November 14, 2023

Vela Operations USA
Larry Rea
Chief Operating Officer
2441 South 3850 West
Salt Lake City, Utah 84120

Re: K232092
Trade/Device Name: Great Basin Toxigenic C. difficile Direct Test (CDF2)
Regulation Number: 21 CFR 866.3130
Regulation Name: Clostridium Difficile Toxin Gene Amplification Assay
Regulatory Class: Class II
Product Code: OZN
Dated: July 5, 2023
Received: July 13, 2023

Dear Larry Rea:

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 (the 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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Natasha Griffin -S

O.B.O.
Ribhi Shawar, Ph.D. (ABMM)
Branch Chief
General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K232092

Device Name
Great Basin Toxigenic C. difficile Direct Test (CDF2)

Indications for Use *(Describe)*
The Great Basin Toxigenic C. difficile Direct Test, performed on the Great Basin PA500 Analyzer, is a qualitative in vitro diagnostic test for the detection of toxigenic Clostridioides difficile in unformed (liquid or soft) stool samples collected from patients suspected of having C. difficile infection (CDI). The automated assay utilizes polymerase chain reaction (PCR) to detect a conserved region of the toxin gene (tcdB) associated with toxin-producing C. difficile.

The Toxigenic C. difficile Direct Test is intended for use as an aid in the diagnosis of CDI in conjunction with clinical and epidemiological risk factors

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(K) SUMMARY – TOXIGENIC C. DIFFICILE DIRECT TEST

A. Submitted By

Vela Operations USA
2441 South 3850 West
Salt Lake City, Utah 84120

Contact Information

Larry Rea
Chief Operating Officer
Vela Operations USA (DBA Great Basin Scientific)

B. Name of Device

Proprietary Name:	Great Basin Toxigenic *C. difficile* Direct Test
Common or Usual Names:	Toxigenic *C. difficile* Direct Test CDF2

C. Regulatory Information:

a.	Regulation:	21 CFR 866.3130 − Clostridium difficile toxin gene amplification assay
b.	Classification:	Class II (Toxigenic *C. difficile* Direct Test; non-exempt) Class II (PA500 Analyzer System)
c.	Panel:	Microbiology (83)
d.	Product Code:	OZN − C. difficile Toxin Gene Amplification Assay

D. Intended use(s)/Indications for Use

The Great Basin Toxigenic *C. difficile* Direct Test, performed on the Great Basin PA500 Analyzer, is a qualitative *in vitro* diagnostic test for the detection of toxigenic *Clostridioides difficile* in unformed (liquid or soft) stool samples collected from patients suspected of having *C. difficile* infection (CDI). The automated assay utilizes polymerase chain reaction (PCR) to detect a conserved region of the toxin gene (*tcdB*) associated with toxin producing *C. difficile*.

The Toxigenic *C. difficile* Direct Test is intended for use as an aid in the diagnosis of CDI in conjunction with clinical and epidemiological risk factors.

E. Device Description

Test Principle:

The Great Basin Toxigenic *C. difficile* Direct Test (CDF2) performed on the PA500 Analyzer utilizes automated, hot-start PCR amplification technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface, in a single-use, self-contained test cartridge.

A swab volume of the specimen (raw stool) is first processed using the Sample Preparation Device (SPD). During processing through the SPD the specimen is infused with a synthetic bacterial sample processing control (SPC). An aliquot (250 μL) of the eluate obtained from the SPD, containing diluted specimen mixed with SPC, is loaded into the sample port of the CDF2 Test Cartridge.

Genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of PCR. During the PCR process, biotin-labeled primers direct the amplification of specific nucleic acid sequences within a conserved region of the *C. difficile* Toxin B (*tcdB*) gene.

Following PCR, biotin-labeled, amplified target DNA sequences are hybridized to sequence specific probes immobilized on the silicon chip surface and incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is washed away, and then tetramethylbenzidine (TMB) is added to produce a colored precipitate at the location of the probe/target sequence complex.

The resulting signal is detected by the automated PA500 Optical Reader within the PA500 Analyzer System. The SPC undergoes the same extraction, amplification, and detection steps as the sample in order to monitor for inhibitory substances, as well as process inefficiency due to instrument or reagent failure. No operator intervention is required once the sample is loaded into the sample port and the Toxigenic *C. difficile* Direct Test cartridge is loaded into the PA500 Analyzer.

Test Device:

The Great Basin System is a fully automated *in vitro* diagnostic system that includes: the PA500 Analyzer, single-use SPDs, single-use Toxigenic *C. difficile* Direct Test Cartridges, and the PA500 Data Analysis Software Program. The PA500 Analyzer is designed to perform automated sample preparation, target amplification via PCR, chip-based (optical) target detection, and integrated data analysis in less than two hours. The PA500 Analyzer has been granted 510(k) clearance for the Portrait Toxigenic *C. difficile* Assay (DEN120013/K113358), Portrait GBS Assay (K143312), Staph ID/R Blood Culture Panel (K152470), Shiga Toxin Direct Test (K152955), Stool Bacterial Pathogens Panel (K163571), and the Bordetella Direct Test (K170284).

F. Substantial Equivalence Information

A comparison of the Toxigenic *C. difficile* Direct Test (CDF2) and the predicate device, Meridian Bioscience® GenePOC® CDiff Assay (K172569), is provided in Table 1.

Table 1. Predicate Comparison

Similarities		
Features/ Characteristics	**Great Basin Toxigenic *C. difficile* Direct Test (CDF2)**	**Predicate Device GenePOC® CDiff Assay (K172569)**
Manufacturer	Vela Operations USA	Meridian Bioscience
Trade Name	Great Basin Toxigenic *C. difficile* Direct Test	GenePOC® CDiff Assay
510(k) Number	K232092	K172569
Classification	II	II
Product Code	OZN	OZN
Intended Use/ Indications for Use	The Great Basin Toxigenic C. difficile Direct Test, performed on the Great Basin PA500 Analyzer, is a qualitative *in vitro* diagnostic test for the detection of toxigenic *Clostridioides difficile* in unformed (liquid or soft) stool samples collected from patients suspected of having *C. difficile* infection (CDI). The automated assay utilizes polymerase chain reaction (PCR) to detect a conserved region of the toxin gene (*tcdB*) associated with toxin-producing *C. difficile*. The Toxigenic *C. difficile* Direct Test is intended for use as an aid in the diagnosis of CDI in conjunction with clinical and epidemiological risk factors.	The GenePOC CDiff assay performed on the revogene instrument is a qualitative *in vitro* diagnostic test that utilizes automated sample processing and real-time polymerase chain reaction (PCR) to detect the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The GenePOC CDiff assay is intended to aid in the diagnosis of CDI.
Qualitative/ Quantitative	Qualitative	Qualitative

Instrument	PA500 Analyzer	revogene®
Specimen Type	Unformed (liquid or soft) stool specimens	Same
Assay Target	toxin B gene (*tcdB*)	Same
Sample Lysis and DNA Extraction	Automated sample lysis and DNA extraction in a self-contained cartridge	Same
Amplification Technology	Multiplex polymerase chain reaction (PCR)	Same
Result Interpretation	Automated	Same
Test Principle	Automated and integrated sample preparation, nucleic acid amplification, and detection of the target sequence in a single-use, disposable test cartridge	Same
Controls	Integrated Sample Processing Control (SPC) to control for adequate processing of the target bacteria and monitor the PCR amplification reaction for the presence of inhibitors as well as microfluidic cartridge, instrument or reagent failure. Fiducial and Hybridization Controls (HC) to control for cartridge selection, cartridge integrity, and to verify detection chemistry.	Integrated Processing Control (PrC) to verify sample processing and amplification steps. The PrC allows for the verification of potential inhibitor substances as well as microfluidic, instrument or reagent failure.

Differences		
Features/ Characteristics	**Great Basin Toxigenic *C. difficile* Direct Test (CDF2)**	**Predicate Device GenePOC® CDiff Assay (K172569)**
Detection Probes	Hybridization probes	TaqMan probes
Detection Technology	Colorimetric target specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation.	Real-time PCR (fluorescent signals) and embedded detection algorithm
Sample Input	1 Swab Volume	1 Disposable Transfer Loop Volume (5 µL)
Samples per Run	1	Up to 8
Time to Result	~2 hours	~70 min

G. Performance Summary – Analytical Studies

a. Specimen Stability

A room temperature and refrigerated Specimen Stability study was conducted to determine the allowable time and temperature storage conditions for clinical specimens. The study was conducted using fresh, never frozen *C. difficile* ATCC 43255 cells spiked into a pooled, clinical negative stool matrix at a concentration of approximately 2.5X LoD. A true negative sample was also evaluated. Stability samples were tested in triplicate in a time course study. The Specimen Stability Study demonstrated that the *C. difficile* target remains stable up to and beyond 72 hours when specimens are stored refrigerated (2° - 8°C). The results also demonstrate that the CDF2 test performance is consistent and stable for low positive samples stored up to 24 hours at room temperature (18° - 22°C).

b. Analytical Sensitivity

The limit of detection (LoD) of the Toxigenic *C. difficile* Direct Test was measured for two (2) toxigenic *Clostridioides difficile (tcdB*+) strains: *Clostridioides difficile* ATCC 43255 (Toxinotype

0, *tcdA*+, *tcdB*+) and *Clostridioides difficile* ATCC BAA-1805 (Toxinotype IIIb, NAP1/027, *tcdA*+, *tcdB*+).

For limit of detection (LoD) assessment, *C. difficile* strains were freshly cultured in liquid medium, serially diluted, and plated on agar to determine the cell concentration (i.e., CFU/mL). Enriched broths were then diluted into a pooled clinical negative stool to create a dilution series that was utilized for preliminary range finding on the CDF2 test. Results of the preliminary range finding were analyzed via Probit analysis to calculate the theoretical LoD at 95% agreement. The Probit estimated LoD was then confirmed with an additional round of testing from frozen cultures. The LoD for each strain tested is provided in Table 2.

Table 2. Toxigenic C. difficile Direct Test Limit of Detection

Strain	Limit of Detection (LoD)
Clostridioides difficile (ATCC 43255)	2.6 x 10^4 CFU/mL
Clostridioides difficile (ATCC BAA-1805)	7.1 x 10^4 CFU/mL

c. Analytical Reactivity (Inclusivity)

The Analytical Reactivity of the Toxigenic *C. difficile* Direct Test was evaluated by testing an additional twenty-two (22) toxigenic *Clostridioides difficile* strains covering six (6) unique toxinotypes. *C. difficile* strains were grown as described in the LoD studies, diluted into pooled, clinical negative stool to a concentration of 2X LoD (9.7 x 10^4 CFU/mL) and tested in triplicate. All additional strains tested were detected in CDF2.

The CDF2 test correctly identified every replicate of all 22 strains evaluated, indicating that the test can detect multiple strains and toxinotypes of *Clostridioides difficile* strains that harbor the *tcdB* toxin gene. Results of Analytical Reactivity (Inclusivity) testing are provided in Table 3.

Table 3. *Clostridioides difficile* Strains Tested for Analytical Reactivity (Inclusivity)

***Clostridioides difficile* Strain**	**Toxinotype, Toxin**
ATCC 9689	0, A+, B+
ATCC 17857	
ATCC 17858	
ATCC 43594	
ATCC 43596	
ATCC 43599	
ATCC 43600	
ATCC 51695	
ATCC 700792	
ATCC BAA-1382	
ATCC BAA-1804	
ATCC BAA-1808	
ATCC BAA-1871	
ATCC BAA-1872	
ATCC BAA-1874	
ATCC BAA-2156	
ATCC BAA-1803	IIIc, NAP1, A+, B+
ATCC BAA-1875	V, A+, B+
ATCC 43598	VIII, A-, B+
ATCC BAA-1812	XII, A+, B+
ATCC BAA-1814	XXII, A+, B+
ATCC BAA-2155	

d. Analytical Specificity (Exclusivity)

The potential for cross-reactivity to non-target organisms was evaluated in an Exclusivity Study, by testing microorganisms commonly found in stool, with the CDF2 test. The study included 83 microorganisms phylogenetically related to *C. difficile*, as well as other bacteria, fungi/yeast, parasites, viruses, and human genomic DNA (69 unique bacterial strains, 1 yeast, 3 parasites, 9 viruses, and human genomic DNA). Genomic DNA was tested in place of whole organism, for those isolates that were classified as Biosafety Level III, or unable to be cultured via standard clinical microbiology techniques.

Contrived samples were formulated for Exclusivity testing by spiking previously frozen, enumerated cell culture stocks, commercially sourced genomic templates, or commercially sourced viruses into pooled, clinical negative stool matrix. Apart from a single strain (*Clostridium butyricum* ATCC 19398 tested at an input of 1.0 x 10^5 CFU/mL), all bacterial and yeast strains were tested at concentrations ≥ 1x10^6 CFU/mL. Genomic templates and viral strains were tested at ≥ 1x10^6 copies/mL or ≥ 1x10^6 $TCID_{50}$/mL, respectively. Each organism was tested in triplicate.

All Exclusivity organisms (see Table 4) gave the expected 'NEGATIVE, Toxin NOT DETECTED' result indicating no cross-reactivity was observed in CDF2. The one exception was *Salmonella enterica* ATCC 6962 which was "Detected" in the CDF2 test in a single replicate. Suspecting contamination, the strain was re-grown, and testing was repeated. Upon retesting all replicates of *Salmonella enterica* ATCC 6962 yielded the expected 'Negative' result.

Table 4. Analytical Specificity (Exclusivity) Study Results

Species	Strain ID	Input Tested
Bacteria and Fungi		
Abiotrophia defectiva	ATCC 49176	4.64 x 10^8 CFU/mL
Acinetobacter baumannii	ATCC19606	1.39 x 10^8 CFU/mL
Aeromonas hydrophila	ATCC 35654	9.70 x 10^7 CFU/mL
Alcaligenes faecalis	ATCC 15554	6.20 x 10^7 CFU/mL
Bacillus cereus	ATCC 14579	6.10 x 10^6 CFU/mL
Bacteroides fragilis	ATCC 23745	1.16 x 10^8 CFU/mL
Campylobacter coli	ATCC 49941	4.84 x 10^8 CFU/mL
Campylobacter fetus	ATCC 25936	3.30 x 10^6 CFU/mL
Campylobacter jejuni	ATCC 49943	1.50 x 10^9 CFU/mL
Candida albicans	ATCC 14053	1.40 x 10^6 CFU/mL
Citrobacter freundii	ATCC 8090	7.40 x 10^7 CFU/mL
Clostridioides difficile (tcdA-, tcdB-)	ATCC 43593	3.00 x 10^7 CFU/mL
Clostridioides difficile (tcdA-, tcdB-)	ATCC 43601	2.20 x 10^7 CFU/mL
Clostridium bifermentans	ATCC 638	1.61 x 10^7 CFU/mL
Clostridium butyricum	ATCC 19398	1.00 x 10^5 CFU/mL
Clostridium novyi	ATCC 19402	≥ 1 x 10^6 CFU/mL
Clostridium perfringens	ATCC 13124	1.10 x 10^7 CFU/mL
Clostridium scindens	ATCC 35704	7.02 x 10^7 CFU/mL
Clostridium septicum	ATCC 12464	≥ 1 x 10^6 CFU/mL
Clostridium sporogenes	ATCC 15579	5.80 x 10^6 CFU/mL
Edwardsiella tarda	ATCC 15947	1.10 x 10^8 CFU/mL
Enterobacter aerogenes	ATCC 15038	1.20 x 10^9 CFU/mL
Enterobacter cloacae	ATCC 13047	1.40 x 10^8 CFU/mL
Enterococcus faecalis	ATCC 29212	2.14 x 10^8 CFU/mL
Enterococcus faecalis (vanB)	ATCC 51299	5.65 x 10^7 CFU/mL
Enterococcus faecium	ATCC 19434	7.10 x 10^7 CFU/mL
Escherichia coli	ATCC 4157	6.80 x 10^7 CFU/mL
Escherichia coli (stx1+, stx2+, eae+)	ATCC BAA-2221	4.70 x 10^7 CFU/mL
Escherichia coli (stx1+, stx2+, O157:H7)	ATCC 43895	8.60 x 10^6 CFU/mL
Escherichia coli (stx2+)	ATCC BAA-2326	5.90 x 10^7 CFU/mL
Escherichia fergusonii	ATCC 35469	3.20 x 10^7 CFU/mL
Escherichia hermanii	ATCC 33650	5.60 x 10^6 CFU/mL
Flavonifractor plautii (Clostridium orbiscindens)	ATCC 49531	4.00 x 10^6 CFU/mL
Hathewaya histolytica (Clostridium histolyticum)	ATCC 19401	4.00 x 10^7 CFU/mL
Helicobacter pylori	ATCC 43504	2.55 x 10^7 CFU/mL
Klebsiella oxytoca	ATCC 49131	1.21 x 10^8 CFU/mL
Klebsiella pneumoniae	ATCC 13883	6.70 x 10^7 CFU/mL
Lactobacillus acidophilus	ATCC 4356	1.70 x 10^7 CFU/mL

Species	Strain ID	Input Tested
Lactobacillus lactis	ATCC 49032	3.64 x 10^8 CFU/mL
Listeria monocytogenes	ATCC 19115	1.34 x 10^8 CFU/mL
Paeniclostridium (Clostridium) sordellii	ATCC 9714	6.40 x 10^7 CFU/mL
Peptostreptococcus anaerobius	ATCC 27337	1.40 x 10^7 CFU/mL
Plesiomonas shigelloides	ATCC 51903	8.00 x 10^6 CFU/mL
Porphyromonas asaccharolytica	ATCC 27908	1.32 x 10^8 CFU/mL
Prevotella melaninogenica	ATCC 700524	2.25 x 10^6 CFU/mL
Proteus mirabilis	ATCC 25933	2.14 x 10^8 CFU/mL
Proteus vulgaris	ATCC 6896	4.90 x 10^7 CFU/mL
Providencia alcalifaciens	ATCC 9886	1.25 x 10^8 CFU/mL
Providencia rettgeri	ATCC 9250	1.25 x 10^8 CFU/mL
Pseudomonas aeruginosa	ATCC 10145	9.60 x 10^7 CFU/mL
Pseudomonas fluorescens	ATCC 13525	2.18 x 10^7 CFU/mL
Salmonella enterica subsp. Arizonae	ATCC 13314	7.98 x 10^8 CFU/mL
Salmonella enterica subsp. enterica	ATCC 13312	1.38 x 10^8 CFU/mL
Salmonella enterica subsp. enterica	ATCC 29628	4.20 x 10^7 CFU/mL
Salmonella enterica subsp. enterica	ATCC 6962	1.37 x 10^8 CFU/mL 1.40 x 10^8 CFU/mL
Salmonella enterica subsp. enterica	ATCC 6539	2.50 x 10^6 CFU/mL
Salmonella enterica subsp. enterica	ATCC 13311	1.37 x 10^8 CFU/mL
Serratia liquefaciens	ATCC 35551	1.90 x 10^8 CFU/mL
Serratia marcescens	ATCC 13880	1.12 x 10^8 CFU/mL
Shigella boydii	ATCC 29928	9.00 x 10^7 CFU/mL
Shigella dysenteriae	ATCC 9361	1.84 x 10^7 CFU/mL
Shigella flexneri	ATCC 25929	7.20 x 10^7 CFU/mL
Shigella sonnei	ATCC 25931	6.00 x 10^7 CFU/mL
Staphylococcus aureus	ATCC 43300	1.20 x 10^8 CFU/mL
Staphylococcus aureus	ATCC 12598	1.76 x 10^7 CFU/mL
Staphylococcus epidermidis	ATCC 12228	5.80 x 10^7 CFU/mL
Streptococcus agalactiae	ATCC 13813	1.20 x 10^6 CFU/mL
Streptococcus dysgalactiae	ATCC 43078	≥ 1 x 10^6 CFU/mL
Vibrio parahaemolyticus	ATCC 17802	1.71 x 10^7 CFU/mL
Yersinia enterocolitica	ATCC 49397	1.82 x 10^8 CFU/mL
Viruses and Parasites		
Adenovirus type 40	ATCC VR-931D	1.67 x 10^{10} copies/mL
Adenovirus type 41	ATCC VR-930D	1.34 x 10^{13} copies/mL
Coxsackievirus B2	ATCC VR-29	1.10 x 10^7 $TCID_{50}$/mL
Cryptosporidium parvum	ATCC PRA-67D	1.11 x 10^8 copies/mL
Echovirus 4	ATCC VR-1734D	4.25 x 10^9 copies/mL
Entamoeba histolytica	ATCC 30459DQ	5.70 x 10^7 copies/mL
Enterovirus 71	ATCC VR-1775DQ	4.80 x 10^7 copies/mL
Giardia intestinalis	ATCC 50803D	1.52 x 10^7 copies/mL
Herpesvirus 5 (Cytomegalovirus)	ATCC VR-538D	2.64 x 10^9 copies/mL
Norovirus GI	ATCC VR-3234SD	4.70 x 10^7 copies/mL
Norovirus GII	ATCC VR-3235SD	4.80 x 10^7 copies/mL
Rotavirus A	ATCC VR-2018DQ	5.30 x 10^7 copies/mL
Human genomic DNA	Promega G147A	4.99 x 10^6 copies/mL

e. Microbial Interference (Competitive Inhibition)

The CDF2 test was evaluated for interference from mixed microbial populations using 82 microorganisms tested in the Exclusivity Study. Potentially interfering microorganisms were pooled into seventeen (17) unique pools, each containing 3-5 non-target organisms (Table 5).

Test samples were contrived for Microbial Interference testing by first spiking previously frozen cultures of each non-target organism into pooled, negative clinical stool matrix. Each non-target organism was spiked into the Microbial Interference pool at the same input concentration tested in the Analytical Specificity (Exclusivity) Study (see Table 4 above). The Microbial Interference pool was then contrived to a final concentration of 2X LoD *C. difficile* ATCC 43255 (5.2 x 10^4 CFU/mL) with previously frozen cell stocks and tested in triplicate. Details of the Microbial Interference Study pools are shown in Table 5.

All Microbial Interference pool replicates gave the expected 'POSITIVE, Toxin DETECTED' result (100% agreement) indicating no interference or competitive inhibition from non-target microbes was observed.

Table 5. Microbial Interference (Competitive Inhibition) Study Results

Potentially Interfering Microbe		
Species	**Strain ID**	**Pool**
Aeromonas hydrophila	ATCC 35654	1
Bacteroides fragilis	ATCC 23745	
Campylobacter coli	ATCC 49941	
Campylobacter fetus	ATCC 25936	
Campylobacter jejuni	ATCC 49943	
Citrobacter freundii	ATCC 8090	2
Clostridioides difficile	ATCC 43593	
Clostridioides difficile	ATCC 43601	
Clostridium perfringens	ATCC 13124	
Paeniclostridium (Clostridium) sordellii	ATCC 9714	
Enterobacter cloacae	ATCC 13047	3
Enterococcus faecalis	ATCC 29212	
Enterococcus faecium	ATCC 19434	
Escherichia coli	ATCC 4157	
Escherichia coli (O157:H7)	ATCC 43895	
Escherichia fergusonii	ATCC 35469	4
Escherichia hermanii	ATCC 33650	
Helicobacter pylori	ATCC 43504	
Klebsiella pneumoniae	ATCC 13883	
Peptostreptococcus anaerobius	ATCC 27337	
Salmonella enterica	ATCC 13312	5
Salmonella enterica	ATCC 29628	
Salmonella enterica	ATCC 6962	
Salmonella enterica	ATCC 6539	
Salmonella enterica	ATCC 13311	
Serratia liquefaciens	ATCC 35551	6
Serratia marcescens	ATCC 13880	
Shigella boydii	ATCC 29928	
Shigella flexneri	ATCC 25929	
Shigella sonnei	ATCC 25931	
Candida albicans	ATCC 14053	7
Staphylococcus aureus	ATCC 43300	
Staphylococcus aureus	ATCC 12598	
Staphylococcus epidermidis	ATCC 12228	
Yersinia enterocolitica	ATCC 49397	
Klebsiella oxytoca	ATCC 49131	8
Proteus mirabilis	ATCC 25933	
Salmonella enterica	ATCC 13314	
Shigella dysenteriae	ATCC 9361	
Streptococcus agalactiae	ATCC 13813	
Acinetobacter baumannii	ATCC 19606	9
Bacillus cereus	ATCC 14579	
Edwardsiella tarda	ATCC 15947	
Enterobacter aerogenes	ATCC 15038	
Pseudomonas fluorescens	ATCC 13525	
Abiotrophia defectiva	ATCC 49176	10
Alcaligenes faecalis	ATCC 15554	
Clostridium bifermentans	ATCC 638	
Clostridium butyricum	ATCC 19398	
Clostridium novyi	ATCC 19402	
Hathewaya histolytica (Clostridium histolyticum)	ATCC 19401	11
Lactobacillus lactis	ATCC 49032	
Listeria monocytogenes	ATCC 19115	
Pseudomonas aeruginosa	ATCC 10145	
Vibrio parahaemolyticus	ATCC 17802	
Clostridium sporogenes	ATCC 15579	12
Lactobacillus acidophilus	ATCC 4356	
Plesiomonas shigelloides	ATCC 51903	
Proteus vulgaris	ATCC 6896	
Providencia rettgeri	ATCC 9250	

Flavonifractor plautii (Clostridium orbiscindens)	ATCC 49531	13
Providencia alcalifaciens	ATCC 9886	
Salmonella enterica	ATCC 6962	
Escherichia coli	ATCC BAA-2221	
Escherichia coli	ATCC BAA-2326	
Adenovirus type 40	ATCC VR-931D	14
Enterovirus 71	ATCC VR-1775DQ	
Norovirus GI	ATCC VR-3234SD	
Norovirus GII	ATCC VR-3235SD	
Rotavirus A	ATCC VR-2018DQ	
Clostridium scindens	ATCC 35704	15
Clostridium septicum	ATCC 12464	
Enterococcus faecalis (vanB)	ATCC 51299	
Porphyromonas asaccharolytica	ATCC 27908	
Prevotella melaninogenica	ATCC 700524	
Adenovirus type 41	ATCC VR-930D	16
Coxsackievirus B2	ATCC VR-29	
Herpesvirus 5 (Cytomegalovirus)	ATCC VR-538D	
Echovirus 4	ATCC VR-1734D	
Giardia intestinalis	ATCC 50803D	
Cryptosporidium parvum	ATCC PRA-67D	17
Entamoeba histolytica	ATCC 30459DQ	
Human gDNA	Promega G147A	

f. Interfering Substances (Chemical Interference)

The CDF2 test was evaluated for potential endogenous and exogenous Chemical Interference against a panel of thirty (30) substances that are common stool contaminants, or likely present in patients with diarrhea. Each substance was tested in the background of 2X LoD contrived positive stools and true negative stool. Two (2) *C. difficile* strains were evaluated in the Interfering Substances study at 2X LoD, ATCC 43255 (5.2×10^4 CFU/mL) and ATCC BAA-1805 (1.4×10^5 CFU/mL). The same clinical negative (i.e., un-spiked) stool matrix was also tested as a true negative to evaluate potential interference with the internal assay control in the absence of target analyte.

Each substance was tested in triplicate against both *C. difficile* strains and a negative sample for a total of nine (9) CDF2 tests per substance. During testing if any substance resulted in less than 100% agreement, the input concentration of the potentially interfering substance was reduced by 10-20% and re-tested stepwise until 100% agreement was achieved. The highest, non-inhibitory input is reported. No CDF2 interference was observed for any of the endogenous or exogenous substances at the concentrations listed in Table 6.

Table 6. Interfering Substances Study Results

Interfering Substance (Brand Evaluated)	**Input Tested**	***C. difficile* ATCC 43255 2X LoD**	***C. difficile* ATCC BAA-1805 2X LoD**	**Negative**
		POSITIVE CDF2 Result		**NEGATIVE CDF2 Result**
Bismuth subsalicylate	50% v/v	3/3	3/3	3/3
Liquid glycerin suppository	50% v/v	3/3	3/3	3/3
Water-based personal lubricant	50% v/v	3/3	3/3	3/3
Silicon-based personal lubricant	50% v/v	3/3	3/3	3/3
Benzalkonium chloride wipe solution	50% v/v	3/3	3/3	3/3
Aloe leaf juice flushable wipes	50% v/v	3/3	3/3	3/3
Nonoxynol-9 condom lubricant	40% w/v	3/3	3/3	3/3
Hemorrhoidal cream	20% w/v	3/3	3/3	3/3
Hydrocortisone cream	30% w/v	3/3	3/3	3/3
Miconazole nitrate anti-fungal cream	30% w/v	3/3	3/3	3/3
Petroleum jelly	30% w/v	3/3	3/3	3/3
Antacid tablet	38% w/v	3/3	3/3	3/3
Loperamide anti-diarrheal	50% v/v	3/3	2/3	3/3
	44% v/v	Not Tested	3/3	Not Tested
Senna glycoside natural laxative	17% w/v	3/3	3/3	3/3
NSAID, Naproxen Sodium	10% w/v	3/3	3/3	3/3

Interfering Substance (Brand Evaluated)	Input Tested	*C. difficile* ATCC 43255 2X LoD	*C. difficile* ATCC BAA-1805 2X LoD	Negative
		POSITIVE CDF2 Result		NEGATIVE CDF2 Result
Mineral oil	50% v/v	3/3	3/3	3/3
Biotin supplement	51% w/v	3/3	3/3	3/3
Barium sulfate	25% w/v	3/3	3/3	3/3
Stearic acid	1.1% w/v	2/3	3/3	3/3
	1% w/v	3/3	Not Tested	Not Tested
Palmitic acid	1.5% w/v	3/3	3/3	3/3
Whole Blood	40% v/v	3/3	3/3	3/3
Biotin	0.5 w/v	3/3	3/3	3/3
Nonoxynol-9	48% v/v	3/3	2/3	3/3
	42% v/v	Not Tested	3/3	Not Tested
Mesalazine	2.5% w/v	3/3	3/3	3/3
Mucin from Porcine Stomach	2.5% w/v	3/3	3/3	3/3
Topical Antibiotic	50% w/v	3/3	3/3	3/3
Nystatin	10% w/v	3/3	3/3	3/3
Metronidazole	0.5% w/v	3/3	3/3	3/3
Erythromycin	10% w/v	3/3	3/3	3/3
Vancomycin (100 mg/mL in DMSO)	5% w/v	0/3	3/3	3/3
	0.4% w/v	3/3	Not Tested	Not Tested

g. Reproducibility

A between-site Reproducibility Study was conducted at one (1) internal and two (2) external sites. The study incorporated several variables including multiple operators (n=6) across the three (3) study sites, multiple non-consecutive testing days (n=5), multiple Analyzers (n=24), and cartridge lots (n=2).

The Reproducibility Panel consisted of five (5) panel members. Each panel contained two (2) toxigenic *Clostridioides difficile* strains: ATCC 43255 (toxinotype 0, ribotype 087) and ATCC BAA-1805 (toxinotype IIIb, NAP1/ribotype 027). Each strain was evaluated at both low positive (1.5X LoD) and moderate positive (3X LoD) concentrations. A single negative sample was also included in the panel. Contrived Reproducibility Panel samples were prepared by spiking previously enumerated and frozen *Clostridioides difficile* cultures into pooled negative clinical stool matrix. The contrived panels were frozen, shipped to sites frozen, and thawed at the test site prior to testing on the Toxigenic *C. difficile* Direct Test.

Results of the Reproducibility Study are provided by site in Table 7.

Table 7. Reproducibility Study Results

	Agreement (Actual/Expected Outcomes)				
***C. difficile* Strain**	**ATCC 43255**		**ATCC BAA-1805**		**N/A**
Concentration (LoD)	**1.5X**	**3X**	**1.5X**	**3X**	**Negative**
Clinical Site 1	100% (30/30)	100% (30/30)	100% (30/30*)	100% (30/30*)	100% (30/30)
Clinical Site 2	93.3% (28/30)	100% (30/30)	100% (30/30)	100% (30/30)	96.7% (29/30)
Internal Site	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)
Total (All Sites) [95% CI]	**97.8% (88/90)** [92.2-99.7%]	**100% (90/90)** [96-100%]	**100% (90/90)** [96-100%]	**100% (90/90)** [96-100%]	**98.9% (89/90)** [94-100%]

* This dataset contained a single (1) 'Invalid' result that was resolved upon retest.

Analysis of Positive Results: A total of four (4) positive samples were tested in triplicate by each of the 6 operators over 5 days. Therefore, the study contains a total of 60 positive results per operator and 360 total positives.

Two (2) low positive (test concentration = 1.5X LoD) Reproducibility panel samples yielded 'NEGATIVE' results in a single replicate. Both false negative replicates are assumed to be due to assay variability and stochastic sampling of the contrived sample for this analyte at concentrations very near the limit of detection. Although both false negatives occurred at Site 2, there was no commonality between the operator or the test day. Even with these two missed replicates the overall reproducibility for this analyte was still acceptable (≥ 95%).

Analysis of Negative Results: To assess negative results, aliquots of the same pooled negative matrix that was used to contrive the panel were tested. A single (1) negative sample was included in each panel, tested in triplicate by each of 6 operators over 5 days each, therefore a total of 15 negative results per operator and 90 total negative results in the study.

A single negative Reproducibility panel sample yielded a 'POSITIVE' result in a single replicate. The false positive was most likely caused by unintentional sample contamination by the operator.

Overall agreement for positive and negative Reproducibility panel samples are provided in Table 8. The agreement of positive results for toxigenic *C. difficile* was 98.9% at low positive concentrations (1.5X LoD) and 100% at moderate positive concentrations (3X LoD). The overall agreement of positive results was 99.4% [CI_{95} 98.0 – 99.9%]. The overall agreement of negative results was 98.9% [CI_{95} 94.0 – 100%]. Given the number of variables included in this study (cartridge lots, analyzers, operators, sites, days) the study demonstrated acceptable reproducibility of the Toxigenic *C. difficile* Direct Test.

Table 8. Summary of Reproducibility Results

Analyte	Concentration	Agreement (Actual/Expected Outcomes) [95% CI]	
Clostridioides difficile (tcdB+)	1.5X LoD	98.9% (178/180) [96-99.9%]	**99.4%** (358/360) [98-99.9%]
	3X LoD	100% (180/180) [98-100%]	
Negative	N/A	98.9% (89/90) [94-100%]	**98.9%** (89/90) [94-100%]

H. Performance Data – Clinical Studies

A prospective multi-center method comparison study was conducted to compare the performance of the Toxigenic *C. difficile* Direct Test (CDF2) to an FDA-cleared molecular reference. The study was conducted at three, geographically diverse, U.S. clinical study sites (Midwest, Northeast, West) from June 2022 - February 2023.

Specimens enrolled in the study were excess remnants of unpreserved stool specimens that were prospectively collected from symptomatic individuals ≥ 2 years of age suspected of *C. difficile* infection (CDI), processed according to routine standard of care testing, and would have otherwise been discarded. All specimens were enrolled under an Institutional Review Board (IRB) approved protocol and testing was performed by trained laboratory personnel.

A total of 829 specimens met the inclusion criteria and were included in the final dataset. The dataset included specimens collected from 431 females (52%), 393 males (47.4%), and 5 (0.6%) individuals of undeclared or unknown gender. The age distribution of study participants is shown in Table 9.

Table 9. Study Population Distribution by Age Group

Age Group (Years)	Number of Specimens
<2	Excluded
2-12	37 (4.5%)
13-18	32 (3.9%)
19-64	442 (53.3%)
65-88	299 (36.1%)
≥ 89	19 (2.3%)
Total	829 (100.0%)

Results of the prospective method comparison study were analyzed for concordance by site and in total using a 2x2 table. The positive percent agreement (PPA), negative percent agreement (NPA), disease prevalence, positive predictive value (PPV), negative predictive value (NPV) along with the 95% confidence intervals (CI) were then calculated. Results of Prospective Specimen testing by site and in total are shown in Table 10.

Table 10. Prospective Study Results

Site 1		Molecular Comparator		
		Positive	Negative	Total
Toxigenic *C. difficile* Direct Test	Positive	71	7	78
	Negative	2	320	322
	Total	73	327	400

PPA = 97.3% (90.5% - 99.7%)
NPA = 97.9% (95.6% - 99.1%)
Prevalence = 18.3%
PPV = 91.0% (82.4% - 96.3%)
NPV = 99.4% (97.8% - 99.9%)

Site 2		Molecular Comparator		
		Positive	Negative	Total
Toxigenic *C. difficile* Direct Test	Positive	60	2	62
	Negative	4	318	322
	Total	64	320	384

PPA = 93.8% (84.8% - 98.3%)
NPA = 99.4% (97.8% - 99.9%)
Prevalence = 16.7%
PPV = 96.8% (88.8% - 99.6%)
NPV = 98.8% (96.9% - 99.7%)

Site 3		Molecular Comparator		
		Positive	Negative	Total
Toxigenic *C. difficile* Direct Test	Positive	7	1	8
	Negative	0	37	37
	Total	7	38	45

PPA = 100.0% (59.0% - 100.0%)
NPA = 97.4% (86.2% - 99.9%)
Prevalence = 15.6%
PPV = 87.5% (47.4% - 99.7%)
NPV = 97.8% (88.2% - 99.9%)

All Sites		Molecular Comparator		
		Positive	Negative	Total
Toxigenic *C. difficile* Direct Test	Positive	138	10	148
	Negative	6	675	681
	Total	144	685	829

PPA = 95.8% (91.2% - 98.5%)
NPA = 98.5% (97.3% - 99.3%)
Prevalence = 17.4%
PPV = 93.2% (87.9% - 96.7%)
NPV = 99.1% (98.1% - 99.7%)

Discrepant results were defined as results obtained from the CDF2 test that were not in agreement with the standard of care molecular reference method. Specimens with discrepant results were investigated by testing the specimens on a third molecular test.

As shown in Table 11, five (5) of six (6) false negative specimens also tested negative when evaluated with a second FDA-cleared molecular *C. difficile* test and are therefore concordant with the CDF2 result. The remaining false negative (1) specimen tested positive on the BD Max and found to be in concordance with the reference test.

All ten (10) CDF2 false positive results tested negative when evaluated with a second FDA-cleared molecular *C. difficile* test and found to be in concordance with the reference test.

Results of discrepant resolution testing are summarized in Table 11.

Table 11. Prospective Study Discrepant Testing Results

Resolved False Negatives	Resolved False Positives
5/6	0/10

Established clinical performance, positive (PPA) and negative (NPA) percent agreement along with 95% Confidence Intervals, of the Toxigenic *C. difficile* Direct Test is shown in Table 12. Overall, the results from the clinical evaluation demonstrated acceptable performance of the Great Basin Toxigenic *C. difficile* Direct Test and support the Intended Use of this product.

Table 12. Summary Table - Clinical Performance of the Toxigenic C. difficile Direct Test

	n	PPA (95% CI)	NPA (95% CI)
Toxigenic *C. difficile* Direct Test (CDF2)	829	95.8% (91.2 - 98.5) 138/144	98.5% (97.3 - 99.3) 675/685

I. Overall Conclusion

The information submitted in this 510(k) pre-market notification is complete and supports substantial equivalence.