettor tip for each specimen or reagents.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.

- Additional controls may be tested according to guidelines or requirements of Local, State, Provincial and/or Federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated or segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating the reagents.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with County, Federal, Provincial, State and Local regulations.
- Wear suitable protective clothing, gloves, eye and face protection when using this kit.
- For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- MSDS is available upon request or can be accessed on the product website.

STORAGE AND HANDLING OF KIT REAGENTS

Store assay reagents and detection cassettes as indicated on their individual labels.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Specimen Type: unformed stool samples indicative of CDAD.

Using a sterile container:

1. Transfer the liquid or soft stool into the sterile container, taking care not to transfer toilet paper, urine, water or soap.
2. Label the container according to hospital standard operating procedures.
3. Transport the labeled specimen to the laboratory. If specimens can be processed within 3 to 4 hours after collection, transport at room temperature is adequate. Specimens delayed to the laboratory should be promptly cooled and kept between 2°C to 8°C during transport.

Storage: Specimens may be stored between 2°C to 8°C or –20°C for up to 7 days prior to testing.

ASSAY PROCEDURE

Heat Lysis

1. 25 minutes prior to the heat lysis step, warm the heating block to 95°C.
2. Vortex liquid stool specimen for 15 seconds to ensure complete mixing.
3. Collect sample by using the sterile swab provided. Dip the swab into the liquid or unformed stool sample being aware to not oversample – the swab head should only be lightly coated with stool.
4. Place swab in a patient identified Dilution Buffer tube (Blue Cap) and release the specimen by swirling the tip rapidly for 5 to 10 seconds to remove stool; remove swab and discard as appropriate for your laboratory. Vortex approximately 10 seconds to mix.
   Note: Samples in Dilution Buffer are stable up to 24 hours at room temperature.
5. Transfer 50 µL of the diluted stool specimen to a labeled Lysis Buffer tube and vortex for 10 seconds.
   Note: Samples in Lysis Buffer are stable up to 24 hours at room temperature prior to heating.
6. Heat the Lysis Buffer tube at 95°C ± 2°C for 10 minutes ± 2 minutes and then vortex for 10 seconds.
   Note: Begin 10 minute lysis procedure after placing tubes in block and waiting until block returns to 95°C.
   Note: Samples in Lysis Buffer are stable up to 24 hours at room temperature after heating.
   Note: Unused Dilution Buffers and Lysis Buffers must be stored at 2°C to 8°C.

Amplification

1. 15 minutes prior to the amplification step, warm a heating block with a heated lid to 64°C.
2. Transfer 50 µL of lysed sample to a labeled Reaction Tube and rehydrate lyophilized reagents by pipetting up and down 3 to 5 times to mix. Close the lid tightly and proceed to the next step.
3. Incubate the Reaction Tube at 64°C ± 2°C for 60 minutes ± 2 minutes in a heating block with a heated lid.
   Note: To avoid laboratory contamination, once the tube has been closed, and the amplification reaction started, **DO NOT** open the Reaction Tube.
   Note: Unused Reaction Tubes must be stored at 2°C to 8°C.

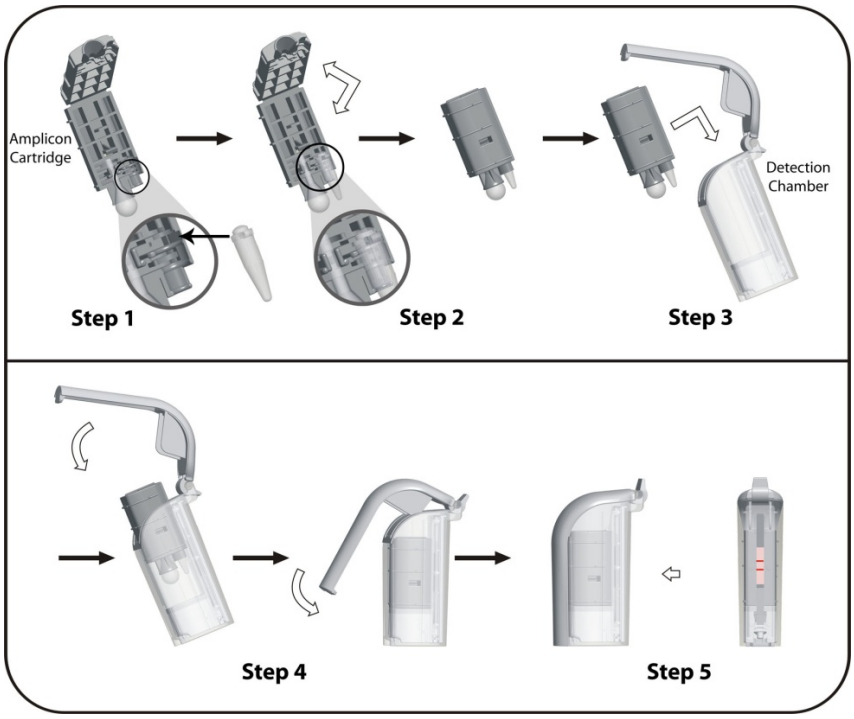
Detection

1. Tear open a new Detection Cassette package. Label the Cassette appropriately.
   Make sure a buffer bulb is attached in the Amplicon Cartridge.
2. Place the Reaction Tube into the Amplicon Cartridge (Figure 1, step 1). Be sure to place the HINGE of the Reaction Tube cap into the largest slot adjacent to the buffer bulb.
3. Close the Amplicon Cartridge (Figure 1, step 2) ensuring that it snaps shut.
   If the cartridge does not snap shut, reposition the tube within the cartridge.
4. Insert the closed Amplicon Cartridge into the Detection Cassette (Figure 1, step 3).
   Make sure the arrow faces the detection strip (Reaction Tube should face the razor blade and the plastic bulb containing the running buffer should face the pin). Identify the cassette on the top and/or side of the outer casing.
5. Keep the device upright and press the handle of the outer casing to close the device (Figure 1, step 4). The handle will lock into place when closed completely (Figure 1, step 5).
6. Read results at 10 minutes. Results are stable up to 30 minutes after the Cassette has been snapped shut.
7. Discard the used Detection Chambers in sealed bags and as appropriate for your laboratory.

Warning

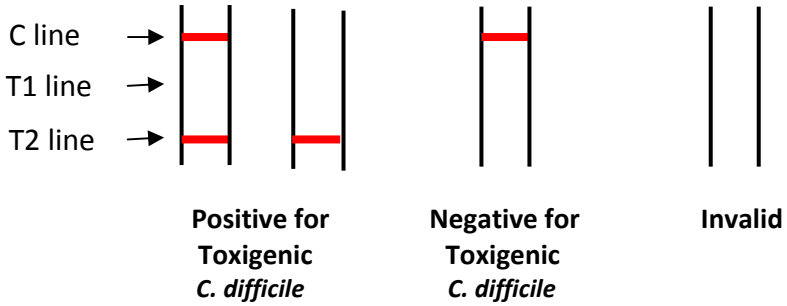
1. DO NOT open the AmpliVue Detection Cassette after use. Opening the Cassette after use may result in amplicon contamination of the test area.
2. Remove the required number of Reaction Tubes from the protective pouch, remove the excess air and reseal the bag.

Figure 1



INTERPRETATION OF RESULTS

- Any pink to red colored visible line should be recorded as positive (+) and no line should be recorded as (–); for example, "T+" = Visible T line and "T–" = No T line (See diagram below).
- The T2 line detects toxigenic *C. difficile* DNA.
- The C line detects the process control DNA in the absence of the target toxigenic *C. difficile* DNA. In the presence of the target toxigenic *C. difficile* DNA, the C line detects amplified products from both the toxigenic *C. difficile* DNA and the process control DNA. The control line intensity may vary with each test. Any pink to red colored visible line in the control signifies a valid test.



The interpretation of the assay results is done according to the following criteria:

Test line (T) Reading	Control line (C) Reading	Interpretation of result
T2+	C+	Toxigenic *C. difficile* DNA detected (Positive)
T2+	C–	Toxigenic *C. difficile* DNA detected (Positive)
T2–	C+	No toxigenic *C. difficile* DNA detected (Negative)
T2–	C–	Invalid: failure due to inhibitory specimen, reagent failure, or device failure. Repeat test with original stool specimen.
Note 1: The T1 line is for triplex assays. The T1 line is not used on this assay. **Note 2:** The absence of a C line (control) in conjunction with a positive test line (T2) means that target material was successfully amplified. This occurs because of the over abundance of amplicons that generates competition with the test targets.		

QUALITY CONTROL

The AmpliVue C. difficile Assay incorporates several controls to monitor assay performance.

1. The process control is used to monitor sample processing, to detect HDA inhibitory specimens and to confirm the integrity of assay reagents and Cassette detection. The process control is included in the lysis buffer tube.
2. External positive controls may be treated as a patient specimen. Dip the provided swab into the external positive control ensuring liquid covers the tip. Identify the Dilution Buffer tube as the positive control and proceed with processing as described above in the Assay Procedure. The external positive control is intended to monitor substantial Reagent and Cassette failure.
3. External negative controls may be treated as a patient specimen. Dip the provided swab into the external negative control ensuring liquid covers the tip. Identify the Dilution Buffer tube as the negative control and proceed with processing as described above in the Assay Procedure. The external negative control is used to detect reagent or environmental contamination (or carry-over) by *C. difficile* DNA or amplicon.

It is recommended that the reactivity of each new lot and each new shipment of the AmpliVue C. difficile Assay be verified on receipt and before use. External control tests should be performed thereafter in accordance with appropriate Federal, State and Local guidelines. The AmpliVue C. difficile Assay should not be used in patient testing if the external controls do not produce the correct results.

LIMITATIONS

- A negative *C. difficile* result should not be used as the sole basis for diagnosis, treatment, or patient management decisions.
- Although there is no need for reagent preparation, the main laboratory technique required is pipetting; good laboratory technique is essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown *C. difficile* variants and may result in a false negative result with the AmpliVue C. difficile Assay.
- A positive test result does not necessarily indicate the presence of viable organisms.
- This test detects but does not differentiate hypervirulent strains from other toxigenic *C. difficile* genotypes.
- This test does not indicate the susceptibility of detected *C. difficile* strains to various antimicrobial agents.
- Negative test results may occur from improper specimen collection, handling or storage, presence of inhibitors, technical error, sample mix-up or because the number or organisms in the specimen is below the analytical sensitivity of the test. Careful compliance with the instructions given in this insert is necessary to avoid erroneous results. Use of this assay should be limited to personnel trained on the procedure.
- The AmpliVue C. difficile Assay procedure must be carried out in an environment that does not exceed 30°C.

CLINICAL PERFORMANCE

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 (4) 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. The Quidel AmpliVue C. difficile Assay achieved a sensitivity and specificity of 93.6% and 94.1%, respectively, in this study.

		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				
	Total	109	726	835				

* 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.

ANALYTICAL PERFORMANCE

Limit of Detection

The analytical sensitivity (limit of detection or LOD) of the AmpliVue C. difficile Assay was determined using quantified (CFU/mL) cultures of two (2) *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.

Analytical Reactivity (Inclusivity)

The reactivity of the AmpliVue C. difficile Assay was evaluated against an additional twenty-four (24) strains of *Clostridium difficile* representing multiple toxinotypes. The testing was performed near the level of detection for the assay (2 to 3x LOD). The testing was performed using three (3) separate production lots of the AmpliVue C. difficile Assay. All twenty-four (24) strains were detected by the AmpliVue C. difficile Assay in this study and the highest observed LOD was 15.67 CFU/assay.

Strain	Toxinotype	CFU/Assay	AmpliVue *C. difficile* (Detected/Total)
ATCC 43255	0	4.2	19/20
CCUG 8864	X	0.7	19/20
ATCC BAA-1870	IIIb	8.15	3 / 3
CCUG 37770	IV	2.84	3 / 3
ATCC BAA-1875	V	10.92	3 / 3
ATCC 43598	VIII	2.13	3 / 3
ATCC 37774	XXIII	1.52	3 / 3
CCUG 9004	Unknown	1.63	3 / 3
ATCC BAA-1874	0	5.75	3 / 3
ATCC 43600	0	3.92	3 / 3
ATCC BAA-1871	0	3.61	3 / 3
ATCC BAA-1803	IIIc	0.7	3 / 3
ATCC BAA-1872	0	7.97	3 / 3
ATCC 700792	0	1.65	3 / 3
ATCC 43599	0	1.55	3 / 3
CCUG 60276	Unknown	15.67	3 / 3
CCUG 60275	Unknown	1.99	3 / 3
CCUG 37778	Unknown	9.29	3 / 3
CCUG 37777	Unknown	7.97	3 / 3
CCUG 37776	Unknown	1.08	3 / 3
CCUG 37773	Unknown	1.29	3 / 3
ATCC 17857	0	0.72	3 / 3
ATCC 43594	0	0.54	3 / 3
ATCC 43596	0	1.27	3 / 3

Reproducibility Study

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 was tested at three (3) test sites (two (2) clinical sites). Each site tested a reproducibility panel and Assay Controls for 5 days in triplicate. 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. A total of five hundred and forty (540) specimens were tested (including controls). The AmpliVue C. difficile Assay generated reproducible results in this study.

Reproducibility of the Quidel AmpliVue C. difficile Assay									
Category	**Site #1**		**Site #2**		**Site #3**		**Overall Percent Agreement**		**95% Confidence Interval**
	#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. A "high negative/low positive" sample is a sample with a concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 20%- 80% of the time.[5]									

Analytical Specificity – Cross-reactivity and Microbial Interference

The analytical specificity of the 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.

The table below summarizes the data from these studies. There was no evidence of cross reactivity or interference with any of the panel members and the AmpliVue C. difficile Assay.

Organisms ID	**Identification**	**Concentration tested (CFU/mL or PFU/mL)**	***C. difficile* Results**		
			Cross-reactivity	**Microbial Interference (Strain 1)**	**Microbial Interference (Strain 2)**
Abiotrophia defective *	CCUG 27280	4.30E+08	Negative	Positive	Positive
Acinetobacter baumannii	ZM 081597	5.27E+08	Negative	Positive	Positive
Aeromonas hydrophila	ATCC 7966	2.09E+10	Negative	Positive	Positive
Alcaligenes faecalis subspecies faecalis	ATCC 15554	4.65E+09	Negative	Positive	Positive
Bacillus cereus	ATCC 13472	1.00E+07	Negative	Positive	Positive
Bacteroides fragilis	CCUG 4856	1.77E+08	Negative	Positive	Positive
*Campylobacter coli**	CCUG 36995	5.30E+08	Negative	Positive	Positive
Campylobacter jejuni sub sp .jejuni	ATCC 33292	1.72E+07	Negative	Positive	Positive
Candida albicans	ATCC 10231	3.00E+07	Negative	Positive	Positive
Citrobacter freundii	ATCC 8090	2.38E+09	Negative	Positive	Positive
Clostridium bifermentans	ATCC 638	2.05E+07	Negative	Positive	Positive
Clostridium botulinum	*In silico* analysis		No *in silico* cross reactivity observed		
Clostridium butyricum	CCUG 47601	1.75E+07	Negative	Positive	Positive
Clostridium difficile (non-toxigenic)	ATCC 43601	4.58E+06	Negative	Positive	Positive
Clostridium difficile (non-toxigenic)	ATCC 43593	1.13E+06	Negative	Positive	Positive
*Clostridium haemolyticum**	ATCC 9650	3.43E+09	Negative	Positive	Positive
Clostridium novyi	CCUG 57219	6.50E+06	Negative	Positive	Positive
Clostridium orbiscindens	ATCC 49531	5.30E+06	Negative	Positive	Positive
Clostridium perfringens (Strain: Type A)	ZM 0801585	3.37E+07	Negative	Positive	Positive
Clostridium scindens	ATCC 35704	1.62E+07	Negative	Positive	Positive

Organisms ID	Identification	Concentration tested (CFU/mL or PFU/mL)	*C. difficile* Results		
			Cross-reactivity	Microbial Interference (Strain 1)	Microbial Interference (Strain 2)
Clostridium septicum	ATCC 12464	6.60E+09	Negative	Positive	Positive
Clostridium sordellii	ATCC 9714	1.94E+06	Negative	Positive	Positive
Clostridium sordellii	Z077	2.07E+08	Negative	Positive	Positive
Clostridium sordellii	CCUG 6329	9.85+E07	Negative	Positive	Positive
Clostridium sordellii	CCUG 9284	6.50E+07	Negative	Positive	Positive
Clostridium sordellii	CCUG 33098	2.00E+07	Negative	Positive	Positive
Clostridium sordellii	CCUG 36938	5.55E+07	Negative	Positive	Positive
Clostridium sordellii	CCUG 43123	2.50E+07	Negative	Positive	Positive
Clostridium sordellii	CCUG 47545	1.36E+07	Negative	Positive	Positive
Clostridium sordellii	CCUG 59819	7.00E+06	Negative	Positive	Positive
Clostridium sporogenes	ATCC 11437	3.55E+07	Negative	Positive	Positive
Edwardsiella tarda	ATCC 15947	2.03E+09	Negative	Positive	Positive
Enterobacter aerogenes	ATCC 13048	1.31E+10	Negative	Positive	Positive
Enterobacter cloacae	ATCC 13047	5.95E+08	Negative	Positive	Positive
Enterococcus faecalis vanB	ATCC 51299	3.45E+09	Negative	Positive	Positive
Escherichia coli	ATCC 23511	1.92E+09	Negative	Positive	Positive
Escherichia coli O157:H7	ZM 0801622	2.20E+09	Negative	Positive	Positive
Helicobacter pylori	ZM 0801486	3.57E+06	Negative	Positive	Positive
Klebsielia oxytoca	ATCC 33496	1.63E+09	Negative	Positive	Positive
Lactobacillus acidophilus	ATCC 4356	6.82E+07	Negative	Positive	Positive
Listeria monocytogenes(Serotype 1/2b)	ZM 0801534	1.18E+10	Negative	Positive	Positive
Peptostreptococcus anaerobius	ATCC 27337	5.80E+08	Negative	Positive	Positive
Plesiomonas shigelloides	ATCC 14029	1.40E+08	Negative	Positive	Positive
Porphyromonas asaccharolytica	CCUG 7834	1.30E+07	Negative	Positive	Positive
Prevotella melaninogenica	ATCC 25845	5.10E+08	Negative	Positive	Positive
Proteus mirabilis	ATCC 25933	1.06E+09	Negative	Positive	Positive
Providencia alcalifaciens	ATCC 9886	9.60E+08	Negative	Positive	Positive
Pseudomonas aeruginosa	ATCC 35554	2.60E+10	Negative	Positive	Positive
Salmonella cholerasuis (typhimurium)	ATCC 14028	3.55E+10	Negative	Positive	Positive
Salmonella enterica subspecies *Arizonae (formerly Choleraesuis arizonae)*	ATCC 13314	4.22E+09	Negative	Positive	Positive
Salmonella enteric subspecies *enterica* (formally *Salmonella choleraesuis*)	ATCC 7001	6.80E+09	Negative	Positive	Positive
Serratia liquefaciens	ATCC 27592	3.79E+10	Negative	Positive	Positive
Serratia marcescens	ZM 0801723	6.10E+08			Positive
Shigella boydii	ATCC 9207	8.16E+08	Negative	Positive	Positive
Shigella dysenteriae	ATCC 49557	1.26E+10	Negative	Positive	Positive
Shigella sonnei	ATCC 29930	3.36E+08	Negative	Positive	Positive
Staphylococcus aureus	ATCC 43300	6.00E+07	Negative	Positive	Positive
Staphylococcus epidermidis	ATCC 14990	4.00E+08	Negative	Positive	Positive
Streptococcus agalactiae (Group B Streptococcus)	ATCC 12386	2.75E+08	Negative	Positive	Positive
Vibrio parahaemolyticus	ATCC 17802	9.50E+06	Negative	Positive	Positive
Adenovirus 1 VR-1*	DHI 62207	5.67E+05	Negative	Positive	Positive
Rotavirus (Strain: WA)*	ZM NATROTA-ST	2.32E+08	Negative	Positive	Positive
Norovirus GII	ZM NATNOVII-ST	3.92E+08	Negative	Positive	Positive

Organisms ID	Identification	Concentration tested (CFU/mL or PFU/mL)	*C. difficile* Results		
			Cross-reactivity	Microbial Interference (Strain 1)	Microbial Interference (Strain 2)
Enterovirus 71	DHI 80406	4.82E+05	Negative	Positive	Positive
Echovirus 6	DHI 121506	1.05E+09	Negative	Positive	Positive
Coxsackievirus B4	DHI 92206	2.43E+07	Negative	Positive	Positive
Cytomegalovirus Towne VR-977	DHI 201006	1.48E+06	Negative	Positive	Positive
Human Genomic DNA	Promega G3041	184 µg/mL	Negative	Positive	Positive
* Purified nucleic acid was used in the testing of these organisms. Cell counts were approximated based on nucleic acid concentration and genome size.					

Analytical Specificity – Interfering Substances

The performance of AmpliVue C. difficile Assay was evaluated with potentially interfering substances that may be present in stool specimens. The potentially interfering substances were evaluated using two *C. difficile* strains (ATCC 43255 {toxinotype 0} and CCUG 8864 {toxinotype X}) at a concentration of 2 to 3x LOD. There was no evidence of interference caused by the substances tested.

Substance Name	Concentration Tested	C difficile Result	Substance Name	Concentration Tested	C difficile Result
Nystatin	10,000 USP U/mL	Positive	Esomeprazole magnesium hydrate	0.5 mg/mL	Positive
Hydrocortisone (aka cortisone)	1% w/v	Positive	Barium sulfate	5 mg/mL	Positive
Phenylephrine hydrochlorine	2% w/v	Positive	Witch hazel	100%	Positive
Calcium carbonate	0.2 mg/mL	Positive	Petroleum jelly	100%	Positive
Aluminum hydroxide	0.1 mg/mL	Positive	Vancomycin HCl	12.5 mg/mL	Positive
Magnesium hydroxide	0.1 mg/mL	Positive	Methicillin	12.5 mg/mL	Positive
5-aminosalicylic acid/Mesalazine	2 mg/mL	Positive	Sennosides	0.1 mg/mL	Positive
Mineral oil	2% v/v	Positive	triclosan	0.1% v/v	Positive
Nonoxynol-9	7%	Positive	Naproxen sodium	14 mg/mL	Positive
Loperamide hydrochloride	1 mg/mL	Positive	Benzalkonium chloride	0.12%	Positive
Bismuth subsalicylate	0.87 mg/mL	Positive	Ethanol	10%	Positive
Blood	5% v/v	Positive	Glucose	1 mg/mL	Positive
Mucus (Mucin)	3 mg/mL	Positive	Human IgA	1.6 mg/mL	Positive
Miconazole nitrate	2%	Positive	Human serum albumin	10 mg/mL	Positive
Zinc oxide	13% w/v	Positive	Palmitic acid	1.3 mg/mL	Positive
Aluminum hydroxide / Magnesium carbonate	0.1 mg/mL	Positive	Stearic acid	26 mg/mL	Positive
Cimetidine	0.5 mg/mL	Positive	Human hemoglobin	3.2 mg/mL	Positive

Carryover – Cross Contamination

Four (4) runs of twenty-four (24) samples consisting of twelve (12) negative and twelve (12) high *C. difficile* positive samples were tested in an alternating pattern by a total of two (2) operators on two (2) assay lots (operators were blinded to the expected results). All positive samples were reported as positive (T2+/C+) and all negative samples were reported as negative (T2–/C+). No carry over contamination was seen when performing the Quidel AmpliVue C. difficile Assay according to product instructions for use.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact custserv@quidel.com or technicalsupport@quidel.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

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REF M201 – AmpliVue C. difficile Assay kit

IVD

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REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
Use by	Manufacturer
Temperature limitation	Intended use
Rx ONLY Prescription use only	Consult e-labeling instructions for use
WARNING: Harmful if swallowed (oral)	IVD For *In Vitro* diagnostic use
Σ 16 Contains sufficient for XX determinations	CONT Contents/Contains