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CE

Campylobacter Fecal Antigen

REF

Catalog No.E23-281

IVD

FOR INVITRO DIAGNOSTIC USE ONLY

Intended Use

Immunospec microwell enzyme linked immunoabsorbant assay (ELISA) detection kit is an *in vitro* diagnostic (IVD) 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.

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.

Principle of the Test

The *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

Item	Description
Test Strips	Microwells containing anti-*Campylobacter* polyclonal antibodies: 96 Test Wells.
Reagent 2	One (1) bottle containing 11 ml of anti-*Campylobacter* polyclonal antibody conjugated to peroxidase with red dye and preservative.
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 1 M phosphoric acid.

Precautions

Do not use solutions if they precipitate or become cloudy. Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
Do not add azides to the samples or any of the reagents. Some reagents contain a preservative.
Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
Though and complete washing steps is critical to proper performance of the test.

Storage Conditions

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

Reagent Preparation

Wash Buffer - 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.

Sample Preparation

Fresh or frozen (unpreserved) fecal samples may be used. The assay has not been validated on preserved specimens.
If the sample is a free flowing liquid, add two drops of the sample directly to the test wells. If the sample is solid or semi-solid, add just enough wash buffer (the diluted Wash Concentrate) to produce a free flowing liquid, then add two drops of the sample to the appropriate test well.

Test Procedure

1. Break off the required number of wells (number of samples plus 2 and place in strip holder.
2. Add 100 µl (2 drops) of the negative control to well #1 and 100 µl of the positive control to well #2.

3. Add 100 µl (2 drops) of the test sample to the appropriate well.
4. Incubate at room temperature (15 to 25 °C) for 30 minutes, then wash.*
5. Add 2 drops of Enzyme Conjugate (red solution) to each well.
6. Incubate for 15 minutes, then wash.
7. Add 2 drops of Chromogen to each well.
8. Incubate for 5 minutes.
9. Add 2 drops of stop solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
10. Read results visually or at 450/620-650 nm.

* Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) and using the diluted wash buffer to fill in each well, shaking out the contents and refilling the wells for a total of three to five times. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

Only one set of controls is required per run.
Read results within 4 hours from addition of Stop Solution.
All incubations are at room temperature (15-25 °C).

Interpretation of Results - Visual

Positive: Any sample well that has significant and obvious yellow color.
Negative: Any sample well that does not have significant and obvious yellow color.

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 - OD Readings

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

Positive: Absorbance reading of 0.15 or greater.
Negative: Absorbance reading less than 0.15.

Specific Performance Characteristics

Study #1

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

	Culture +	Culture -
Immunospec ELISA +	10	0
Immunospec ELISA -	3	15

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

Analytical Sensitivity

This assay can detect approximately 104 to 105 CFU per ml of feces.

Quality Control

The Positive and Negative Controls must be run each time the assay is performed.

For a valid run, the Negative Control must be below 0.10 ODs and the Positive Control greater than 0.5 OD units. If either Control is out of range, do not use the kit and contact Immunospec Research's Technical Service at (818) 717-1840.

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

References

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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

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