# *CAMPYLOBACTER CHEK*™

An Enzyme Immunoassay for the Qualitative Detection of a *Campylobacter*-Specific Antigen in Human Fecal Specimens
Catalog #T5052 (96 Tests)

IVD *In Vitro* Diagnostic Medical Device

## INTENDED 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 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.

**Caution**: **U.S. Federal Law restricts this device to sale by or on the order of a physician**

## EXPLANATION

Worldwide, *Campylobacter* species are the most common cause of bacterial gastroenteritis, with 400-500 million cases of diarrhea each year (1). Infants in developing countries are at even greater risk, as are travelers to those countries (2). *Campylobacter*–associated gastroenteritis is estimated to affect nearly 1 million people a year in the USA (3). In approximately 1 of 1000 cases, *Campylobacter jejuni* is closely linked to the subsequent development of Guillian-Barre Syndrome, an acute auto-immune paralysis (4). *C. jejuni* infection has also been associated with reactive arthritis in both children and adults (4, 5). When individuals with severe symptoms of gastroenteritis seek medical help, the clinician is faced with multiple possible causes that can present with similar clinical features (e.g., diarrhea, nausea, vomiting, fever, abdominal pain) but that require very different, often conflicting, types of treatment (4).

For *Campylobacter*, the current standard for identification is bacterial culture followed by microscopic examination of the organisms (6). Although this traditional method is straightforward, it has two major limitations. First, pathogenic species of *Campylobacter* are microaerophilic or strictly anaerobic, so that exposure of culture or feces to environmental oxygen leads to death or inactivation of the bacteria (7, 8). Thus, during transport or storage of specimens under aerobic conditions, the number of viable organisms can decrease, leading to potentially inaccurate culture results (9). Second, *Campylobacter* species are slow-growing, requiring from 48-72 hr before reaching a point where the culture can safely be reported as negative. Such delays can leave the clinician in a quandary and the patient with non-specific, ineffective, or even inappropriate treatment.

The *CAMPYLOBACTER CHEK*™ test allows detection of *Campylobacter jejuni* and *Campylobacter coli*, the species most commonly associated with human disease, in less than 60 minutes. Furthermore, the *CAMPYLOBACTER CHEK*™ test does not rely on bacterial viability, and can be performed on the bench-top with samples that have been exposed to air.

## PRINCIPLE OF THE TEST

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 *Substrate*, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of antigen.

## MATERIALS PROVIDED

***Microassay Plate*** – 12 strips, each consisting of 8 wells coated with monoclonal antibodies to a *Campylobacter*-specific antigen (stored with desiccant)

***Conjugate* (7 mL)** – Antibodies to a *Campylobacter*-specific antigen coupled to horseradish peroxidase in a buffered protein solution containing 0.05% ProClin® 300*

***Diluent* (40 mL)** – Buffered protein solution containing 0.05% ProClin® 300*. *Diluent* is also to be used as the negative control solution (see TEST PROCEDURE).

***Positive Control* (3.5 mL)** - *Campylobacter*-specific antigen in a buffered protein solution containing 0.05% ProClin® 300*

*Signal Word: Warning – 0.05% ProClin® 300
H317: May cause an allergic skin reaction
P261, P272, P280, P302, P352, P333, P313, P321, P362, P364, P501

***Stop Solution* (7 mL)** – 0.6 N sulfuric acid. CAUTION: Avoid contact with skin or eyes; flush with water immediately if contact occurs

Signal Word: Danger
H314: Causes severe skin burns and eye damage
P260, P264, P280, P301, P330, P331, P303, P361, P353, P363, P304, P340, P310, P321, P305, P351, P338, P501

***Substrate* (14 mL)** – solution containing tetramethylbenzidine and peroxide
***Wash Buffer Concentrate* (50 mL)** – 20X concentrate containing phosphate buffered saline, detergent, and 0.2% thimerosal

Signal Word: Warning
H373: May cause damage to organs through prolonged or repeated exposure P260, P314, P501

Contains mercury


**ACCESSORIES**

100 *Disposable plastic transfer pipettes* 2 *Plastic adhesive sheets* 1 *Wash Solution Label*
*50 Wooden Applicator sticks*

**MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED**

*Squirt bottle for wash reagent* *Vortex mixer* *Timer*
*950 mL distilled water for diluting wash reagent* *Discard container* *Absorbent paper*
*ELISA plate reader capable of reading at 450 nm, 450/620 nm, or 450/630 nm* *Gloves*
*Small tubes for dilution of fecal specimens (e.g., microcentrifuge tubes)*

**SHELF LIFE AND STORAGE**

The expiration date of this kit is given on the box label. Expiration dates for each component are listed on the individual labels. The kit should be stored between 2°C and 8°C and should be returned to the refrigerator as soon as possible after use.

**PRECAUTIONS**

1. Rx Only – Prescription Only
2. Each component in the kit should be inspected for any signs of leakage. Upon arrival, the kit should be inspected to ensure that components are not frozen or warm to the touch due to improper shipping conditions.
3. Reagents from different kits should not be mixed. Do not use a kit past the assigned expiration date.
4. Bring all components to ROOM TEMPERATURE BEFORE USE!
5. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
6. Caps and tips are color-coded; DO NOT mix or interchange!
7. When handling assay wells, avoid scratching the bottom of the wells as this may result in inaccurate absorbance readings.
8. Unused microwells must be placed back inside of the resealable pouch with the desiccant to protect them from moisture.
9. Hold reagent bottles vertically when dispensing to ensure proper drop size and correct volume.
10. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
11. All reagents, except for the *Wash Buffer Concentrate*, are supplied in ready-to-use bottles. Reagents can be dispensed directly from the dropper bottles or decanted for use with multichannel pipettes. If excess reagent has been decanted, the excess should be discarded. Do not pour back into the bottle. The *Substrate* should be stored in and used from the light-protected bottle in which it is supplied. If an aliquot is removed from the original bottle for any reason, do not return unused *Substrate* to the original bottle. The *Substrate* is light sensitive and should be protected from direct sunlight or UV sources.
12. Perform the washing procedure as directed to avoid high background reactions.
13. The test has been optimized for sensitivity and specificity. Do not deviate from the specified procedure. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test.
14. Fecal specimens may contain potentially infectious agents and should be handled at "Biosafety Level 2" as recommended in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories."
15. Handle specimens and used microassay wells as if capable of transmitting infectious agents. Do not place specimens or used microassay wells in trash. Wear disposable gloves when doing the test.
16. Reagents contain 0.05% ProClin® 300 as a preservative. Although the concentration is low, ProClin® 300 is known to be harmful. If skin irritation or rash occurs, get medical advice/attention. Take off contaminated clothing and wash it before reuse. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.
17. The 20X *Wash Buffer Concentrate* contains 0.2% Thimerosal as a preservative. Once diluted to normal use concentration this solution is classified as non-hazardous. The *Stop Solution* contains 0.6N sulfuric acid. Flush with water immediately if contact occurs. Take off contaminated clothing and wash it before reuse. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.
18. Follow your national, regional, and local ordinances accordingly for waste disposal regulations. Do not place in trash, dispose of as hazardous waste.

**PRELIMINARY PREPARATIONS**

**1. All reagents must be at room temperature prior to use in the assay.**

2. **Prepare *1X Wash Solution*.** The *Wash Buffer Concentrate* is supplied as a 20X concentrate (a precipitate may be noticed). It should be diluted to a total volume of 1 liter by adding 50 mL of the concentrate to 950 mL of distilled water. Label the bottle. Store any unused 1X *Wash Solution* between 2°C and 8°C for up to the expiration date of the kit.
3. **Assay Strip Preparation.** Each strip contains 8 wells coated with antibodies specific to *Campylobacter* antigen. Each specimen or control will use one of these coated wells. Determine the number of wells to be used. Avoid contact with the base of the wells. Assay wells not used must be returned to the foil pouch and carefully resealed with desiccant.

**COLLECTION, HANDLING, AND STORAGE OF FECAL SPECIMENS**

| Acceptable Sample Type |
|---|
| Fresh Fecal Specimen |
| Frozen Fecal Specimen |
| Specimen in Transport Media (C&S, Cary Blair) |

| Do Not Use |
|---|
| Fecal specimens in Formalin-based fixative (e.g., sodium acetate formalin, 10% formalin) |
| Fecal specimens in alcohol-based fixative (e.g., polyvinyl alcohol) |
| Concentrated Fecal Specimens |

1. Standard collection and handling procedures used in-house for fecal specimens are appropriate. Fresh fecal specimens should be collected in clean, leak-proof containers, stored between 2° and 8°C, and tested within 96 hours of collection. Specimens that cannot be tested within this time should be stored at ≤ -10°C. Fecal specimens that are stored frozen may be thawed up to 5 times. If using frozen specimens, thaw at room temperature.
2. Specimens in transport media should be stored according to the manufacturer's recommendations.
3. Storing fecal specimens in the *Diluent* is NOT recommended.
4. Set up and label one test tube for each sample as necessary.
5. **Add 200 µL *Diluent* to each tube for unpreserved specimens. For specimens in Cary Blair or C&S Transport media, add 100 µL *Diluent* to each tube.**
6. Obtain one disposable plastic transfer pipette (supplied with the kit) for each sample.

***Transfer Pipette***


7. **Mix all specimens and cultures thoroughly regardless of consistency - it is essential that the samples be evenly suspended before sampling.** For **fresh or frozen/thawed** specimens, using the disposable plastic transfer pipette, add 50 µL (first graduation mark) of fecal specimen to the tube containing *Diluent* and mix well. If the specimen cannot be pipetted, use an applicator stick to transfer approximately 0.05 g of feces. This is about the size of a cooked grain of rice (about 2mm in diameter). For specimens in **Cary Blair** or **C&S Transport media,** add 100 µL (second graduation mark) of fecal specimen to the tube containing *Diluent* and mix well.
8. Close each tube of diluted sample and mix thoroughly. Proper mixing can be achieved by vortexing for 5 – 20 seconds.
9. If using semi-automated or automated washing equipment, well-mixed specimens, once diluted, must be centrifuged (5000 x g for 10 minutes) to remove any particulate matter from the supernatant before transfer to assay wells.

**TEST PROCEDURE**

1. Bring all reagents and the required number of test strips to room temperature before use.
2. **Add 1 drop (50 µL) of *Conjugate* (red cap) to each well.** Gently mix the *Conjugate* in the bottle by inverting several times. Be sure to hold each bottle vertically when adding the drops. Use 1 well for each fecal specimen, 1 well for the *Positive Control* and 1 well for the negative control. Identification marks may be written directly on side of well.
3. **Using a transfer pipette, transfer 100 µL of diluted specimen (or supernatant from the centrifuged diluted sample if using automated washing equipment) to the assay well.** Add 1 drop (50 µL) of the *Positive Control* (black cap) to the positive control well and 100 µL of the *Diluent* (negative control) to the negative control well. Tap the sides of the plate to mix.
4. Cut the adhesive plastic sheet to the size necessary to cover the wells. **Cover the wells and incubate them at 37°C ± 2°C for 50 minutes.**
5. Shake out contents of assay wells into a discard pan.
6. **Wash each well using the 1X *Wash Solution* in a squirt bottle with a fine-tipped nozzle**, directing the *Wash Solution* to the bottom of the well with force. Fill the wells, and then shake the ***Wash Solution*** out of the well into a discard pan. Slap the inverted plate on a dry paper towel.
   Note: If using semi-automated or automated washing equipment, add 350 µL of 1X *Wash Solution* to each well. Wash for a total of 5 times.

7. Repeat step 6 four additional times using a dry paper towel each time. If any particulate matter is seen in the wells, continue washing until all the particulate matter is removed.
8. After washing, completely remove any residual liquid in the wells by striking the plate onto a dry paper towel until no liquid comes out. Dispose of paper towels and specimen containers properly. Wipe the underside of each well.
9. **Add 2 drops (100 µL) of *Substrate* (blue cap) to each well**. Gently tap the wells to mix the substrate. Incubate the wells at room temperature for 10 minutes. Gently tap the wells at 5 minutes.
10. **Add 1 drop (50 µL) of *Stop Solution* (yellow cap) to each well.** Gently tap the wells and wait 2 minutes before reading. The addition of the *Stop Solution* converts the blue color of the Substrate to a yellow color which can be quantitated by measuring the optical density at 450 nm on a microplate ELISA reader. The instrument should be blanked against air. If a dual wavelength reader is used, blank against air at 620 or 630 and read at 450 nm. Wipe the underside of each well before measuring the optical density. If an ELISA reader is unavailable, the test may be read visually in good light against a white background. Read within ten minutes after adding *Stop Solution.*

**QUALITY CONTROL**

1. Positive and negative controls must be run with each series of test specimens. The positive controls demonstrate that the assay is functioning properly for the detection of *Campylobacter* antigen in fecal specimens. The negative control demonstrates that the assay is not reacting nonspecifically.
2. Positive and negative controls must fall within their respective ranges (below) or the test results are not valid. If these results are not observed, call Technical Services.
   a) **Positive Control must be a visible yellow color**. If read on a spectrophotometer, the OD at 450 nm or using dual wavelength at 450/620 nm or 450/630 nm must be ≥ 0.500. Any well that gives a positive reading without visible color should be repositioned, wiped on the underside of the well, and read again.
   b) **Negative Control must be visually clear**. If read on a spectrophotometer, the OD at 450 nm must be <0.120. If read at 450/620 nm or 450/630 nm the absorbance must be < 0.080. If not, the test is invalid and should be repeated, paying attention to the wash procedure.
3. Visual readings must be taken in good light against a white background.

**INTERPRETATION OF RESULTS**

| | **Spectrophotometric Reading** | |
|---|---|---|
| | **Single Wavelength at 450 nm** | **Dual Wavelength at 450/620 nm or 450/630 nm** |
| **Negative Samples** | OD < 0.120 | OD < 0.080 |
| **Positive Samples** | OD ≥ 0.120 | OD ≥ 0.080 |

**Visual Interpretation**

The negative control well should be colorless or have only a faint yellow color (less than the 1+ yellow color according to the Visual Interpretation guide provided with the kit). The positive control well should give a visible yellow color. If these results are not observed, call Technical Services. A test sample is considered positive if it has an obvious yellow color when compared to the negative control well. It may be less yellow or more yellow than the color observed in the positive control well. A test sample is considered negative if the reaction is colorless or less yellow than the negative control well.

A positive result indicates *Campylobacter* antigen is present in the specimen. A negative result indicates that *Campylobacter* antigen is absent or the level is below the detection limit of the test.

**Limitations**

1. The *CAMPYLOBACTER CHEK*™ test is used to detect a *Campylobacter*-specific antigen in human fecal specimens. The test confirms the presence of antigen in feces and this information should be taken under consideration by the physician in light of the clinical history and physical examination of the patient.
2. Negative results should not definitively rule-out the presence of *Campylobacter* species in suspected patients. Levels of organism may be present in feces beneath the limit of detection for the *CAMPYLOBACTER CHEK*™ test, and therefore, if *Campylobacter* is suspected, alternative testing should be conducted.
3. 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.
4. Transferring too little specimen, or failure to mix and completely suspend the specimen in the *Diluent/Conjugate* mixture, may result in a false-negative test result. The addition of too much fecal specimen may cause invalid results.
5. 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.
6. The *CAMPYLOBACTER CHEK*™ test is qualitative. The intensity of the color should not be interpreted quantitatively.

7. 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.

**EXPECTED VALUES**

The *CAMPYLOBACTER CHEK*™ test detects the presence of a *Campylobacter*-specific antigen in human fecal specimens. Expected values for a particular population should be established by each laboratory, and will vary depending on local food safety practices, sanitation of water sources, country, and season of year (10). FoodNet, the U.S. Food-Borne Diseases Active Surveillance Network, reported an annual incidence of 13.45 per 100,000 population for *Campylobacter* infection between 1996 to 2012 (11). Globally, incidence rates can reach >400 per 100,000 (12, 13). Reported annual incidence rates in fecal samples submitted for testing range from 1-2% (14, 15). Higher incidence rates (up to 7%) are seen in the summer months and in preschool-aged children (10, 15).

**PERFORMANCE CHARACTERISTICS**

**Prospective Study**

The performance of the *CAMPYLOBACTER CHEK*™ test was evaluated at 4 independent sites. Prospective incoming fecal specimens were collected and tested by culture and the *CAMPYLOBACTER CHEK*™ test. The following table shows a summary of the clinical performance of the *CAMPYLOBACTER CHEK*™ test for all 4 sites combined. 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.

**Age and Gender Distribution**

Age information was available for 1552 patients. The ages ranged from less than 1 year to 100 years. Of the 1552 patients, 15.7% were ≤ 18 years. The gender identification was 38.7% females and 61.3% males. No difference in test performance was observed based on patient age or gender.

***CAMPYLOBACTER CHEK*™ test versus Culture**

| N = 1552 | Culture Positive | Culture Negative |
|---|---|---|
| ***CAMPYLOBACTER CHEK*™ Positive** | **32** | **14*** |
| ***CAMPYLOBACTER CHEK*™ Negative** | **3**** | **1503** |

| | | 95% Confidence Limits |
|---|---|---|
| **Sensitivity** | 91.4% | 77.6% - 97.0% |
| **Specificity** | 99.1% | 98.5% - 99.5% |

The 17 discrepant specimens were further characterized by additional testing at TECHLAB. This testing included an FDA-cleared commercial Microassay well EIA, an FDA-cleared commercial molecular test, in-house PCR (detecting the 16s rRNA gene of *Campylobacter* spp., and species-specific identification), and bidirectional sequencing.

* Eight of the 14 specimens that were culture negative and *CAMPYLOBACTER CHEK*™ test positive were confirmed to be positive with all tests.
Two of the 14 specimens that were culture negative and *CAMPYLOBACTER CHEK*™ test positive were confirmed to be positive with the commercial EIA, in-house PCR, and bidirectional sequencing.
Four specimens that were culture negative and *CAMPYLOBACTER CHEK*™ test positive were confirmed to be positive for *C. upsaliensis* (an important pathogen) by species-specific PCR and sequencing.

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

**Retrospective Study**

Supplemental 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 Microassay well EIA, an FDA-cleared commercial molecular test, 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.

**REPRODUCIBILITY**

The reproducibility of the *CAMPYLOBACTER CHEK*™ test was determined using 8 human fecal samples coded to prevent their identification during testing. Testing was performed at 2 independent laboratories and on-site at TECHLAB, Inc. The samples were tested twice a day over a 5-day period by multiple technicians at each site using 2 different kit lots. Positive and negative controls were run with each panel of the masked samples. The results from each laboratory were

submitted to TECHLAB, Inc. and compared with in-house results. The results were consistent among the different locations and exhibited a correlation of 100%. The samples produced the expected results 100% of the time.

**CROSS REACTIVITY**

The *CAMPYLOBACTER CHEK*™ test was evaluated for cross-reactivity with common intestinal organisms and viruses listed below. None of the organisms or viruses were shown to interfere with the performance of the *CAMPYLOBACTER CHEK*™ test.

| | | |
|---|---|---|
| *Acinetobacter baumannii* | *Aeromonas hydrophila* | *Bacillus cereus* |
| *Bacillus subtilis* | *Bacteroides fragilis* | *Campylobacter concisus* |
| *Campylobacter fetus* | *Campylobacter hyointestinalis* | *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* | *Prevotella melaninogenica* |
| *Proteus vulgaris* | *Pseudomonas aeruginosa* | *Pseudomonas fluorescens* |
| *Salmonella enterica typhimurium* | *Serratia marcescens* | *Shigella dysenteriae* |
| *Shigella flexneri* | *Shigella sonnei* | *Staphylococcus aureus* |
| *Staphylococcus aureus (Cowan's)* | *Streptococcus agalactiae* | *Staphylococcus epidermidis* |
| *Vibrio parahaemolyticus* | *Yersinia enterocolitica* | |

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

*Campylobacter* species that were shown to be reactive with the *CAMPYLOBACTER CHEK*™ test. *C. helveticus* (strain 54661) was found to be positive at 3.14 x $10^6$ CFU/mL (2 x LoD of *C. coli*), *C. lari* (strain 23947) was found to be positive at 1.26 x $10^7$ CFU/mL (8 x LoD of *C. coli*), and *C. upsaliensis* (strain 14913) was found to be positive at 3.14 x $10^6$ CFU/mL (2 x LoD of *C. coli*).

**INCLUSIVITY STUDY**

The specificity of the *CAMPYLOBACTER CHEK*™ test was evaluated using several strains of *Campylobacter jejuni* and *Campylobacter coli.* All strains listed generated positive results when tested.

*C. coli* CCUG strains: 11283, 10956, 17755, 36994, 53138*
*C. jejuni* sub-species *jejuni* CCUG strains: 11284, 6951, 12081, 29411, 38106
*C. jejuni* sub-species *doylei* CCUG strain: 24567

*Strain 53138 was positive at 4 x LoD.

**INTERFERING SUBSTANCES (U.S. FORMULATION)**

The following substances had no effect on positive or negative *CAMPYLOBACTER CHEK*™ test results analyzed at the concentrations indicated:

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), Hydrocortisone (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), Steric Acid/Fecal Fat (40% w/v), Tagamet® (5 µg/mL), TUMS® (50 µg/mL), Human Urine (5% v/v), and Vancomycin (0.25% w/v).

**PRECISION – INTRA-ASSAY**

For the determination of intra-assay performance, 8 fecal samples were analyzed by the *CAMPYLOBACTER CHEK*™ test. The samples included 2 negative, 2 high negative, 2 low positive, and 2 moderate positive samples. Each sample was assayed a total of five times using two different kit lots. Positive specimens consistently tested positive and negative and high negative specimens consistently tested negative. No difference was observed between the results for the single wavelength, dual wavelength and visual reading results. There was 100% agreement between the two kit lots.

**PRECISION – INTER-ASSAY**

For the determination of inter-assay performance, 8 fecal samples were analyzed by the *CAMPYLOBACTER CHEK*™ test. The samples included 2 negative, 2 high negative, 2 low positive, and 2 moderate positive samples. The samples were tested twice a day by multiple technicians over a 12-day period using 2 different kit lots. All positive samples remained

positive and all negative samples remained negative. Visual interpretation of results gave a correlation of 100% with spectrophotometric interpretation. Both kit lots exhibited a correlation of 100%.

**ANALYTICAL SENSITIVITY**

The analytical sensitivity of the test was determined by using *C. jejuni* and *C. coli* whole organism culture preparations in a sample matrix. The concentration of *C. jejuni* and *C. coli* organisms in fecal matrix at which specimens are positive by the *CAMPYLOBACTER CHEK*™ test 95% of the time is the assay Limit of Detection (LoD).

The Limit of Detection (LoD) for the *CAMPYLOBACTER CHEK*™ test with raw fecal samples was established at 2.10 x $10^5$ CFU/mL (4203 CFU/test) for *C. jejuni*. For specimens in Protocol™ Cary Blair media, the LoD was established at 8.06 x $10^5$ CFU/mL (10072 CFU/test) for *C. jejuni*. For specimens in Protocol™ C&S media, the LoD was established at 5.09 x $10^5$ CFU/mL (6357 CFU/test) for *C. jejuni*. The limits of detection are equivalent for both single and dual wavelength readings.

The Limit of Detection (LoD) for the *CAMPYLOBACTER CHEK*™ test with raw fecal samples was established at 1.57 x $10^6$ CFU/mL (31324 CFU/test) for *C. coli*. For specimens in Protocol™ Cary Blair media, the LoD was established at 3.77 x $10^6$ CFU/mL (47077 CFU/test) for *C. coli*. For specimens in Protocol™ C&S media, the LoD was established at 5.36 x $10^6$ CFU/mL (66974 CFU/test) for *C. coli*. The limits of detection are equivalent for both single and dual wavelength readings.

**PROZONE**

To ensure that a high concentration of *Campylobacter* antigen does not interfere with a positive reaction in the *CAMPYLOBACTER CHEK*™ test, high samples were prepared by spiking a negative fecal pool at a concentration possibly observed in clinical specimens. A total of 5 different dilutions of *C. jejuni* and *C. coli* whole organism culture preparation, up to and including the clinically observed high concentration, were prepared and tested in triplicate. The results demonstrated that there was no overall prozone effect, that elevated levels of antigen did not affect the detection of the antigen.

**REFERENCES**


1. Ruiz-Palacios, G. M. 2007. The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. Clin Infect Dis 44:701-703.
2. Kendall, M. E., S. Crim, K. Fullerton, P. V. Han, A. B. Cronquist, B. Shiferaw, L. A. Ingram, J. Rounds, E. D. Mintz, and B. E. Mahon. 2012. Travel-Associated Enteric Infections Diagnosed After Return to the United States, Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009. Clinical Infectious Diseases 54:S480-S487.
3. Friedman, C. R., R. M. Hoekstra, M. Samuel, R. Marcus, J. Bender, B. Shiferaw, S. Reddy, S. D. Ahuja, D. L. Helfrick, F. Hardnett, M. Carter, B. Anderson, R. V. Tauxe, and E. I. P. F. W. Group. 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin Infect Dis 38:S285-96.
4. Guerrant, R. L., T. Van Gilder, T. S. Steiner, N. M. Thielman, L. Slutsker, R. V. Tauxe, T. Hennessy, P. M. Griffin, H. DuPont, R. Bradley Sack, P. Tarr, M. Neill, I. Nachamkin, L. B. Reller, M. T. Osterholm, M. L. Bennish, and L. K. Pickering. 2001. Practice Guidelines for the Management of Infectious Diarrhea. Clinical Infectious Diseases 32:331-351.
5. Young, K. T., L. M. Davis, and V. J. Dirita. 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nature Reviews Microbiology 5:665-679.
6. Hurd, S., M. Patrick, J. Hatch, P. Clogher, K. Wymore, A. B. Cronquist, S. Segler, T. Robinson, S. Hanna, G. Smith, and C. Fitzgerald. 2012. Clinical Laboratory Practices for the Isolation and Identification of *Campylobacter* in Foodborne Diseases Active Surveillance Network (FoodNet) Sites: Baseline Information for Understanding Changes in Surveillance Data. Clinical Infectious Diseases 54:S440-S445.
7. Bessede, E., A. Delcamp, E. Sifre, A. Buissonniere, and F. Megraud. 2011. New Methods for Detection of *Campylobacters* in Stool Samples in Comparison to Culture. Journal of Clinical Microbiology 49:941-944.
8. Lastovica, A. J., and E. le Roux. 2000. Efficient Isolation of Campylobacteria from Stools. Journal of Clinical Microbiology 38:2798-2799.
9. Couturier, B. A., M. R. Couturier, K. J. Kalp, and M. A. Fisher. 2013. Detection of non-*jejuni* and -*coli Campylobacter* Species from Stool Specimens with an Immunochromatographic Antigen Detection Assay. Journal of Clinical Microbiology 51:1935-1937.
10. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. 2015. Global epidemiology of *Campylobacter* infection. Clin Microbiol Rev. 28:687-720.
11. Crim SM, Griffin PM, Tauxe R, Marder EP, Gilliss D, Cronquist AB, Cartter M, Tobin-D'Angelo M, Blythe D, Smith K, Lathrop S, Zansky S, Cieslak PR, Dunn J, Holt KG, Wolpert B, Henao OL; Centers for Disease Control and Prevention (CDC).2015. Preliminary incidence and trends of infection with pathogens transmitted commonly through food - Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2006-2014. MMWR Morb Mortal Wkly Rep. 64:495-499.
12. Fischer Walker CL, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. 2012. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC Public Health. 12:220-220.
13. Hall G, Yohannes K, Raupach J, Becker N, Kirk M. 2008. Estimating community incidence of *Salmonella*, *Campylobacter*, and Shiga toxin-producing *Escherichia coli* infections, Australia. Emerg Infect Dis. 14:1601-1609.
14. Hindiyeh M, Jense S, Hohmann S, Benett H, Edwards C, Aldeen W, Croft A, Daly J,Mottice S, Carroll KC. 2000. Rapid detection of *Campylobacter jejuni* in stool specimens by an enzyme immunoassay and surveillance for *Campylobacter upsaliensis* in the greater Salt Lake City area. Journal of Clinical Microbiology 38:3076-3079.
15. Nielsen HL, Ejlertsen T, Engberg J and Nielsen H. 2013. High incidence of *Campylobacter concisus* in gastroenteritis in North Jutland, Denmark: a population-based study. Clin Microbiol Infect 19: 445–450.


**Technical Support**


**Tel.: (540) 953-1664 (800) 832-4522 USA**
**Fax: (540) 953-1665**


**Developed and Manufactured By:**


**TECHLAB, Inc.**
**2001 Kraft Drive**
**Blacksburg, VA 24060-6358 USA**
Made in the USA