510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K173219

B. Purpose for Submission:

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

C. Measurand:

Campylobacter jejuni and *Campylobacter coli* antigens

D. Type of Test:

Enzyme Immunoassay

E. Applicant:

TechLab Inc.

F. Proprietary and Established Names:

CAMPYLOBACTER CHEK

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3110, *Campylobacter fetus* serological reagents

2. Classification:

   I

3. Product code:

   LQP

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The *CAMPYLOBACTER CHEK*™ test is an enzyme immunoassay for the qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. The *CAMPYLOBACTER CHEK*™ test is designed to detect *C. jejuni* and *C. coli* from patients with symptoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved human fecal specimens. Test results should be considered in conjunction with clinical findings and patient history.

2. Indication(s) for use:
   The *CAMPYLOBACTER CHEK*™ test is an enzyme immunoassay for the qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. The *CAMPYLOBACTER CHEK*™ test is designed to detect *C. jejuni* and *C. coli* from patients with symptoms of gastroenteritis. The test is intended for use with preserved fecal specimens in transport media and unpreserved human fecal specimens. Test results should be considered in conjunction with clinical findings and patient history.

3. Special conditions for use statement(s):

   - For prescription use only.
   - Optimal results with the *CAMPYLOBACTER CHEK* test are obtained with specimens that are less than 96 hours old. If specimens are not assayed within this time period, they may be frozen.
   - The *CAMPYLOBACTER CHEK* test 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.
   - The *CAMPYLOBACTER CHEK* test is qualitative. The intensity of the color should not be interpreted quantitatively.
   - No data exists on the effects of colonic washes, barium enemas, laxatives, or bowel preparations on the performance of the *CAMPYLOBACTER CHEK* test. All of these procedures can result in extensive dilution or the presence of additives that may affect test performance.

4. Special instrument requirements:

   None

I. Device Description:

The *CAMPYLOBACTER CHEK*™ test is an enzyme immunoassay for the qualitative detection of *Campylobacter* spp. in human fecal specimens. The *CAMPYLOBACTER CHEK* test uses antibodies that recognize a *Campylobacter*-specific antigen. The Microassay Plate in the kit contains immobilized capture monoclonal antibodies against a *Campylobacter*-specific antigen. The Conjugate consists of polyclonal antibodies to a *Campylobacter*-specific antigen conjugated to horseradish peroxidase. In the assay, an aliquot of a diluted fecal specimen is transferred to a microassay well containing the Conjugate. If the antigen is present in the specimen, it will bind to the Conjugate and to the immobilized capture antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of the Substrate, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigen.

The assay kit contains the following:

1. Microassay Plate – 12 strips, each consisting of 8 wells coated with monoclonal antibodies to a Campylobacter-specific antigen (stored with dessicant)
2. Conjugate (7 mL) – Antibodies to a *Campylobacter*-specific antigen coupled to horseradish peroxidase in a buffered protein solution containing 0.05% ProClin® 300
3. Diluent (40 mL) – Buffered protein solution containing 0.05% ProClin® 300. The Diluent is also to be used as the negative control solution
4. Positive Control (3.5 mL) – Recombinant *Campylobacter*-specific antigen in a buffered protein solution containing 0.05% ProClin® 300
5. Stop Solution (7 mL) – 0.6 N sulfuric acid
6. Substrate (14 mL) – solution containing tetramethylbenzidine and peroxide
7. Wash Buffer Concentrate (50 mL) – 20X concentrate containing phosphate buffered saline, detergent, and 0.2% thimerosal
8. Accessories: 100 Disposable plastic transfer pipettes
   2 Plastic adhesive sheets
   1 Wash Solution Label
   50 Wooden Applicator sticks

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Premier CAMPY Enzyme Immunoassay (EIA)

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

   K083464

3. Comparison with predicate:

Table 1. Comparison of the *CAMPYLOBACTER CHEK* to Premier CAMPY EIA

Similarities		
Item	Device K173219	Predicate K083464
Indications for use	The *CAMPYLOBACTER CHEK* test is an enzyme immunoassay for the qualitative detection of a *Campylobacter*-specific antigen in human fecal specimens. The *CAMPYLOBACTER CHEK* test is designed to detect *C. jejuni* and *C. coli* from patients with symptoms of gastroenteritis.The test is intended for use with preserved fecal specimens in transport media and unpreserved human fecal specimens. Test results should be considered in conjunction with clinical findings and patient history.	Premier CAMPY enzyme immunoassay (EIA) is an *in vitro* qualitative procedure for the detection of specific Campylobacter antigens in stool samples from patients with signs and symptoms of gastroenteritis. Premier CAMPY detects *C. jejuni* and *C. coli* in human stool that may be either unpreserved or preserved in Cary Blair-based transport media. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures. Premier CAMPY is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.
Measured analyte	Detection of *Campylobacter*-specific antigens (*C. jejuni* and *C. coli*)	Same
Specimen Type	Fecal specimens in Cary-Blair and C&S Transport Media	Same
Type of Test	Qualitative	Same
Controls	Positive and negative control included in the kit	Same
Target Population	Persons suspected of having *Campylobacter* infection	Same
Storage (kit)	Refrigerated (2°C – 8°C)	Same
Reading Method	Visual, Spectrophotometric	Same

Differences		
Item	Device K173219	Predicate K083464
Time to Result	~1 hour	~2 hours
Antibody Format	Monoclonal/Polyclonal	Monoclonal/Monoclonal
Endpoint determinations (dual wavelength)	≥ 0.120	≥ 0.100
Endpoint determinations (single wavelength)	≥ 0.080	≥ 0.150

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

CLSI EP07-A2 Interference Testing In Clinical Chemistry; Approved Guideline - Second Edition

CLSI EP15-A3 User Verification of Precision And Estimation Of Bias; Approved Guideline - Third Edition

CLSI EP17-A2 Evaluation Of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition

L. Test Principle:

Enzyme-linked Immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the *CAMPYLOBACTER CHEK* test was determined using an eight member masked fecal specimen panel. The panel consisted of two negative, two high negative (just below C5), two low positive (1-2x higher than C95), and two moderate positive (2-3x higher than C95) specimens. Each fecal specimen was spiked using known concentration of *C. jejuni* whole organism to achieve the desired level. Testing was performed at two independent laboratories and on-site at TECHLAB, Inc. The Specimens were tested twice a day in triplicate over a five day period by multiple technicians at each site using two different kit lots. Positive and negative controls were run with each sample panel of masked specimens. The result from each laboratory were submitted to TECHLAB Inc. and compared. The results were consistent among all three locations with 100% reproducibility for all test panels.

b. Linearity/assay reportable range:

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The effect of specimen storage on antigen stability was evaluated for both fresh samples and samples in transport media. For the analysis, a total of 20 fecal specimens were tested with the *CAMPYLOBACTER CHEK* test. The fecal specimens consisted of 10 *C. jejuni* low positive fecal samples (1-2x higher than C95), 5 *C. jejuni* moderate positive fecal samples (2-3x higher than C95), and 5 *C. jejuni* high positive fecal samples (4-5x higher than C95). Both fresh samples and samples stored in Cary Blair and C&S transport media were tested.

For fresh samples, samples were stored between 2°C and 8°C and tested at 24 hour intervals up to 168 hours. At the same time positive and negative controls were tested. The following transport media were used for the study: Thermo Scientific *Protocol* Cary Blair media and Meridian Bioscience Inc., *Para-Pak* C&S. As recommended in the package insert, Cary Blair and C&S samples were stored between 20°C and 30°C. Samples stored in transport media were tested at 24 hour intervals up to 168 hours.

Positive samples remained positive throughout the study. Storage in transport media did not affect the stability of the samples. Based on these results, the recommended storage time for fresh samples is up to 96 hours between 2°C and 8°C, Cary Blair transport media is up to 96 hours between 20°C and 30°C and C&S transport media is up to 96 hours between 20°C-30°C based on the data and the package insert recommendation.

Freeze/Thaw:

Freeze/Thaw stability for raw specimens was demonstrated for six freeze/thaw cycles over a 9 day period. A panel consisting of 20 masked raw stool samples were prepared and tested in duplicate; three true negative, two high negative (1-2x below C5), five low positives (1-2x higher than C95), six moderate positives (2-3x higher than C95), and four high positives (4-5x higher than C95). All spiked samples yielded positive results after the freeze/thaw cycles. Based on the data submitted, the freeze-thaw cycle neither enhances nor impairs the performance of the *CAMPYLOBACTER CHEK* test.

d. *Detection limit:*

The limit of detection (LoD) for the *CAMPYLOBACTER CHEK* test was determined by spectrophotometry at both single (450 nm) and dual wavelength (450/620 nm) using whole organisms of both *C. jejuni* and *C. coli* spiked into unpreserved (raw stool) and preserved (Cary Blair and C&S media). The concentrations were calculated in colony forming units per milliliter (CFU/mL) and their equivalent in CFU/test by factoring in the dilutions and the final volume used in the assay. The LoD is the concentrations of organisms that yields a positive result 95% of the time and a negative result 5% of the time. The LoDs that were determined using single wavelength readings for both species were equivalent to those obtained using dual wavelength readings. See Table 2 for LoDs determined at dual wavelength.

Table 2. LoD for *C. jejuni* and *C. coli* Read at Dual Wavelength

C. jejuni		*C. coli*	
CFU/mL	CFU/test	CFU/mL	CFU/test
Fecal Matrix			
2.10×10^5	4,203	1.57×10^6	31,324
Cary Blair			
8.06×10^5	10,072	3.77×10^6	47,077
C & S			
5.09×10^5	6,357	5.36×10^6	66,974

e. Analytical specificity:

Cross-reactivity Study

The *CAMPYLOBACTER CHEK* test was evaluated for cross-reactivity with the bacteria and viruses listed below. *C. helveticus* and *C. upsaliensis* were found to be cross-reactive at 2 times the LoD of *C. coli* (3.14×10^6 CFU/mL). In addition, *C. lari* was found to be cross-reactive at 8 times the LoD of *C. coli* (1.26×10^7 CFU/mL). All other strains were shown to not interfere with the performance of the *CAMPYLOBACTER CHEK* test. Bacteria was spiked at concentration of $>10^8$ CFU/mL and viruses at a range from $10^{1.75}$ to $10^{8.25}$ $TCID_{50}$ units per 0.2 mL.

Bacteria

Acinetobacter baumannii
Aeromonas hydrophila
Bacillus cereus
Bacillus subtilis
Bacteroides fragilis
Campylobacter concisus
Campylobacter fetus
Campylobacter helveticus
Campylobacter hyointestinalis
Campylobacter lari
Campylobacter upsaliensis
Candida albicans
Citrobacter freundii
Clostridium bifermentans
Clostridium difficile
Clostridium perfringens
Edwardsiella tarda
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Escherichia coli EIEC
Escherichia coli EPEC
Escherichia coli ETEC
Escherichia coli O157:H7 (non-toxigenic)
Escherichia coli O157:H7 (toxigenic)
Escherichia fergusonii
Escherichia hermanii
Helicobacter pylori
Klebsiella pneumoniae
Lactobacillus acidophilus
Lactococcus lactis
Listeria monocytogenes
Peptostreptococcus anaerobius
Plesiomonas shigelloides
Porphyromonas asaccharolytica

Proteus vulgaris
Pseudomonas areuginosa
Salmonella enterica typhimurium
Shigella dysenteriae
Shigella sonnei
Staphylococcus aureus (Cowan's)
Streptococcus agalactiae
Yersinia enterocolitica
Prevotella melaninogenica
Pseudomonas fluorescens
Serratia marcescens
Shigella flexneri
Staphylococcus aureus
Staphylococcus epidermidis
Vibrio parahaemolyticus

Viruses

Adenovirus, 1, 2, 3, 5, 40, 41
Coxsackievirus B2, B3, B4, B5
Enterovirus 68, 69, 70, 71
Human Rotavirus
Coronavirus
Echovirus 9, 11, 18, 22, 33
Norovirus

Interfering Substances Study

The *CAMPYLOBACTER CHEK* test was evaluated for interfering substances with the substances and concentrations listed in Table 3. None of the substances where shown to interfere with the performance of the *CAMPYLOBACTER CHEK* test.

Table 3. Interfering Substances

Barium sulfate (5% w/v)	Benzalkonium Chloride (1% w/v)
Ciprofloxacin (0.25% w/v)	Ethanol (1% w/v)
Hog gastric mucin (3.5% w/v)	Human blood (40% v/v)
Hydrocortizone (1% w/v)	Imodium® (5% v/v),
Kaopectate® (5% v/v)	Leukocytes (0.05% w/v)
Maalox® Advanced (5% v/v)	Mesalazine (10% w/v)
Metronidazole (0.25% w/v)	Mineral Oil (10% w/v)
Mylanta® (4.2 mg/mL)	Naproxen Sodium (5% w/v)
Nonoxynol-9 (40% w/v)	Nystatin (1% w/v)
Palmitic Acid/Fecal Fat (40% w/v)	Pepto-Bismol® (5% v/v)
Phenylephrine (1% w/v)	Polyethylene glycol 3350 (10% w/v)
Prilosec OTC® (5 µg/mL)	Sennosides (1% w/v)
Simethicone (10% w/v)	Stearic Acid/Fecal Fat (40% w/v)
Tagamet® (5 µg/mL)	TUMS (50 µg/mL)
Human Urine (5% v/v)	Vancomycin (0.25% w/v)

Strain Reactivity Study

The sponsor tested the reactivity of the following *Campylobacter* stock cultures from different sources at 2x, 4x, and 8x the LoD of each species: *C. coli* strains 11283, 10956, 17755, 36994, 53138 and *C. jejuni* strains 11284, 6951, 12081, 29411, 38106, and 24567. All results were read at dual-wavelength and were positive with the exception of *C. coli* strain 53138 which was negative at 2x LoD but positive at 4x LoD.

f. Assay cut-off:

Interpretation of Results
Visual Reading
Negative = Colorless to very faint yellow
Positive = Definitive yellow color

Spectrophotometric Single Wavelength (450 nm)
Negative: <0.120
Positive: ≥0.120
Negative Control: <0.120
Positive Control: ≥0.500

Spectrophotometric Dual Wavelength (450/620 nm)
Negative: <0.080
Positive: ≥0.080
Negative Control: <0.080
Positive Control: ≥0.500

A positive result indicates that *Campylobacter* antigens were detected. A negative result indicates that no *Campylobacter* antigens were detected, or that the antigen levels are below what can be detected by the assay.

g. Prozone:

The purpose of this study was to demonstrate that a high concentration of *Camplobacter* (antigen) does not interfere with a positive reaction in the *CAMPYLOBACTER CHEK* test. High samples were prepared by spiking a negative fecal pool with 5 X 10^7 whole organisms of *C. jejuni* and 2.4 X 10^8 whole organisms of *C. coli*. A total of 5 different dilutions of *C. jejuni* and *C. coli* whole organisms, 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*.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Prospective Study

The performance of the *CAMPYLOBACTER CHEK* test was evaluated at four independent sites. The sensitivity and specificity for the *CAMPYLOBACTER CHEK* device was compared directly to culture results that were obtained from three of the sites. Prospective testing consisted of 1552 stool specimens tested in clinical microbiology laboratories at the Medical College of Wisconsin, Penn State Milton S. Hershey Medical Center, TriCore Reference Laboratories, and at TechLab, Inc. The ages of patients ranged from less than 1 year to 100 Years with, 15.7% of the specimens coming from patients that were ≤ 18 years. Of the 1552 patients tested 38.7% were female and 61.3% were male. No difference in test performance was observed based on patient age or gender. The results of the study show that the *CAMPYLOBACTER CHEK* test exhibited a sensitivity of 91.4%, and a specificity of 99.1% with culture. The results are shown in Table 4.

All samples with discrepant results were tested using an FDA cleared EIA assay, an FDA cleared molecular assay, in-house PCR testing (16s rRNA), and bidirectional sequencing. Of the three false negative results, one sample was confirmed to be negative using these additional tests.

Table 4. Clinical Perfomance of the CAMPYLOBACTER CHEK

	Culture Positive	Culture Negative	Total
CAMPYLOBACTER CHEK Positive	32	14*	46
CAMPYLOBACTER CHEK Negative	3**	1503	1506
Total	35	1517	1552
Sensitivity (95% C.I.)	91.4% (75.8% - 97.8%)		
Specificity (95% C.I.)	99.1% (98.4% - 99.5%)		

*Of the 14 culture negative and *CAMPYLOBACTER CHEK* test positive, 8 were confirmed to be positive with all follow-up testing, 2 were confirmed to be positive with commercial EIA, in-house PCR, and bidirectional sequencing, and 4 were confirmed to be positive for *C. upsaliensis* by species specific PCR and sequencing. Earlier analytical studies have shown that *C. upsaliensis* yields cross-reactive results with the *CAMPYLOBACTER CHEK*.

**One of the three specimens that were culture positive and *CAMPYLOBACTER CHEK* test negative was confirmed to be negative with all tests.

Retrospective Study

Additional testing was performed on 30 retrospective positive specimens. The patient ages ranged from less than 11 months to 74 years. All retrospective specimens were *Campylobacter* spp. culture positive and were further characterized as *Campylobacter*

spp. positive by an FDA-cleared commercial EIA assay, an FDA-cleared commercial molecular assay, in-house PCR (detecting the 16s rRNA gene of *Campylobacter* spp., and species-specific identification), and bidirectional sequencing. These specimens were then tested in the *CAMPYLOBACTER CHEK* test. All 30 specimens tested positive for *Campylobacter* spp. by all methods, yielding 100% correlation with all test methods

*b. Clinical specificity**:***

See section M3a. above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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