Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

CE

# AccuDiag™ Campylobacter (Fecal) ELISA Kit

**REF** 8320-3

**IVD** See External Label 2°C 8°C Σ 96 Tests

| Campylobacter (Fecal) ELISA | |
|---|---|
| Method | Enzyme Linked Immunosorbent Assay |
| Principle | Sandwich Complex |
| Detection Range | Qualitative Positive; Negative control |
| Sample | 1 gm stool sample |
| Specificity | 100% |
| Sensitivity | 77% |
| Incubation Time | 50 minutes |
| Shelf Life | 12 Months from the manufacturing date |

## PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

## INTENDED USE

This Diagnostic Automation Inc. microwell enzyme linked immunoabsorbant assay (ELISA) detection kit is an *in vitro* diagnostic immunoassay for the detection of antigen to *Campylobacter* species in human feces using peroxidase as the indicator enzyme. The assay may be read visually or with an ELISA reader. This ELISA is intended to be used with stools that are fresh or frozen.

## SIGNIFICANCE AND SUMMARY

Infection by thermophilic *Campylobacter* species is a leading cause of human gastroenteritis. Of the various species of *Campylobacter*, *C. jejuni*, *C. coli* and *C. lari*, are the species most often associated with human illness. *Campylobacter* are often passed to humans through the handling or consumption of contaminated food, particularly foods of animal origin.

Recently, human infection with *Campylobacter* has been implicated in the induction of Guillain-Barré Syndrome (GBS) and reactive arthritis. GBS is a debilitating and potentially fatal neurological disease that produces paralysis.

*Campylobacter* species are gram negative, motile curved or spiral rods that require highly specialized growth conditions. Typical cultivation entails pre-enrichment and enrichment steps in broth, followed by isolation on a selective solid medium. Of particular importance in the cultivation of *Campylobacter* is the requirement for a microaerobic atmosphere.

## ASSAY PRINCIPLE

The DAI *Campylobacter* ELISA is a double antibody (sandwich) immunoassay utilizing specific anti-*Campylobacter* antibodies coated to microwells. After addition of the sample and the enzyme conjugate, a positive reaction (indicating the presence of *Campylobacter* antigen) produces a deep blue color. Addition of the Stop Solution ends the assay and turns the blue color to yellow. The results may be read visually or with an ELISA reader.

## REAGENTS

**Materials provided with the test kit**

| Item | Description |
|---|---|
| Test Strips | Microwells containing anti-*Campylobacter* polyclonal antibodies: 96 Test Wells. |
| Enzyme Conjugate | One (1) bottle containing 11 ml of anti-*Campylobacter* antibodies conjugated to horseradish peroxidase with Thimerosal. |
| Positive Control | One (1) vial containing 2 ml of *Campylobacter* antigen in a buffered base. |
| Negative Control | One (1) vial containing 2 ml of buffered base. |
| Chromogen | One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB). |
| Wash Concentrate (20X) | Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal. |
| Stop Solution | One (1) bottle containing 11 ml of 5% phosphoric acid solution. |

**Materials Required But Not Provided**

1. Transfer Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Graduated Cylinder
4. Reagent grade (DI) water
5. Sample dilution tubes

**Suggested Equipment**

ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

## PRECAUTIONS

1. **Do not deviate from the specified procedures when performing this assay.**

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All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.

2. For In Vitro Diagnostic Use Only.
3. Do not interchange reagents between kits with different lot numbers.
4. Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
5. Unused microwells should be stored in the desiccated pouch to protect them from moisture.
6. Do not use solutions if they precipitate or become cloudy.
   **Exception**: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
7. Do not add azides to the samples or any of the reagents.
8. Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
9. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
10. Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
11. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

## STORAGE

Reagents, strips and bottled components: Store between 2-8°C. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

## REAGENT PREPARATION

1. Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
2. (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. ***Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration***. To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

## SPECIMEN COLLECTION

- Stools should be collected in clean containers.
- Unpreserved samples should be kept at 4°C and tested within 24 hours of collections. Samples that cannot be tested within this time should be frozen at -20°C until used. Freezing specimens does not adversely affect the test however, avoid repeated freeze/thaw cycles.
- All dilutions must be made with the diluted wash buffer.

## SAMPLE PREPARATION

**Fresh/Frozen Stools**

Thaw sample if needed. Prepare a 1:4 dilution in tubes using 0.3 ml of diluted wash buffer and one swab of fecal specimen (approximately 0.1 g). Coat swab with specimen and transfer into the diluted wash buffer, expressing as much liquid as possible and mix well. For watery specimens, add 0.1 ml of sample to 0.3 ml diluted wash buffer in tubes.
Note: Do not formalin fix samples prior to use.

## ASSAY PROCEDURE

**Proper Temperature**

All incubations are at room temperature (15 to 25°C)

**Test Procedure Notes:**

- Ensure all samples and reagents are at room temperature (15-25°C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided prediluted. DO NOT dilute further.

**Procedure**

1. Break off the required number of wells (number of samples plus 2) and place in strip holder.
2. Add 100 µl of the negative control to well #1
3. Add 100 µl of the positive control to well #2.
4. Add 100 µl of the test sample to the appropriate well.
5. Incubate at room temperature (15 to 25°C) for 30 minutes, then wash.* After last wash, slap wells out on a clean absorbent towel to remove excess wash buffer.
6. Add **100 µl** of Enzyme Conjugate to each well.
7. Incubate at room temperature for **15 minutes**, then wash.* After last wash, slap wells out on a clean absorbent towel to remove excess wash buffer.
8. Add **100 µl** of Chromogen to each well.
9. Incubate at room temperature for **5 minutes**.
10. Add **100 µl** of stop solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
11. Read results visually or using an ELISA plate reader (see instructions below).

**** Washings consist of vigorously filling each well to overflowing and decanting contents five (5) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.***

## RESULTS

**Interpretation of Results - Visual**

**Positive:** Any sample well that is obviously more yellow than the negative control well.
**Negative:** Any sample well that is not obviously more yellow than the negative control well.

***NOTE:*** The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result.

**Interpretation of Results – ELISA Plate Reader**

Zero reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.
**Positive:** Absorbance reading of 0.15 OD and above indicates the sample contains *Campylobacter* antigen.
**Negative:** Absorbance reading less than 0.15 OD indicates the sample does not contain detectable levels of Campylobacter antigen.

## PERFORMANCE CHARACTERISTICS

**Study #1**

A total of 28 stools were tested against culture. The following results were obtained.

| | Culture + | Culture - |
|---|---|---|
| **DAI ELISA +** | 10 | 0 |
| **DAI ELISA -** | 3 | 15 |

Sensitivity: 77% (10/13)
Specificity: 100% (15/15)

## ANALYTICAL SENSITIVITY

This assay can detect approximately $10^4$ to $10^5$ CFU per ml of feces.

## LIMITATIONS OF THE PROCEDURE

1. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
2. DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
3. A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for *Campylobacter*.

## EXPECTED VALUES

Normal healthy individuals should be free of *Campylobacter* and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of *Campylobacter* antigen. Incidence of *Campylobacter* infection varies significantly between populations, season of the year, and geographic regions. No expected prevalence level can be assumed.

## QUALITY CONTROL

The positive and negative controls must be included each time the kit is run. The use of a positive and negative control allows easy validation of the kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

**Troubleshooting**

**Problem:** Negative control has excessive color after development.
**Reason:** Inadequate washings
**Correction:** Wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

## REFERENCES

1. Beuchat, Larry, 1985. Efficacy of Media and Methods for Detecting and Enumerating *Campylobacter jejuni* in Refrigerated Chicken Meat. Appl and Environ Micro Vol. 50, No. 4 pp.934-939.
2. Nachamkin, Irving, 1997. Microbiologic Approaches for Studying *Campylobacter* Species in Patients with Guillain-Barré Syndrome. J of Infect Dis Vol 176 (Suppl 2) pp. S106-114
3. Stern, Norman J. et. Al. 19XX. Campylobacter – Chapter 29. Indicator Microorganisms and Pathogens. Pp. 475-495
4. Bolton, FJ and Robertson, L. 1982. A Selective medium for isolating *Campylobacter jejuni/coli*. J Clin Pathol Vol 35, pp. 462-467.
5. BAM 1998

## MANUFACTURER AND BRAND DETAILS

**ISO 13485:2016**

ISO 13485 Quality Management for Medical Devices CERTIFIED

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|---|---|
| **Date Adopted** | **2023-07** |
| **Brand Name** | **AccuDiag™** |
| **REF 8320-3** | **AccuDiag™ - Campylobacter (Fecal) ELISA** |
| **EC REP** | **CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu** |
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