QUIDEL

Sofia 2
C. difficile FIA

For use with Sofia 2

For export only – Not for sale in the U.S.
For *in vitro* diagnostic use.
A symbols glossary can be found at quidel.com/glossary.

INTENDED USE

The Sofia 2 C. difficile FIA employs immunofluorescence for the qualitative detection of *Clostridiodes difficile* glutamate dehydrogenase (GDH) antigen and Toxins A/B. The test detects *C. difficile* antigen, glutamate dehydrogenase, as a screen for the presence of *C. difficile* and confirms the presence of toxigenic *C. difficile* by detecting Toxins A/B in human fecal specimens from persons suspected of having *C. difficile* disease. The test can be used with unpreserved fecal specimens and fecal specimens preserved in transport media. Test results should be taken into consideration by the physician in conjunction with patient history and symptoms.

SUMMARY AND EXPLANATION

Clostridioides difficile is the most frequently identified enteric pathogen in patients with antibiotic-associated diarrhea and colitis. Each year in the United States, *C. difficile* infection results in approximately half a million infections among patients in the United States.[1] These infections account for considerable increases in the length of hospital stays and more than $1.1 billion in health care costs.[2] Recently, the incidence and severity of *C. difficile*-associated disease corresponding to short-term hospital stays has been on the rise.[3,4]

The majority of *C. difficile* infections are acquired nosocomially, and many patients remain asymptomatic following acquisition. Exposure to antibiotics disrupts the flora of the intestine, allowing an opportunistic colonization by *C. difficile*. The virulence of *C. difficile* is mediated by the production of two toxins (Toxin A/B).[5]

PRINCIPLE OF THE TEST

The Sofia 2 C. difficile FIA employs immunofluorescence technology that is used with Sofia 2 for the rapid qualitative detection of glutamate dehydrogenase (GDH), Toxin A, and Toxin B in fecal samples.

The patient's sample is placed in the Specimen Tube containing the Specimen Diluent, making the antigenic components more accessible to the specific antibodies. An aliquot of the diluted sample is dispensed through a filter to remove particulates (making them more compatible for testing) into the Test Cassette sample well. From the sample well, the sample migrates through a test strip containing various unique chemical environments. If GDH, Toxin A/B are present, they will be bound by antibodies coupled to fluorescent microparticles that migrate through the test strip. The fluorescent microparticles containing bound proteins will be captured by antibodies at a defined location on the test strip where they are detected by Sofia 2. If GDH, Toxin A/B are not present, the fluorescent microparticles will not be trapped by the capture antibodies nor detected by Sofia 2.

The Test Cassette is placed inside of Sofia 2 for automatically timed development (WALK AWAY Mode), or pre-incubated on the bench top prior to loading into Sofia 2 (READ NOW Mode), where Sofia 2 will scan, measure,

and interpret the immunofluorescent signal using method-specific algorithms. Sofia 2 will display the test results (Positive, Negative, or Invalid) on the screen.

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Test Cassettes (25): Antibodies to *C. difficile* GDH, *C. difficile* Toxin A and Toxin B, anti-Mouse IgG and Mouse IgG
- Specimen Diluent Tubes containing 1.07mL of diluent containing 0.05% of ProClin 300 ⚠
- 80 µm Top Filters (Purple) (Dropper Tips) (25)
- Multi-Volume Pipettes (Flared) (25)
- One (1) Bottle Positive Control: (1) Recombinant GDH and recombinant Toxin in a protein solution containing 0.05% ProClin 300 ⚠
- One (1) Bottle Negative Control: (1) Protein solution containing 0.05% ProClin 300 ⚠
- Package Insert (1)
- Quick Reference Instructions (1)
- QC Card (located on kit box)

MATERIALS NOT SUPPLIED IN KIT

- Timer or watch
- Sofia 2
- Calibration Cassette (supplied with Sofia 2)
- Clean, dry container for specimen collection

WARNINGS AND PRECAUTIONS

- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.[6]
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.[6]
- Do not reuse any used Test Cassettes, Specimen Diluent Tubes, or solutions.
- The Calibration Cassette must be kept in the provided storage pouch between uses.
- To obtain accurate results, the Package Insert instructions must be followed.
- Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
- Sample collection and handling procedures require specific training and guidance.
- Use the Multi-Volume pipette, provided with this assay, to collect samples.
- The user should never open the foil pouch of the Test Cassette exposing it to the ambient environment until the Test Cassette is ready for immediate use.
- Discard and do not use any damaged Test Cassette or material.
- Do not pour samples from the Specimen Diluent Tube into the Test Cassette sample well. Use the provided Dropper Tip when adding the sample to the Test Cassette.
- Do not write on the barcode or top of the Test Cassette. This is used by Sofia 2 to identify the type of test being run.
- Do not attempt to scan a Test Cassette more than one time. The barcode on the Test Cassette contains a unique identifier that will prevent Sofia 2 from performing a second read on a previously scanned Test Cassette. An error message will be displayed if a Test Cassette is scanned more than once on the same Sofia 2.
- As the detection reagent is a fluorescent compound, no visible results will form on the test strip. Sofia 2 must be used for result interpretation.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local requirements.

- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

KIT STORAGE AND STABILITY

Store the kit at room temperature, 59°F to 86°F (15°C to 30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

QUALITY CONTROL

There are three types of Quality Control for Sofia 2 and the Test Cassette: Sofia 2 Calibration Check Procedure, Built-in Procedural Control features, and External Controls.

Sofia 2 Calibration Check Procedure

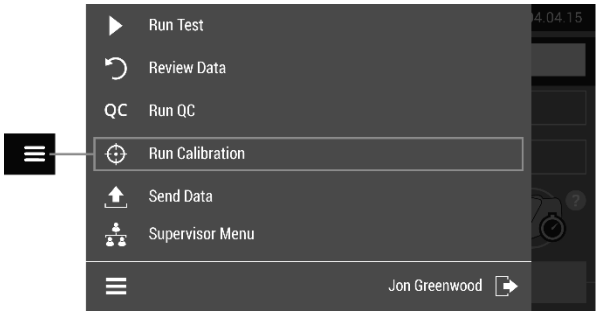
Note: This is a "Calibration Check" procedure.

The Calibration Check Procedure should be performed every 30 days. Sofia 2 can be set to remind the user to complete the Calibration Check Procedure.

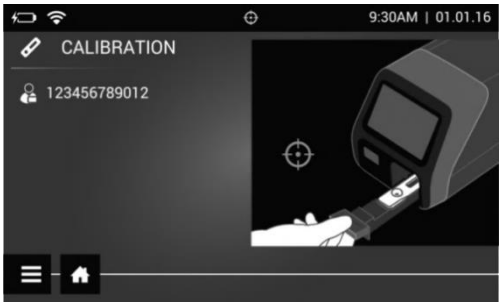
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses to protect from exposure to light.

1. To check the calibration of Sofia 2, select "Run Calibration" from the Main Menu.



2. Following the prompts, insert the Calibration Cassette into Sofia 2 and close the drawer. Sofia 2 performs the Calibration Check automatically within one minute with no user input required.



Sofia 2 indicates when the Calibration Check is completed, ✔ or ✖. Select ⌂ to return to the Run Test screen.

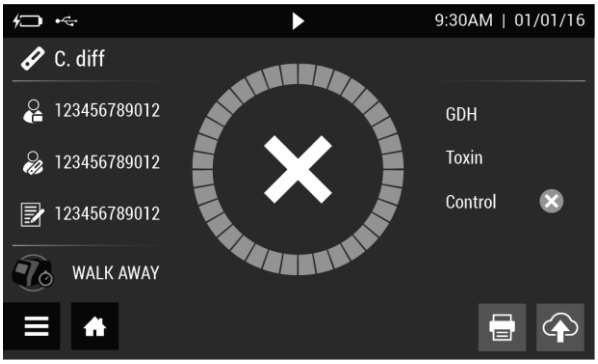
NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support); or contact your local distributor.

Built-in Procedural Controls

The Sofia 2 C. difficile FIA contains a built-in procedural control feature. Each time a test is run, the procedural control area is scanned by Sofia 2 and the result is displayed on the Sofia 2 screen.

The manufacturer's recommendation for daily control is to document the results of these built-in procedural controls for the first sample tested each day. This documentation is automatically logged in Sofia 2 with each test result.

A ✓ result obtained from the procedural control demonstrates that the test flowed correctly, and the functional integrity of the Test Cassette was maintained. **The procedural control is interpreted by Sofia 2 after the Test Cassette has developed for 15 minutes. If the test does not flow correctly, Sofia 2 will indicate that the result is ⊗.** Should this occur, review the procedure and repeat the test with a new aliquot of the same sample.



For example: This display shows an invalid result.

External Quality Control

External Controls may also be used to demonstrate that the reagents and assay procedure perform properly.

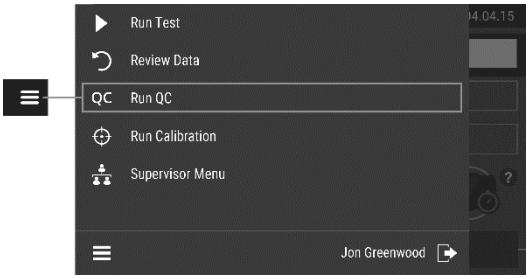
Quidel recommends that Positive and Negative External Controls be run:

- Once for each untrained operator.
- Once for each new shipment of kits – provided that each different lot received in the shipment is tested.
- As deemed additionally necessary by your internal quality control procedures, and in accordance with Local, State and Federal regulations or accreditation requirements.

To test External Controls, follow the instructions below.

External Quality Control Test Procedure

1. From the main menu, select Run QC.



2. Follow the prompts on the screen. Scan the QC Card (located on the kit box).

3. Sofia 2 prompts the user to select the desired mode (WALK AWAY or READ NOW) and then to run the External Controls.

4. Use the following procedure to test each of the control solutions. **The Positive Control must be run first, followed by the Negative Control.**

    a. Prepare a **Positive Control Cassette** by adding **3 drops** of the Positive Control solution (red cap) to the round Test Cassette sample well. Then follow the Sofia 2 screen instructions for developing and analyzing the Positive Control Cassette.

    b. Prepare a **Negative Control Cassette** by adding **3 drops** of the Negative Control solution (blue cap) to the round Test Cassette sample well. Then follow the Sofia 2 screen instructions for developing and analyzing the Negative Control Cassette.

5. After both the Positive and Negative Controls have been run, the results will be displayed as ✓ or ✗.

Do not perform patient tests or report patient test results if either of the QC test results are ✗. If a control is ✗, repeat the test Step 1 and a new Test Cassette or contact Quidel Technical Support before testing patient specimens.

If both the Positive and Negative Controls fail, repeat testing with new Positive and Negative Controls a second time. If only a single Control fails, the user has the option of repeating both the Positive and Negative Controls OR to repeat only the Control that failed. The user may select » on the Sofia 2 display to skip the Control test that previously passed. The QC Results will show a skipped Control test as » on Sofia 2.

Additional External Controls may be obtained separately by contacting Quidel Customer Support Services at 800.874.1517 (in the U.S.) or 858.552.100 (outside the U.S.).

SPECIMEN COLLECTION AND HANDLING

Collect fecal specimen in a clean, dry specimen collection container per standard procedures. Neat, unpreserved fecal specimens may be stored at room temperature (15°C to 30°C) for up to three (3) days (72 hours). Neat samples may be stored frozen at ≤-10°C for up to thirteen (13) days prior to use. The frozen neat, unpreserved samples may be thawed up to three times. Alternatively, specimens may be stored in Thermo Scientific Protocol™ Cary Blair or Thermo Scientific Protocol™ C&S transport media for up to three (3) days (72 hours) prior to use when refrigerated (2°C to 8°C) or at room temperature (15°C to 30°C).

TEST PROCEDURE

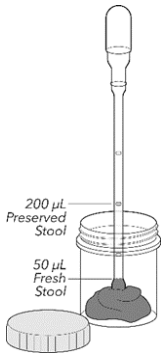
Important:

- DO NOT open the foil pouch containing the Test Cassette until ready to test the sample. Place the Test Cassette on a clean and level surface.
- **All clinical samples and test materials must be at room temperature before beginning the test.**
- **All stool samples must be mixed prior to testing.**
- **Expiration Date:** Check expiration date on each individual test package or outer box before using. Do not use any test past the expiration date on the label.
- **Samples should be handled with appropriate Personal Protective Equipment, to include lab coat, facemask, gloves, and safety glasses.**

1. Verify that Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**.
   See the "Using Sofia 2" section for more information.

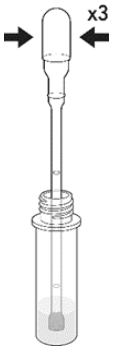
2. Collect 50 µL (top of flared tip) of Specimen using the Multi-Volume Pipette provided in the kit.

   Note: For Specimens in Transport Media (preserved), collect 200 µL (2nd Graduation) using the Multi-Volume Pipette provided in the kit.



3. Transfer the Specimen to the Specimen Diluent Tube and mix the solution by squeezing and releasing the top bulb of the Multi-Volume Pipette 3 times.

   Remove the Multi-Volume Pipette from the Specimen Diluent Tube.



4. Screw the purple Dropper Tip to Specimen Diluent Tube and mix well.

5. Remove the small clear cap, hold the Specimen Diluent Tube in a vertical position and dispense **5 drops** into the Test Cassette sample well.

6. Proceed to the "Using Sofia 2" section to complete the test.

USING SOFIA 2

WALK AWAY/READ NOW Modes

Refer to the Sofia 2 User Manual for operating instructions.
Sofia 2 may be set to two different modes (WALK AWAY and READ NOW). The procedures for each mode are described below.

WALK AWAY MODE

In WALK AWAY Mode, the user **immediately** inserts the Test Cassette into Sofia 2. Sofia 2 will automatically time the test development, and the results will be displayed in 15 minutes.

READ NOW MODE

Critically important: Allow the test to develop for the FULL 15 minutes BEFORE placing it into Sofia 2.

The user must first place the Test Cassette onto the counter or bench top for 15 minutes (outside of Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia 2. In READ NOW Mode, Sofia 2 will scan and display the test result within 1 minute.

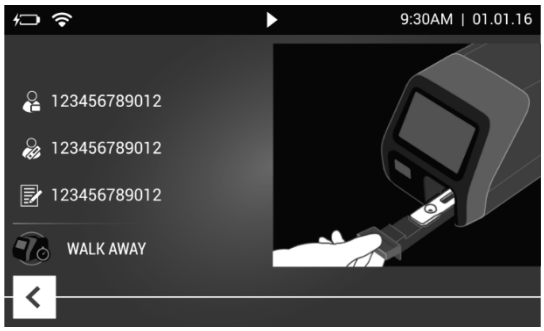
Run Test

1. Input the User ID using the barcode scanner or manually enter the data using the touchscreen.

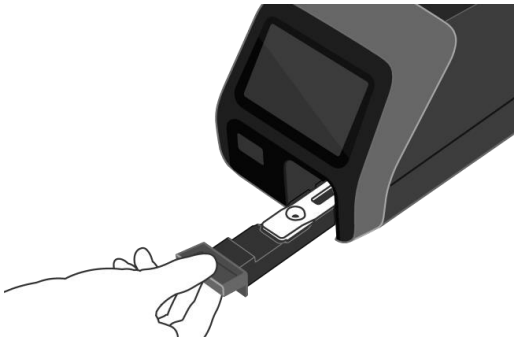
   NOTE: *If you mistakenly scan the incorrect barcode, select the field again to re-highlight it. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*

2. Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the touchscreen.

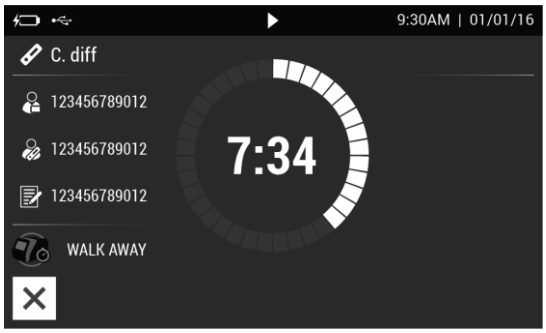
3. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



4. Insert the prepared patient Test Cassette into the drawer of Sofia 2 and gently close the drawer.



5. Sofia 2 will start automatically and display the progress as shown in the example below. In WALK AWAY Mode, Sofia 2 will automatically time the test development and the test results will be displayed on the screen in 15 minutes. In READ NOW Mode, the test results will be displayed on the screen within 1 minute. See Interpretation of Results section.



For example: This display shows that the test has 7 minutes, 34 seconds remaining.

CLEANING PROCEDURE

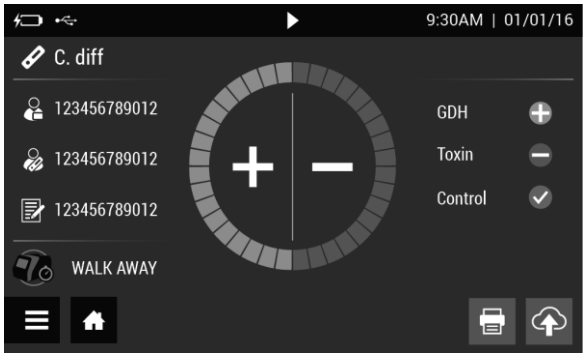
Important: Use 10% bleach to disinfect spills, if any. The 70% alcohol or 0.6% bleach solution recommended in the Sofia 2 User Manual will not suffice for cleaning up spills.

INTERPRETATION OF RESULTS

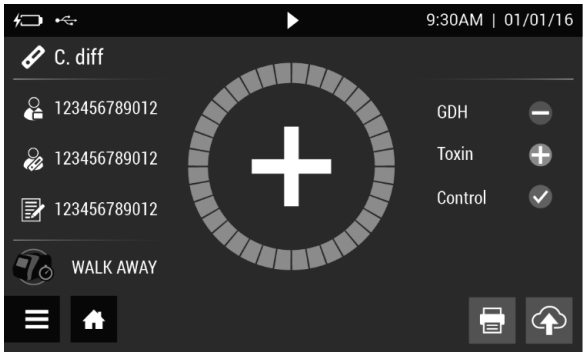
When the test is complete, the results will be displayed on the Sofia 2 screen. Test Lines, which are fluorescent, cannot be seen with the naked eye.

The Sofia 2 screen will display results for the procedural control as being ✓ or ✗, and will provide a ⊕ or ⊖ result for both *C. difficile* GDH antigen and Toxins A/B. If the procedural control is ✗, a repeat test should be performed with a new aliquot of the same sample.

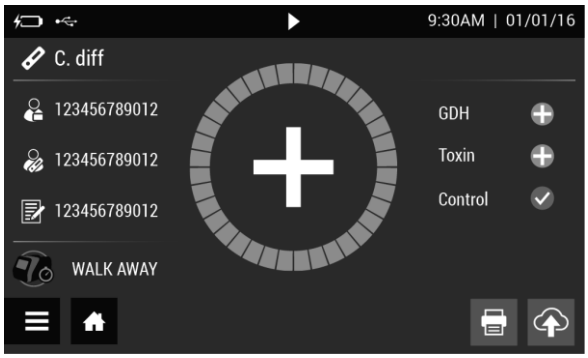
Valid Results:



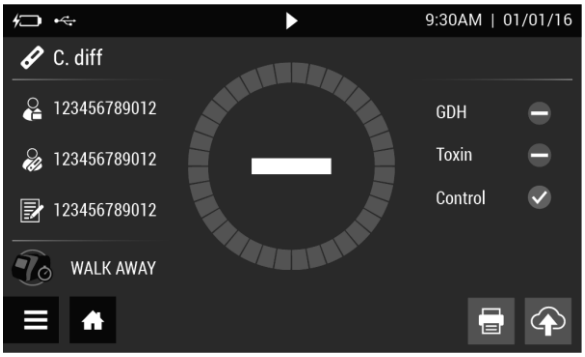
This display shows a valid positive result for C. difficile GDH antigen, but negative result for C. difficile Toxins A/B.



This display shows a valid positive result for C. difficile Toxins A/B but negative result for C. difficile GDH antigen.

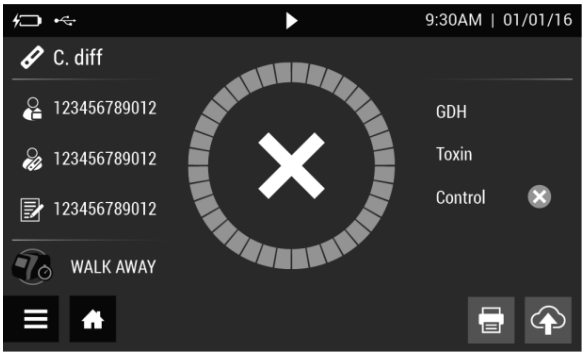


This display shows a valid positive result for C. difficile GDH antigen and Toxins A/B.



This display shows a valid negative result for C. difficile GDH antigen and Toxins A/B.

Invalid Results:



This result shows an invalid result.

Invalid Result: If the test is invalid, a repeat test should be performed with a new aliquot of the same sample.

LIMITATIONS

- The Sofia 2 C. difficile FIA does not differentiate between Toxin A and Toxin B.
- The contents of this kit are to be used for the qualitative detection of *C. difficile*-specific antigens and toxins from fecal specimens.
- The test detects both viable and nonviable *C. difficile* bacteria and may yield a positive result in the absence of living organisms.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected, transported, or stored improperly.
- Additional follow-up testing using a culture method or Nucleic Acid Amplification Test (NAAT) should be performed if the result is negative and the patient is suspected of *C. difficile* infection or clinical symptoms persist.
- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Negative test results do not rule out other possible infections.
- Positive test results do not rule out co-infections with other pathogens.

EXPECTED VALUES

Clinical studies determined the prevalence to be 17.3% (273/1582) when assessed versus CCFA Bacterial Culture and 6.8% (107/1571) versus Cytotoxic Tissue Culture.

PERFORMANCE CHARACTERISTICS

The following studies were performed with Sofia 2 C. difficile FIA and Sofia 2.

Limit of Detection

The limit of detection (LoD) for each analyte (GDH, Tox A and Tox B) was determined in fecal matrix and in Cary Blair and C&S transport media. The LoD values are as described in Table 1. The concentration of *C. difficile* in the patient specimen is shown below as the LoD. The concentration of *C. difficile* in the specimen after it is added to the Specimen Diluent and before it is added to the test cassette is shown as LoD post-dilution.

Table 1
Limits of Detection

Analyte	Specimen Matrix	LoD (ng/mL)	LoD Post-dilution (ng/mL)
GDH	Fecal Matrix (Neat)	8.54	0.41
	Cary Blair	1.97	0.08
	C&S	2.78	0.12
Toxin A	Fecal Matrix (Neat)	10.86	0.52
	Cary Blair	10.93	0.46
	C&S	17.06	0.71
Toxin B	Fecal Matrix (Neat)	1.02	0.05
	Cary Blair	1.39	0.06
	C&S	1.87	0.08

Analytical Reactivity

Analytical reactivity for Sofia 2 C. difficile FIA was demonstrated using 27 strains of *C. difficile* (both Toxin producing and non-Tox strains) of varying ribotypes (Table 2). Test samples were prepared to concentrations between 1x -5x LoD of GDH of the reference strain. Each strain produced positive results in the assay.

Table 2
Analytical Reactivity

C. difficile strain	ATCC Strain Number (if applicable)	Toxinotype	Ribotype	Toxins Produced
CTH 205	BAA 1810	Nontox	9	None
Bartlett 234	BAA 1801	Nontox	10	None
RMA 10790	43602	Nontox	31	None
VPI 11186	700057	Nontox	38	None
ATCC 43593	43593	Nontox	60	None
PITT 02	51695	0	1	AB
UVA 10	BAA 1874	0	2	AB
VPI 10463	43255	0	3/87	AB
RMA 15187	700792	0	5	AB
630	BAA 1382	0	12	AB
PUC 25	43600	0	14/20	AB
F1470	43598	VIII	17	B
HMC 8271	BAA1812	XII	24	AB

C. difficile strain	ATCC Strain Number (if applicable)	Toxinotype	Ribotype	Toxins Produced
Pitt 45	-	III	27	ABC
R20291	BAA 1803	III	27	ABC
8864	-	X	36	BC
BAA 1873	BAA 1804/ BAA 1873	0	53	AB
VPI 13071	17858	0	54	AB
PITT 46	BAA 1811	0	57	AB
UVA 049 (K049) or Summa 093	-	IX	19	AB
PITT 07	BAA 1875	V	78	AB
NCTC 13404	-	0	106	AB
BAA 2156	BAA 2156	0	118	AB
RMA 9401	-	V	126	ABC
CCL 19010	BAA 1806	0	220	AB
CCL 19917	BAA1814	XXII	251	ABC
PUC 40	N/A	0	54	AB

Analytical Specificity

The cross reactivity of the Sofia 2 C. difficile FIA was evaluated with a total of 62 bacterial and fungal microorganisms and 25 viral isolates. None of the microorganisms or viruses below in Table 3 showed cross reactivity in the assay at the concentrations listed. *C. histolyticum* showed GDH cross reactivity above concentrations of 1.46E+04 cells/mL and *C. sporogenes* showed GDH cross reactivity at concentrations above 2.93E+04 cells/mL as listed in Table 3.

Table 3
Cross Reactivity / Microbial Interference Testing

Virus / Bacteria / Fungi	Tested Concentration	Units
Acinetobacter baumannii	6.00E+07	cells/mL
Aeromonas hydrophila	6.00E+07	cells/mL
Bacillus cereus	6.00E+07	cells/mL
Bacillus subtilis	3.00E+07	cells/mL
Bacteroides fragilis	6.00E+07	cells/mL
Borrelia burgdorferi	Not Available*	cells/mL
Campylobacter coli	4.80E+07	cells/mL
Campylobacter concisus	3.00E+07	cells/mL
Campylobacter fetus	1.56E+08	cells/mL
Campylobacter helveticus	1.26E+08	cells/mL
Campylobacter hyointestinalis	1.78E+08	cells/mL
Campylobacter jejuni	1.44E+08	cells/mL
Candida albicans	3.00E+07	cells/mL
Citrobacter freundii	6.00E+07	cells/mL
Clostridium bifermentans	6.00E+07	cells/mL

Virus / Bacteria / Fungi	Tested Concentration	Units
Clostridium butyricum	6.00E+07	cells/mL
Clostridium clostridiforme	3.00E+07	cells/mL
Clostridium haemolyticum	3.00E+07	cells/mL
Clostridium histolyticum	1.46E+04	cells/mL
Clostridium novyi	3.00E+07	cells/mL
Clostridium perfringens	3.00E+07	cells/mL
Clostridium septicum	3.00E+07	cells/mL
Clostridium sporogenes	2.93E+04	cells/mL
Edwardsiella tarda	6.00E+07	cells/mL
Enterobacter aerogenes	6.00E+07	cells/mL
Enterobacter cloacae	6.00E+07	cells/mL
Enterococcus faecalis	3.00E+07	cells/mL
Escherichia coli	6.00E+07	cells/mL
Escherichia coli EIEC	6.00E+07	cells/mL
Escherichia coli EPEC	6.00E+07	cells/mL
Escherichia coli ETEC	6.00E+07	cells/mL
Escherichia coli O157:H7 (non-toxigenic)	6.00E+07	cells/mL
Escherichia coli O157:H7 (toxigenic)	6.00E+07	cells/mL
Escherichia fergusonii	6.00E+07	cells/mL
Escherichia hermannii	6.00E+07	cells/mL
Haemophilus influenzae	1.80E+08	cells/mL
Helicobacter pylori	6.00E+07	cells/mL
Klebsiella pneumoniae	6.00E+07	cells/mL
Lactobacillus acidophilus	3.00E+07	cells/mL
Lactococcus lactis	3.00E+07	cells/mL
Listeria monocytogenes	4.87E+08	cells/mL
Paeniclostridium sordellii (non-toxigenic)	3.00E+07	cells/mL
Peptostreptococcus anaerobius	6.00E+07	cells/mL
Plesiomonas shigelloides	3.00E+07	cells/mL
Porphyromonas assaccharolytica	6.00E+07	cells/mL
Prevotella melaninogenica	6.00E+07	cells/mL
Proteus vulgaris	6.00E+07	cells/mL
Pseudomonas aeruginosa	6.00E+07	cells/mL
Pseudomonas fluorescens	6.00E+07	cells/mL
Salmonella typhimurium	6.00E+07	cells/mL
Serratia liquifaciens	3.00E+07	cells/mL
Serratia marcescens	6.00E+07	cells/mL
Shigella dysenteriae	6.00E+07	cells/mL
Shigella flexneri	6.00E+07	cells/mL
Shigella sonnei	6.00E+07	cells/mL
Staphylococcus aureus	6.00E+07	cells/mL
Staphylococcus aureus (Cowan)	3.00E+07	cells/mL

Virus / Bacteria / Fungi	Tested Concentration	Units
Staphylococcus epidermidis	6.00E+07	cells/mL
Streptococcus agalactiae	6.00E+07	cells/mL
Vibrio cholerae	3.00E+07	cells/mL
Vibrio parahaemolyticus	6.00E+07	cells/mL
Yersinia enterocolitica	3.00E+07	cells/mL
Adenovirus Type 1	4.40E+06	TCID50/mL
Adenovirus Type 2	5.62E+06	TCID50/mL
Adenovirus Type 3	3.16E+07	TCID50/mL
Adenovirus Type 41	3.20E+07	TCID50/mL
Adenovirus Type 5	3.20E+08	TCID50/mL
Coxsackievirus B1	3.20E+08	TCID50/mL
Coxsackievirus B2	5.62E+07	TCID50/mL
Coxsackievirus B3	4.82E+02	TCID50/mL
Coxsackievirus B4	3.20E+06	TCID50/mL
Coxsackievirus B5	1.00E+08	TCID50/mL
Coxsackievirus B6	5.62E+06	TCID50/mL
Echovirus 11	1.78E+06	TCID50/mL
Echovirus 18	4.68E+05	TCID50/mL
Echovirus 33	2.00E+04	TCID50/mL
Echovirus 9	3.20E+07	TCID50/mL
Enterovirus 68	3.20E+06	TCID50/mL
Enterovirus 69	2.00E+06	TCID50/mL
Enterovirus 70	1.00E+06	TCID50/mL
Enterovirus 71	1.78E+06	TCID50/mL
Human Coronavirus	8.90E+06	TCID50/mL
Human mastadenovirus F (formerly Adenovirus Type 40)	3.20E+04	TCID50/mL
Human Parechovirus 1 (formerly Echovirus 22)	3.20E+05	TCID50/mL
Human Rotavirus	1.60E+07	TCID50/mL
Norovirus GI	**	**
Norovirus GII	**	**

*****Concentration of the stock material used to prepare the samples were not available (ATCC 35210)

******Norovirus can only replicate in humans and virus titer is very difficult to measure. The human norovirus samples were provided by collaborator Noah Hull at the Wyoming Public Health Laboratory.

Interfering Substances

Several prescription and over-the-counter (OTC) products and endogenous substances were evaluated with the Sofia 2 C. difficile FIA. None of the substances listed in Table 4 interfered with the assay at the levels tested.

Table 4
Non-Interfering Substances

Product/Substance	Active Ingredient of Substance	Tested Concentration
Antiseptic towelette	Benzalkonium Chloride	1% w/v
Barium Sulfate	Barium sulfate	5% w/v
Ciprofloxacin	Ciprofloxacin	0.25% w/v
Ethanol	Ethanol	1% w/v
Ex-Lax	Sennosides	1% w/v
Hog gastric mucin	Immunoglobulins, Lysozyme, Polymers, etc.	3.5% w/v
Human Blood	Glucose, Hormones, Enzymes, Ions, Iron etc.	40% v/v
Human urine	Urea, Proteins, Hormones, Glucose, Ions	5% v/v
Hydrocortisone	Hydrocortisone	1% w/v
Imodium	Loperamide Hydrochloride	5% v/v
Kaopectate	Bismuth Subsalicylate	5% v/v
Leukocytes	Leukocytes	0.05% w/v
Maalox	Aluminum Hydroxide/Magnesium Hydroxide/Simethicone	5% v/v
Mesalamine	Mesalamine	10% w/v
Metronidazole	Metronidazole	0.25% w/v
Mineral Oil	Mineral Oil	10% w/v
Mylanta Gas	Aluminum hydroxide/Magnesium hydroxide/Simethicone	4.20E+00 mg/mL
Naproxen Sodium	Naproxen Sodium	0.05% w/v
Nystatin	Nystatin	1% w/v
Omeprazole	Omeprazole	5.00E+00 µg/mL
Palmitic Acid	Palmitic Acid	40% w/v
Pepto-Bismol	Bismuth Subsalicylate	5% v/v
Phenylephrine	Phenylephrine	1% w/v
Polyethylene Glycol 3350	Polyethylene Glycol 3350	10% w/v
Simethicone	Simethicone	10% w/v
Stearic Acid	Lipids	40% w/v
Trojan condom with 7% Nonoxynol-9	Nonoxynol-9	1% w/v
TUMS	Calcium Carbonate	5.00E+01 µg/mL
Vancomycin	Vancomycin	0.25% w/v

Hook Effect

To ensure that a high concentrations of C. difficile antigens do not interfere with a positive reaction in the Sofia 2 C. difficile FIA, high positive samples were prepared by spiking a negative fecal pool with high concentrations of GDH, Tox A or Tox B. A total of 7 different dilutions of analytes were prepared and ten devices were tested per sample. The results demonstrated that high concentrations of any analyte did not affect the detection of the others.

Reproducibility

The reproducibility of the Sofia 2 C. Difficile FIA was evaluated at 3 different laboratories over 5 days. On each day of testing, operators at each site performed 2 runs using a prepared test sample panel, and the panel was tested in replicates of three during a run. The series of coded, contrived samples, prepared in negative clinical matrix, ranged from negative (no bacteria) to low positive concentrations (LOD) of rGDH and from negative (no bacteria) to moderate positive concentrations (3x LoD) of Toxin A or B. The interlaboratory agreement (Table 5) was 98.9% to 100.0% for negative samples and 98.9% to 100.0% for positive samples.

Table 5
Sofia 2 C. Difficile FIA Reproducibility Study Inter-laboratory Agreement

Summary of Qualitative Results for 5 Day Reproducibility by Site										
Sample Level	Site ID	n	rGDH				Toxin			
			Invalid	Negative	Positive	% Expected Agreement (95% C.I.)	Invalid	Negative	Positive	% Expected Agreement (95% C.I.)
Negative	1	30	0	30	0	100.0%	0	30	0	100.0%
	2	30	0	30	0	100.0%	0	30	0	100.0%
	3	30	0	30	0	100.0%	0	30	0	100.0%
	Total	90	0	90	0	100.0% (90/90) (95.9% to 100.0%)	0	90	0	100.0% (90/90) (95.9% to 100.0%)
High Negative (rGDH + Toxin A)	1	30	0	29	1	96.7%	0	30	0	100.0%
	2	30	0	30	0	100.0%	0	30	0	100.0%
	3	30	0	30	0	100.0%	0	30	0	100.0%
	Total	90	0	89	1	98.9% (89/90) (94.0% to 99.8%)	0	90	0	100.0% (90/90) (95.9% to 100.0%)
High Negative (rGDH + Toxin B)	1	30	0	29	1	96.7%	0	30	0	100.0%
	2	30	0	30	0	100.0%	0	30	0	100.0%
	3	30	0	30	0	100.0%	0	30	0	100.0%
	Total	90	0	89	1	98.9% (89/90) (94.0% to 99.8%)	0	90	0	100.0% (90/90) (95.9% to 100.0%)
Low Positive* (rGDH + Toxin A)	1	30	0	1	29	96.7%	0	1	29	96.7%
	2	30	0	0	30	100.0%	0	0	30	100.0%
	3	30	0	0	30	100.0%	0	0	30	100.0%
	Total	90	0	1	89	98.9% (89/90) (94.0% to 99.8%)	0	1	89	98.9% (89/90) (94.0% to 99.8%)
Low Positive* (rGDH + Toxin B)	1	30	0	0	30	100.0%	0	0	30	100.0%
	2	30	0	0	30	100.0%	0	0	30	100.0%
	3	30	0	0	30	100.0%	0	0	30	100.0%
	Total	90	0	0	90	100.0% (90/90) (95.9% to 100.0%)	0	0	90	100.0% (90/90) (95.9% to 100.0%)
Moderate Positive** (rGDH + Toxin A)	1	30	0	0	30	100.0%	0	0	30	100.0%
	2	30	0	0	30	100.0%	0	0	30	100.0%
	3	30	0	0	30	100.0%	0	0	30	100.0%
	Total	90	0	0	90	100.0% (90/90) (95.9% to 100.0%)	0	0	90	100.0% (90/90) (95.9% to 100.0%)
	1	30	0	0	30	100.0%	0	0	30	100.0%

Summary of Qualitative Results for 5 Day Reproducibility by Site										
Sample Level	Site ID	n	rGDH				Toxin			
			Invalid	Negative	Positive	% Expected Agreement (95% C.I.)	Invalid	Negative	Positive	% Expected Agreement (95% C.I.)
Moderate Positive** (rGDH + Toxin B)	2	30	0	0	30	100.0%	0	0	30	100.0%
	3	30	0	0	30	100.0%	0	0	30	100.0%
	Total	90	0	0	90	100.0% (90/90) (95.9% to 100.0%)	0	0	90	100.0% (90/90) (95.9% to 100.0%)
* rGDH Concentration is Below LoD (0.9x) ** rGDH Concentration is Low Positive										

CLINICAL PERFORMANCE

Sofia 2 C. Difficile FIA Performance vs. Tissue Culture

The performance of the Sofia 2 C. Difficile FIA was compared to CCFA Bacterial Culture for GDH detection and cytotoxic tissue culture for Toxin detection in a multi-center clinical field study. A fecal specimen was collected from one thousand five hundred eighty-three (1583) subjects suspected of *C. difficile* infection. One portion of the specimen was tested at the clinical site and the remaining specimen was sent to a central reference laboratory for comparator method testing. Additional testing was conducted by the reference laboratory on each discrepant specimen using CCMB-TAL culture for GDH detection and PCR for tcdB for Toxin detection. The results are shown in Table 6 and 7.

Table 6
Performance of Sofia 2 C. difficile FIA (GDH) versus CCFA Bacterial Culture

	CCFA Bacterial Culture		
	Pos	Neg	Total
Sofia 2 C. difficile FIA Pos	236	92	328
Sofia 2 C. difficile FIA Neg	37	1215	1252
Total	273	1307	1580*

*Three (3) samples were invalid in the Sofia 2 C. difficile FIA

Sensitivity = 86.4% (236/273) (95% CI=81.9% to 90.0%)

Specificity = 93.0% (1215/1307) (95% CI=91.4% to 94.2%)

PPV = 72.0% (236/328) (95% CI=66.9% to 76.5%)

NPV = 97.0% (1215/1252) (95% CI=96.0% to 97.8%)

Prevalence = 17.3% (273/1580)

37 CCFA culture positive – Sofia 2 negative results, 7 of the specimens were negative by CCMB-TAL Culture, 1 specimen was unavailable for CCMB-TAL culture. Of the 92 CCFA culture negative – Sofia 2 positive results, 47 of the specimens were positive when tested by the CCMB-TAL culture, 1 specimen was unavailable for CCMB-TAL culture.

Table 7
Performance of Sofia 2 C. difficile FIA (Toxin A/B) versus Cytotoxic Tissue Culture

	Cytotoxic Tissue Culture		
	Pos	Neg	Total
Sofia 2 C. difficile FIA Pos	90	22	112
Sofia 2 C. difficile FIA Neg	17	1442	1459
Total	107	1464	1571*

*Three (3) samples were invalid in the Sofia 2 C. difficile FIA

Sensitivity = 84.1% (90/107)
(95% CI=76.0% to 89.8%)

Specificity = 98.5% (1442/1464)
(95% CI=97.7% to 99.0%)

PPV = 80.4% (90/112)
(95% CI=72.0% to 86.7%)

NPV = 98.8% (1442/1459)
(95% CI=98.1% to 99.3%)

Prevalence = 6.8% (107/1571)

Of the 17 Cytotoxic tissue culture positive – Sofia 2 negative results, 3 of the specimens were negative by PCR for tcdB, 2 specimens were unavailable for PCR. Of the 22 Cytotoxic tissue culture negative – Sofia 2 positive results, 7 of the specimens were positive when tested by the PCR for tcdB, 3 specimens were unavailable for PCR.

ASSISTANCE

If you have any questions regarding the use of this product or to report a product problem, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference quidel.com to see more options for Support.

Country	**Phone**	**E-Mail Address**
Europe, Middle East and Africa	+353 (91) 412 474 (main) 1800 200441 (toll free)	emeatechnicalsupport@quidel.com
Austria	+43 316 231239	
Belgium	+32 (2) 793 0180	
France	0 (805) 371674	
Germany	+49 (0) 7154 1593912	
Netherlands	0 800 0224198	
Switzerland	0 800 554864	
United Kingdom	0 800 3688248	
Ireland	+353 (91) 412 474	
Italy	+39 (800) 620 549	
North America, Asia-Pacific, Latin America	858.552.1100	technicalsupport@quidel.com
Canada	437.266.1704 (main) 888.415.8764 (toll free)	technicalsupport@quidel.com
China	0400 920 9366 or +86 021 3217 8300	chinatechnicalservice@quidel.com

REFERENCES

1. https://www.cdc.gov/media/releases/2015/p0225-clostridium-difficile.html
2. Kyne, L., M.B. Hamel, R. Polavaram, and C.P. Kelly, *Health Care Costs and Mortality Associated with Nosocomial Diarrhea Due to Clostridium difficile*. Clin Infect Dis, 2002. 34(3): p.346-353.
3. Archibald, L.K., S.N. Banerjee, and W.R. Jarvis, *Secular trends in hospital-acquired Clostridium difficile disease in the United States*, 1987-2001. J Infect Dis, 2004. 189(9): p. 1585-9.
4. McDonald, L.C., M. Owings, and D.B. Jernigan, *Clostridium difficile infection in patients discharged from US short-stay hospitals,* 1996-2003. Emerg Infect Dis, 2006. 12(3): p. 409-15.
5. Voth, D.E. and J.D. Ballard, *Clostridium difficile toxins: mechanism of action and role in disease*. Clin Microbiol Rev, 2005. 18(2): p. 247-63.
6. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. U.S. Department of Health and Human Services, CDC, NIH, Washington, DC (2007). https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(16)30025-8/fulltext.

REF 20329 – Sofia 2 C. difficile FIA – 25 Test Kit

IVD

CE

EC REP

MDSS GmbH
Schiffgraben 41 30175
Hannover, Germany

Quidel Corporation
10165 McKellar Court
San Diego, CA 92121
quidel.com

1507200EN00 (12/21)

REF	CE
Catalogue number	CE mark of conformity
EC REP	LOT
Authorized representative in the European Community	Batch code
	
Use-by date	Manufacturer
	
Temperature limit	Consult instructions for use
IVD	
In vitro diagnostic medical device	Keep away from sunlight
	
Health hazards	Contains sufficient for <n> tests
CONTROL +	CONTROL −
Positive control	Negative control