510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K211342

B Applicant

Quidel Corporation

C Proprietary and Established Names

Sofia 2 Campylobacter FIA

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LQP	Class I, reserved	21 CFR 866.3110 - *Campylobacter fetus* Serological Reagents	MI - Microbiology
KHO	Class I, exempt	21 CFR 862.2560 – Fluorometer for Clinical Use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the detection of *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* antigens in human stool.

B Measurand:

Campylobacter jejuni, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* antigens.

C Type of Test:

Lateral flow fluorescent immunoassay (FIA)

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
Sofia 2 Campylobacter FIA employs immunofluorescence for the rapid qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. Sofia 2 Campylobacter FIA is designed to detect *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* from patients with signs and symptoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved fecal specimens. Test results should be considered in conjunction with clinical findings and patient history.

C Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only
- The Sofia 2 Campylobacter FIA was evaluated using only fresh fecal samples and fecal samples stored in Cary Blair media or C&S media. The performance of fecal samples stored in other transport media (e.g., formalin, polyvinyl alcohol) has not been evaluated and therefore, should not be used.
- No data exists on the effects of colonic washes, barium enemas, laxatives, or bowel preparations on the performance of the Sofia 2 Campylobacter FIA. All of these procedures can result in extensive dilution or the presence of additives that may affect test performance.
- The fluorescence signal obtained with this assay is invisible to the unaided eye. The test results can only be obtained with the proper use of Sofia 2

D Special Instrument Requirements:
The Sofia 2 Campylobacter FIA can only be run on the Sofia 2 Instrument.

IV Device/System Characteristics:

A Device Description:
The Sofia 2 Campylobacter FIA is an immunofluorescence-based, lateral flow assay that uses an antibody sandwich design to detect Campylobacter-specific antigens in stool specimens from individuals with signs and symptoms of gastroenteritis.

The patient stool sample is placed in the Specimen Tube, containing Specimen Diluent, to make the antigenic components more accessible to the test antibodies. An aliquot of the diluted sample is dispensed through a filter into the Test Cassette sample well. From the sample well, the sample migrates through a test strip containing various unique chemical environments. If *Campylobacter jejuni, Campylobacter coli, Campylobacter lari*, or *Campylobacter upsaliensis* specific antigens are present, they will be bound by polyclonal antibodies coupled to fluorescent microparticles that migrate through the test strip. The fluorescent microparticles containing bound antigens will be captured by monoclonal antibodies at a defined location on the test strip where they are detected by Sofia 2. If *Campylobacter jejuni, Campylobacter coli, Campylobacter lari,* or *Campylobacter upsaliensis* specific antigens 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. Note that a supervisor can place Sofia 2 into Locked Walk Away mode, which only

allows testing to be performed in Walk Away mode. Once testing is complete, Sofia 2 will display the test results (Positive, Negative, or Invalid) on the screen.

B Principle of Operation:
Immunofluorescence

V Substantial Equivalence Information:

A Predicate Device Name(s):
CAMPYLOBACTER QUIK CHEK

B Predicate 510(k) Number(s):
K191456

C Comparison with Predicate(s):

Similarities		
Item	DEVICE Sofia 2 Campylobacter FIA K211342	PREDICATE *CAMPYLOBACTER QUIK CHEK* K191456
Indications For Use	Sofia 2 Campylobacter FIA employs immunofluorescence for the rapid qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. Sofia 2 Campylobacter FIA is designed to detect *C. jejuni*, *C. coli*, *C. lari,* and *C. upsaliensis* from patietns with signs and symptoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved fecal specimens. Test results should be considered in conjuction with clinical findings and patient history.	The *CAMPYLOBACTER QUIK CHEK* test is a rapid membrane enzyme-linked immunosorbent assay for the qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. The *CAMPYLOBACTER QUIK CHEK* test is designed to detect *C. jejuni*, *C. coli*, *C. lari,* and *C. upsaliensis* from patients with signs and symtpoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved fecal specimens. Test results should be considered in conjuction with clinical findings and patient history.
Classification	I	Same
Product Code	LQP	Same
Regulation	21 CFR 866.3110	Same
Measured Analyte	*Campylobacter*-specific antigen	Same
Antibody Format	Monoclonal/Polyclonal	Same
Type of Test	Qualitative	Same
Sample Matrix	Human fecal specimen	Same
Specimen Type	Unpreserved fecal specimens and fecal specimens in Cary-Blair and C&S transport media	Same

Controls	Positive and negative control included in the kit	Same
Species Detected	*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*	Same
Differences		
Item	DEVICE Sofia 2 Campylobacter FIA K211342	PREDICATE *CAMPYLOBACTER QUIK CHEK* K191456
Test Principle	Immunofluorescence device	Immunochromatographic device
Format	Lateral-flow test cassette	Membrane Enzyme Linked Immunosorbent Assay (ELISA)
Read Result Time	15 minutes	<30 minutes
Result Interpretation Method	Result interpretation automated by Sofia 2 Instrument	Visually Read
Kit Storage	Room Temperature (15˚C-30˚C)	Refrigerated (2˚C-8˚C)

VI Standards/Guidance Documents Referenced:

None referenced

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

As noted above, the test can be used in "Walk Away" mode, or "Read Now" mode. The primary difference between these modes is that in "Read Now" mode, the operator is responsible for the incubation time; in the "Walk Away" mode, the instrument will wait to read the strip until the specified development time has passed. In this submission, all analytical studies were conducted in "Read Now" mode. A validation study was conducted to demonstrate that the analytic study results using the "Read Now" mode can be considered representative. See section **VII.A.3."Read Now" vs. "Walk Away" Mode Study** for more information and the results of this study.

1. Precision/Reproducibility:

The precision of the Sofia 2 Campylobacter FIA was determined using a 3-member masked fecal sample panel that consisted of a negative, low positive (1-2x higher than C_{95}) and a moderate positive (2-3x higher than C_{95}). Each fecal sample was spiked with *C. jejuni* (strain CCUG 11284) whole organism to achieve the desired level. The samples were tested once per day over a 20-day period by multiple operators using three reagent lots. A total of 240 tests per panel member (1 run per day x 4 replicates per run x 20-days x 3 lots) were performed. Positive and negative controls were run with each sample panel of masked samples. All replicates for the negative panel member were negative and all replicates for the positive panel members were positive.

The reproducibility of the Sofia 2 Campylobacter FIA was determined using an 8-member masked fecal sample panel. The panel consisted of 2 negatives, 2 high negatives (just below C_5), 2 low positives (1-2x higher than C_{95}) and 2 moderate positives (2-3x higher than C_{95}). Each fecal sample was spiked with *C. jejuni* (strain CCUG 11284) whole organism to achieve the desired level. Testing was performed at three laboratories, one of which was on-site. The samples were tested by 2 operators per day over 5 non-consecutive days with 2 reagent lots for a total of

90 replicates per panel member (1 run per day x 3 replicates per run x 5-days x 3 sites x 2 lots). In total, 180 measurements were performed for each evaluated *Campylobacter* concentration (e.g. 2 low positive panel members x 90 replicates per panel member = 180 measurements), for a total of 360 expected positive and 360 expected negative measurements. Positive and negative controls were run with each sample panel of masked specimens. The results were consistent among the three locations with 100% reproducibility for all test panel members.

2. Linearity:
Not applicable

3. Analytical Specificity/Interference:

Cross-Reactivity and Microbial Interference
The Sofia 2 Campylobacter FIA was evaluated for cross-reactivity with the bacteria, fungi and viruses listed in **Table 1**. For cross-reactivity testing, bacteria and fungi were spiked at concentrations $>10^7$ CFU/mL and viruses were spiked at concentrations $>10^5$ $TCID_{50}$/mL, unless otherwise noted. For cross-reactivity testing, samples were prepared in the absence of *C. jejuni*. For microbial interference testing, samples were evaluated in the presence of *C. jejuni* (strain CCUG 11284), which was spiked at 2-3x LoD with whole organism. Due to safety concerns with culturing *Listeria monocytogenes*, freeze-dried biomaterial from ATCC was reconstituted in 1 mL of sterile PBS and tested alone (negative sample) or spiked with *C. jejuni* (strain CCUG 11284) whole organism at 2-3x LoD (positive sample). Four (4) clinically positive Norovirus fecal specimens (determined by RT-PCR) were obtained from a clinical repository (Wyoming Public Health Laboratory). The two specimens that were genogroup I (GGI) were pooled, and the two specimens that were genogroup II (GGII) were pooled. The pooled samples served as negative *Campylobacter* samples while the pooled specimens spiked with *Campylobacter jejuni* (strain CCUG 11284) whole organism at 2-3x LoD served as the positive samples.

C. helveticus (strain ATCC 51209) was cross-reactive at $6.3x10^6$ CFU/mL, which is ~3x the LoD of *C. lari* in unpreserved fecal matrix. Additional *C. helveticus* concentrations were tested and cross-reactivity was no longer observed at $1.98x10^5$ CFU/mL. No other tested microorganism exhibited cross-reactivity with the Sofia 2 Campylobacter FIA.

Table 1. Microorganisms Evaluated for Cross-Reactivity/Microbial Interference

Bacteria/Fungi	
Acinetobacter baumannii	*Escherichia hermannii*
Aeromonas hydrophila	*Helicobacter pylori*
Bacillus cereus	*Klebsiella pneumoniae*
Bacillus subtilis	*Lactobacillus acidophilus*
Bacteroides fragilis	*Lactococcus lactis*
Campylobacter concisus	*Listeria monocytogenes*
Campylobacter fetus	*Peptostreptococcus anaerobius*
Campylobacter helveticus	*Plesiomonas shigelloides*
Campylobacter hyointestinalis	*Porphyromonas asaccharolytica*
Candida albicans	*Prevotella melaninogenica*
Citrobacter freundii	*Proteus vulgaris*
Clostridium bifermentans	*Pseudomonas fluorescens*

Bacteria/Fungi	
Clostridiodes difficile	*Salmonella enterica typhimurium*
Clostridium perfringens	*Serratia marcescens*
Edwardsiella tarda	*Shigella dysenteriae*
Enterobacter cloacae	*Shigella flexneri*
Enterococcus faecalis	*Shigella sonnei*
Escherichia coli	*Staphylococcus aureus*
Escherichia coli EIEC	*Staphylococcus aureus* subsp. *aureus* Rosenbach [formerly *Cowan's*]
Escherichia coli EPEC	*Streptococcus agalactiae*
Escherichia coli ETEC	*Staphylococcus epidermidis*
Escherichia coli O157:H7 (non-toxigenic)	*Vibrio parahaemolyticus*
Escherichia coli O157:H7 (toxigenic)	*Yersinia enterolitica*
Escherichia fergusonii	
Viruses	
Adenovirus Type 1	Enterovirus 69
Adenovirus Type 2	Enterovirus 70
Adenovirus Type 3	Enterovirus 71
Adenovirus Type 5	Human coronavirus
Human mastadenovirus F [formerly Adenovirus Type 40][1]	Echovirus 9
Adenovirus Type 41	Echovirus 11
Coxsackie virus B2	Echovirus 18 (ATCC VR-1783)
Coxsackie virus B3	Echovirus 18 (NCPV 0801131v)[3]
Coxsackie virus B4	Human parechovirus 1 [formerly Echovirus 22]
Coxsackie virus B5	Echovirus 33[4]
Human Rotavirus[2]	Norovirus GI
Enterovirus 68	Norovirus GII

[1]Tested at 1.6 x 10^4 $TCID_{50}$/mL
[2]Tested at 5.0 x $10^{0.75}$ $TCID_{50}$/mL
[3]Tested at 5.6 x 10^3 $TCID_{50}$/mL
[4]Tested at 1.0 x 10^4 $TCID_{50}$/mL

Interfering Substances Study
The Sofia 2 Campylobacter FIA was evaluated for interference with the substances and concentrations listed in **Table 2**. Samples were tested in triplicate in both the presence and absence of *C. jejuni* (strain CCUG 11284) whole organism. Positive samples were spiked with *C. jejuni* at 2-3x LoD. At the concentrations tested, none of the substances listed in **Table 2** were shown to interfere with the performance of the Sofia 2 Campylobacter FIA.

Table 2. Interfering Substances

Substance	Concentration	Substance	Concentration
Barium sulfate	5% w/v	Naproxen Sodium	0.05% w/v
Benzalkonium Chloride	1% w/v	Nonoxynol-9	1% w/v

Ciprofloxacin	0.25% w/v	Nystatin	1% w/v
Ethanol	1% w/v	Palmitic Acid/Fecal Fat	40% w/v
Hog gastric mucin	3.5% w/v	Pepto-Bismol®	5% v/v
Human blood	40% v/v	Phenylephrine	1% w/v
Hydrocortisone	1% w/v	MiraLax®	10% w/v
Imodium®	5% v/v	Prilosec OTC®	5 µg/mL
Kaopectate®	5% v/v	Sennosides	1% w/v
Leukocytes	0.05% w/v	Simethicone	10% w/v
Maalox® Advanced	5% v/v	Stearic Acid/Fecal Fat	40% w/v
Mesalazine	10% w/v	TUMS®	50 µg/mL
Metronidazole	0.25% w/v	Human Urine	5% v/v
Mineral Oil	10% w/v	Vancomycin	0.25% w/v
Mylanta®	4.2 mg/mL		

Prozone Study
The purpose of this study was to demonstrate that a high concentration of *Campylobacter* (antigen) does not interfere with a positive reaction in the Sofia 2 Campylobacter FIA. High positive samples were prepared by spiking a negative fecal pool with $5.0x10^7$ CFU/mL whole organisms of *C. jejuni* (strain CCUG 11284), $2.4x10^8$ CFU/mL whole organisms of *C. coli* (strain CCUG 11283), $1.6x10^8$ CFU/mL whole organisms of *C. lari* (strain 2015/1582), and $1.3x10^8$ whole organisms of *C. upsaliensis* (strain 2018/0319H). A total of 5 different dilutions for each *Campylobacter* species were prepared and tested. Testing was performed in triplicate according to the Package Insert instructions. The results demonstrated that there was no overall hook affect, and that elevated levels of whole organism did not affect the detection of *Campylobacter*.

"Read Now" vs. "Walk Away" Mode Study
To demonstrate that performance of the Sofia 2 Campylobacter FIA is not influenced by operating the device in "Read Now" mode under the conditions used for analytical studies, a comparison study was conducted with positive samples prepared by spiking a negative fecal pool with *C. jejuni* (strain CCUG 11284) at 3x LoD. Negative samples were also included. Each sample was tested in replicates of twenty using five Sofia 2 analyzers. Testing was completed once with Sofia 2 analyzers in "Read Now" mode and once with Sofia 2 analyzers in "Walk Away" mode.

There was 100% agreement between the expected and observed results for all positive and negative samples. These results demonstrate that there is no notable difference in performance between "Read Now" and "Walk Away" modes in the analytical testing setting. These study results are acceptable and support performing analytical testing solely in "Read Now" mode.

4. Assay Reportable Range:
   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Specimen Stability
The effect of specimen storage on antigen stability was evaluated for both unpreserved stool samples and samples in Cary Blair and C&S transport media. For the analysis, a total of 22 fecal specimens were tested with the Sofia 2 Campylobacter FIA. The fecal specimens consisted of 10 *C. jejuni* low positive fecal samples (1-2x LoD), 5 *C. jejuni* moderate positive fecal samples (2-

3x LoD), 5 *C. jejuni* high positive fecal samples (4-5x LoD) and 2 negative samples. *C. jejuni* strain CCUG 11284 was used to generate the positive samples.

Unpreserved and preserved stool samples were stored at room temperature (15-30°C) and refrigerated (2-8°C) and tested at 24 hour intervals for 120 hours. Positive and negative controls were also tested. The following transport media were used for the study: ThermoFisher Protocol Cary Blair media and ThermoFisher Protocol C&S. Positive samples remained positive and negative samples remained negative throughout the study. Storage in transport media did not affect the stability of the samples. Based on these results, the recommended storage time for unpreserved and preserved (Cary Blair and C&S) stool samples is up to 96 hours at room temperature (15-30°C) and refrigerated (2-8°C).

Unpreserved stool was also stored frozen (i.e., -10°C) and tested at 0, 5, 10, and 14 days. Positive samples at all concentrations remained positive and negative samples remained negative throughout the study. Based on these results, the recommended storage time for unpreserved stool samples is up to 13 days frozen at ≤-10°C.

Freeze/Thaw Stability
Freeze/Thaw stability for unpreserved specimens was evaluated for five freeze/thaw cycles over a 7-day period. *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* were each tested by spiking whole organisms into unpreserved stool at the following concentrations: high negative (~0.5x LoD), low positive (1-2x LoD), and high positive (3-5x LoD). For *C. jejuni*, 4 high negatives, 15 low positives, and 4 high positive samples were prepared and tested. For each of the remaining *Campylobacter* species, 2 high negatives, 5 low positives, and 2 high positives were tested. All samples were tested in duplicate. For this study, samples were stored at -10°C.

At baseline, 5 of 15 *C. jejuni* low positive samples yielded one negative replicate and one positive replicate. Repeat testing was performed on 4 of these samples in duplicate, and 3 samples yielded two positive results, while one sample yielded 2 negative results. All subsequent timepoints were positive for the *C. jejuni* low positive sample, thus the baseline false negative results were considered acceptable. No other sample converted from positive-to-negative or negative-to-positive after any of the freeze/thaw cycles. The data support a claim for four freeze/thaw cycles and demonstrates that freezing neither enhances nor impairs the performance of the Sofia 2 Campylobacter FIA.

6. Detection Limit:

Limit of Detection Study
The limit of detection (LoD) of the Sofia 2 Campylobacter FIA was determined by spiking whole organism culture preparations of *Campylobacter jejuni* (strain CCUG 11284), *Campylobacter coli* (strain CCUG 11283), *Campylobacter lari* (strain 2015/1582), and *Campylobacter upsaliensis* (strain 2018/0319H) into negative fecal matrix and Cary Blair and C&S transport media. The LoD is defined as the concentration of *Campylobacter* (in CFU/mL) that yields a positive result 95% of the time and negative result 5% of the time. The LoD was determined for two Sofia 2 Campylobacter FIA cassette lots. For each lot, multiple dilutions of *C. jejuni, C. coli, C. lari,* and *C. upsaliensis* were tested in replicates of 20. The LoD values in **Table 3** represent the cassette lot yielding the highest (i.e., least sensitive) LoD. The LoDs were similar between lots.

Table 3. LoD Values for the Sofia 2 Campylobacter FIA

Species	Fecal Matrix		Cary Blair		C&S	
	CFU/mL	CFU/test	CFU/mL	CFU/test	CFU/mL	CFU/test
C. jejuni	$9.82x10^4$	$2.95x10^2$	$1.57x10^5$	$4.38x10^2$	$1.50x10^5$	$4.19x10^2$
C. coli	$1.15x10^6$	$3.45x10^3$	$1.59x10^6$	$4.44x10^3$	$9.02x10^5$	$2.52x10^3$
C. lari	$2.00x10^6$	$6.00x10^3$	$1.75x10^6$	$4.88x10^3$	$2.25x10^6$	$6.28x10^3$
C. upsaliensis	$5.21x10^6$	$1.56x10^4$	$5.21x10^6$	$1.45x10^4$	$2.71x10^6$	$7.56x10^3$

Inclusivity Study

The reactivity (inclusivity) study was conducted using *C. jejuni* and *C. coli* strains from the Culture Collection University of Gothenburg (CCUG). The reactivity of *C. upsaliensis* and *C. lari* were evaluated using clinical isolates obtained from patients with symptoms of gastroenteritis that were isolated at the Centre National de Reference des Campylobacters et Helicobacters – Universite de Bordeaux between 2015 and 2018. The strains are listed in **Table 4**. For reactivity testing, each strain was spiked into negative fecal matrix at 2-3x the LoD of the corresponding reference strain used to confirm the LoD, and then tested with the Sofia 2 Campylobacter FIA. All results were positive.

Table 4. Strains Evaluated for Reactivity with the Sofia 2 Campylobacter FIA

C. jejuni	*C. coli*	*C. upsaliensis*	*C. lari*
CCUG 6951	CCUG 10956	2016/1950	2015/2189
CCUG 12081	CCUG 17755	2016/2826	2015/1657
CCUG 29411	CCUG 36994	2017/0349	2015/2983
CCUG 38106	CCUG 53138	2018/1669	2016/1130H
CCUG 24567[1]			

[1] CCUG 24567 is a *C. jejuni* subspecies *doylei* isolate.

Additional inclusivity testing was performed for select strains of *C. coli, C. lari,* and *C. upsaliensis* to verify the lowest concentration of each strain that is reactive in the Sofia 2 Campylobacter FIA. Four-fold serial dilutions for each of the targeted strains were prepared from stock culture slurries using negative fecal matrix and tested in replicates of three in the assay. The qualitative results were used to determine the lowest detectable level in the series, where 3/3 results were positive (**Table 5**). The results demonstrated that these strains produced positive results in the assay at concentrations below the limit of detection established with the corresponding reference strains. Note: the purpose of this study was not to set a true limit of detection, but the results are used to demonstrate inclusivity of these strains at the specified concentrations detected (**bolded** in **Table 5**).

Table 5. Results of Additional Inclusivity Testing

Species/Strain Tested	Dilution	Concentration (cfu/mL)	N	Pos	Neg	% Pos.
Campylobacter coli CCUG 53138 Stock 2.2×10^7 CFU/mL	Stock slurry	2.2×10^7	3	3	0	100
	1:4	5.5×10^6	3	3	0	100
	1:16	1.375×10^6	3	3	0	100
	1:64	3.438×10^5	3	3	0	100
	1:256	**$8.594x\ 10^4$**	**3**	**3**	**0**	**100**
	1:1024	2.148×10^4	3	0	3	0
	1:4096	5.371×10^3	3	0	3	0

Species/Strain Tested	Dilution	Concentration (cfu/mL)	N	Pos	Neg	% Pos.
Campylobacter lari 2015/2189 Stock 1.55 x 10^7 CFU/mL	Stock slurry	1.55 x 10^7	3	3	0	100
	1:4	3.875 x 10^6	3	3	0	100
	1:16	9.688 x 10^5	3	3	0	100
	1:64	2.422 x 10^5	3	3	0	100
	1:256	**6.055 x 10^4**	**3**	**3**	**0**	**100**
	1:1024	1.514 x 10^4	3	0	3	0
	1:4096	3.784 x 10^3	3	0	3	0
Campylobacter lari 2016/1130H Stock 1.4 x 10^7 CFU/mL	Stock slurry	1.4 x 10^7	3	3	0	100
	1:4	3.5 x 10^6	3	3	0	100
	1:16	8.75 x 10^5	3	3	0	100
	1:64	**2.188 x 10^5**	**3**	**3**	**0**	**100**
	1:256	5.469 x 10^4	3	0	3	0
	1:1024	1.367 x 10^4	3	0	3	0
	1:4096	3.418 x 10^3	3	0	3	0
Campylobacter upsaliensis 2016/1950 Stock 8.4 x 10^7 CFU/mL	Stock slurry	8.4 x 10^7	3	3	0	100
	1:4	2.1 x 10^7	3	3	0	100
	1:16	5.25 x 10^6	3	3	0	100
	1:64	**1.313 x 10^6**	**3**	**3**	**0**	**100**
	1:256	3.281 x 10^5	3	2	1	67
	1:1024	8.203 x 10^4	3	0	3	0
	1:4096	2.051 x 10^4	3	0	3	0

7. Assay Cut-Off:
   To determine the preliminary cut-off the Sofia 2 Campylobacter FIA read on the Sofia 2 Analyzer, a panel of clinical positive, clinical negative, and contrived positive (near LoD) stool samples were evaluated. After the initial cut-offs were established, the values were validated as part of the clinical trial as well as the other analytical studies.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable

2. Matrix Comparison:
   Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Prospective Study
The performance of the Sofia 2 Campylobacter FIA was assessed with specimens prospectively collected from patients with signs and symptoms of gastroenteritis. Specimens were collected from two independent clinical sites. The sensitivity and specificity of the Sofia 2 Campylobacter FIA was calculated by comparing directly to culture results performed at both sites. Prospective testing consisted of 811 stool specimens tested in clinical microbiology laboratories at the Medical College of Wisconsin and TriCore Reference Laboratories. Both "Read Now" and "Walk Away" modes were used for specimen testing.

The ages of patients ranged from 2 years to 93 years with 3% of the specimens coming from patients that were ≤ 18 years. Of the 811 patients tested, 61.9% were female and 38.1% were male. No difference in test performance was observed based on patient age or gender.

A summary of the clinical performance of the Sofia 2 Campylobacter FIA for both sites, combined is shown in **Table 6**.

The results of the study show that the Sofia 2 Campylobacter FIA exhibited a sensitivity of 100% and a specificity of 99.3% compared to culture. The lower bound of the two-sided 95% confidence interval for the sensitivity point estimate was slightly lower than expectations for this type of assay. However, the sensitivity results were deemed acceptable without the need to conduct additional clinical specimen testing for the following reasons: (1) Challenges with performing prospective clinical studies during the COVID-19 pandemic and, (2) The prospective study was supplemented with archived positive specimens (see section **VII.C.Archived Study**, below).

All samples with discrepant results were tested at a third site using an in-house species-specific RT-PCR method and bidirectional sequencing. Of the 6 false positive results, all were confirmed to be positive using these additional tests.

Table 6. Clinical Performance of the Sofia 2 Campylobacter FIA

		Culture		
		Positive	**Negative**	**Total**
Sofia 2 Campylobacter FIA	**Positive**	8	6*	14
	Negative	0	797	797
	Total	8	803	811
	Sensitivity	100% (8/8), 95% CI: (67.6% - 100%)		
	Specificity	99.3% (797/803), 95% CI: (98.4% - 99.7%)		

*All specimens that were culture negative and Sofia 2 Campylobacter FIA positive were confirmed to be *Campylobacter* positive by a species-specific RT-PCR method and bidirectional sequencing.

Archived Study
Additional testing was performed at the third clinical site on 70 retrospectively collected specimens with culture results. The study included 35 positive and 35 negative specimens by *Campylobacter* spp. culture. The patient ages ranged from 2 years to 87 years. All positive specimens were further characterized as *Campylobacter* spp. positive by a validated in-house RT-PCR (targeting species-specific genes) and by bidirectional sequencing (targeting sequences of the 16S rRNA gene of *Campylobacter* spp. and species-specific genes for identification). These

specimens were then tested by the Sofia 2 Campylobacter FIA. With this information, FDA was able to assess if "weak positives" were included in the archived study and determine performance of the Sofia 2 Campylobacter FIA with such specimens. All 35 specimens tested positive for *Campylobacter* spp. by all methods, yielding 100% correlation with all test methods.

Rare Isolate Contrived Study
The performance of the Sofia 2 Campylobacter FIA was further evaluated by testing a panel consisting of five *C. coli,* five *C. upsaliensis*, and five *C. lari* isolates. The *C. coli* strains were obtained from the Culture Collection University of Gothenburg. The *C. upsaliensis* and *C. lari* strains are clinical isolates obtained from the Centre National de Reference des Campylobacters et Helicobacters – Universite de Bordeaux. All samples were prepared by spiking negative, pooled fecal matrix with whole organism at 2x the LoD of the reference strain, which was used to confirm the LoD. Both unpreserved stool and preserved stool (Cary Blair and C&S transport media) were evaluated during the study. Due to availability of a limited number of isolates, each isolate and matrix type were tested in triplicate, twice a day over a period of three days for a total of 18 data points. Ninety datapoints per *Campylobacter* species and matrix were performed (18 data points/isolate x 5 isolates per species = 90 data points). The results of this study demonstrated 100% positive percent agreement (PPA) with expected results for all *C. coli* and *C. upsaliensis* isolates, regardless of matrix. For *C. lari*, 100% PPA with expected results was obtained for all isolates in unpreserved stool and C&S transport media. For *C. lari* in Cary Blair media, 98.9% PPA was obtained due to a single negative result. Upon retesting the sample that yielded a negative result, a positive result was obtained. These results are acceptable.

2. Clinical Specificity:
   See section **VII.C.1.Prospective Study** above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:
Not applicable

VIII Proposed Labeling:
The labeling supports the finding of substantial equivalence for this device.

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