Campylobacter
ELISA [Enzyme-Linked ImmunoSorbent Assay]

REF **7060**

Qualitative assay for the determination of antigen to Campylobacter species in human stool samples

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BIOMERICA

I. INTENDED USE

The Biomerica *Campylobacter* ELISA is intended for the qualitative detection of antigen to *Campylobacter* species in human fecal specimens. This assay is intended FOR *IN VITRO* DIAGNOSTIC USE ONLY.

II. SUMMARY AND EXPLANATION

Campylobacter is a gram-negative, spiral-shaped bacterium which can cause disease in humans and animals. Two species of *Campylobacter*, *C. jejuni*, and *C. coli*, are the species most often associated with human illness, or campylobacteriosis.

Most people who become ill with campylobacteriosis get diarrhea, cramping, abdominal pain, and fever within two to five days after exposure. The diarrhea may be bloody, and can be accompanied by nausea and vomiting. The illness typically lasts for about one week. In immunocompromised persons, *campylobacter* occasionally spreads to the bloodstream, causing a potentially life-threatening situation.

Campylobacteriosis usually occurs in single, sporadic cases, but it can also occur in outbreaks where a number of people get sick at the same time. Most individual cases are associated with eating raw or undercooked poultry, or from cross-contamination of other foods by raw or undercooked poultry.[1] Outbreaks are generally associated with unpasteurized milk or contaminated water.[2] Animals can become infected, and contact with the stool of an infected animal can become a mechanism for spreading infection. Arthritis and Guillain-Barre Syndrome (GBS) have been linked to human infection with *campylobacter* on rare occasions.[3,4]

Typical cultivation methods require fresh stool samples and entail 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.[5,6] The development of ELISA tests specific for *Campylobacter* antigens in stool samples eliminates the need for fresh samples as well as shortens the time-to-result by days.

III. PRINCIPLE OF THE TEST

The Biomerica *Campylobacte*r ELISA is an enzyme immunoassay which detects *Campylobacter* antigens in stool samples. Specific anti-*Campylobacter* antibodies have been immobilized on breakaway microwells. Diluted patient specimens are added to the microwells along with horseradish peroxidase-conjugated antibodies to *Campylobacter*. If Campylobacter antigens are present in the sample, they will bind to the detecting antibody and the immobilized antibody to form a complex, which will remain in the microwell after washing to remove unbound enzyme. After washing, a substrate/chromogen is added which develops a blue color in the presence of the enzyme complex. The stop solution ends the reaction and turns the blue color to yellow.

IV. KIT COMPONENTS

Kit Components	Description	Symbol
Test Strips	96 microwells containing anti-*Campylobacter* polyclonal antibodies.	**PLA**
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-*Campylobacter* polyclonal antibody conjugated to horseradish peroxidase with thimerosal added as preservative.	**CONJ ENZ**
Positive Control	One (1) vial containing 2 ml of *Campylobacter* antigen in a buffered base.	**CTRL +**
Negative Control	One (1) vial containing 2 ml of buffered base.	**CTRL -**
Substrate Solution	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	**SUBS TMB**
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal.	**BUF WASH 20X**
Stop Solution	One (1) bottle containing 11 ml of 5% phosphoric acid.	**STOP**

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader (optional)
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Micropipettes capable of delivering 100 µL.
- Transfer Pipettes.
- Wash Bottle.
- Absorbent Paper.
- Timer
- Reagent grade (DI) water.
- Waste container with disinfectant or biohazard bags.
- Sample Dilution Tubes.
- Parafilm or cover for microwell plate
- Applicator Stick (recommended) or swabs for sample preparation.

V. WARNINGS AND PRECAUTIONS FOR USERS

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- All reagents are for *In Vitro* Diagnostic Use Only.
- Reagents from different kit lots should not be interchanged.
- Do not use reagents that are beyond their expiration dates.
- All reagents should be at room temperature before using.
- If using dropper bottles, hold them vertically to ensure proper drop size.
- Wear gloves when performing the test, and handle specimens and used microwells as if able to transmit infectious agents.
- Unused microwells should be stored in the resealable pouch with dessicant to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy. **Exception**: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- 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 large amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any sample spill.
- Stop solution is a 5% solution of phosphoric acid in water. In case of contact with skin or mucous membranes, flush with water immediately.

VI. SAMPLE COLLECTION AND STORAGE

Stool samples may be used as unpreserved or frozen only. No modification of collection techniques used for standard bacterial examinations is needed. Unpreserved samples should be kept at 2-8°C and tested within 24 hours of collection, if possible. Samples that cannot be tested within 72 hours of collection should be frozen at -20°C or lower until used. Multiple freeze/thaw cycles should be avoided. Samples that are preserved in 10% Formalin, Merthiolate Formalin, Sodium Acetate Formalin, or Polyvinyl Alcohol have not been validated on this assay, and should not be used.

VII. REAGENT PREPARATION AND STORAGE

1. All reagents except the Wash Concentrate are ready-to-use. All reagents should be stored at 2-8° C. The Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
2. Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
3. Wash Concentrate: To prepare a 1X wash solution, add contents of one bottle of Wash Concentrate (25 ml) to 475 mL of distilled or deionized water and mix.

VIII. SAMPLE PREPARATION

1. Prepare sample dilutions in tubes using **300 µl** of diluted wash buffer and approximately **100 mg**, about the size of a small pea (~4mm diameter), of fecal sample using an applicator stick. Mix thoroughly before using.
   -IF USING SWABS, add **600 µl** of diluted wash buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.
2. **For watery, unpreserved specimens**, mix contents, then add **100 µl** of sample to **300 µl** of diluted wash buffer in dilution tubes. Mix thoroughly before using.

NOTES:

- If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.
- All dilutions must be made with the diluted wash buffer.

IX. ASSAY PROCEDURE

NOTES:

- All incubations are at room temperature (15-25°C).
- When running the assay, try to avoid the formation of bubbles in the wells. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder. Return any unused wells to foil pouch with desiccant. Reseal pouch tightly to exclude moisture.
2. Using a micropipette, add **100 µl (or 2 drops)** of the negative control to well #1.
3. Using a micropipette, add **100 µl (or 2 drops)** of positive control to well #2.
4. Add **100 µl** of diluted sample to the appropriate test wells.

NOTE: Place the opening of the transfer pipette just inside the well to avoid splashing into adjacent wells.

5. Cover the plate with parafilm or an appropriate cover and incubate at room temperature for **30 minutes.**
6. Decant the contents of the assay wells.
7. 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 well, and then decant the wash solution out of the well. Repeat this step four more times for a total of five washes.

NOTE: If using semi-automated or automated washing equipment, the specimens must be centrifuged (2000 to 3000 g x 10 minutes) to remove any particulate matter prior to adding to the plate. Add 350 µL of 1X wash solution to each well. Wash a total of five times.

8. Add **100 µl (or 2 drops)** of Enzyme Conjugate to each well.
9. Cover the plate and incubate at room temperature for **30 minute**s.
10. Decant the contents of the wells and wash each well five times as in **Step 6-7**. *
11. Add **100 µl (or 2 drops)** of Substrate Solution to each well.
12. Cover the plate and incubate at room temperature for **10 minutes**.
13. Add **100 µl (or 2 drops)** of Stop Solution to each well. Gently tap the wells to mix. Read reaction within 5 minutes after adding Stop Solution.
14. Read results visually or on a spectrophotometer having a dual wavelength, with the filters set at 450nm and 620-650nm

**** Washings consist of vigorously filling each well to overflowing with diluted wash buffer and decanting contents, banging the wells on a clean absorbent towel after each wash.***

X. CALCULATION OF RESULTS

Interpretation of Results – Visual Method

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 - Spectrophotometric Method:

Zero spectrophotometer against air**. Read all wells at 450/620-650 nm.**

Positive: Absorbance reading of 0.15 O.D. units and above indicates the sample contains campylobacter antigen.
Negative: Absorbance reading less than 0.15 O.D. units indicates the sample does not contain detectable levels of campylobacter antigen.

XI QUALITY CONTROL

Positive and negative controls must be included each time the assay is run.

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

XII. LIMITATION OF PROCEDURE

- Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
- Do <u>not</u> concentrate stool samples. Assay will not give accurate results on a concentrated sample.
- Inadequate washings may lead to the negative control having excessive color development. Care should be taken to perform wash steps in the manner described.
- 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.

XIII. PERFORMANCE CHARACTERISTICS

Clinical Evaluation

A total of 64 stool specimens were tested on the Biomerica *Campylobacter* ELISA and a reference ELISA for comparison. The clinical profile for the Biomerica *Campylobacter* ELISA is shown below.

		Reference *Campylobacter* ELISA		
		POS	NEG	TOTAL
BIOMERICA *Campylobacter* ELISA	POS	20	0	20
	NEG	1	43	44
	TOTAL	21	43	64

Accuracy	98%
Sensitivity	95%
Specificity	100%

In a combined study 200 stool specimens were tested on the Biomerica *Campylobacter* ELISA. 33 of the 200 samples tested were confirmed positive by culture. 167 of the 200 samples tested were confirmed negative by culture The clinical profile for the Biomerica *Campylobacter* ELISA is shown below.

		Culture		
		POS	NEG	TOTAL
BIOMERICA *Campylobacter* ELISA	POS	31	1	32
	NEG	2	166	168
	TOTAL	33	167	200

Clinical Data	Results
Accuracy	99%
Sensitivity	94%
Specificity	99%

Analytical Sensitivity

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

Precision and Reproducibility

The precision (Intra-assay variation) of the Biomerica *Campylobacter* ELISA test was calculated from 24 replicate determinations on each of the two control samples.

Intra-Assay

Sample	Mean O.D	N	% CV
Control 1	1.2	24	3.5%
Control 2	1.5	24	5.6%

The reproducibility (Inter-assay variation) of the Biomerica *Campylobacter* ELISA test was calculated from data on two control samples obtained in 12 different assays by three technicians on two different lots of reagents.

Inter-Assay

Sample	Mean O.D	N	% CV
Control 1	1.1	12	9.1%
Control 2	1.4	12	8.4 %

Cross-Reactivity

The following bacterial strains at a concentration greater than 10^6 cell/mL were spiked into a confirmed negative fecal specimen. No cross-reactivity to the below bacterial strains was observed in the Biomerica *Campylobacter* ELISA.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Negative Sample - No Spike	N/A	0.024
Escherichia coli serotype 055:k59 (B5) - Spike	12014	0.018
Escherichia coli serotype 0111:K58 (B4) - Spike	33780	0.030
Escherichia coli serotype 0111:NM - Spike	43887	0.022
Escherichia coli serotype 0124.NM - Spike	43893	0.022
Escherichia coli serotype 0157.H7 - Spike	43890	0.014
Salmonella typhimurium - Spike	SA972229	0.020
Shigella sonneii - Spike	25931	0.029

Interference

The following bacterial strains at a concentration greater than 10^6 cell/mL were spiked into a confirmed positive fecal specimen. No interference to the below bacterial strains was observed in the Biomerica *Campylobacter* ELISA, as all positive samples remained positive.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Positive Sample - No Spike	N/A	0.458
Escherichia coli serotype 055:k59 (B5) - Spike	12014	0.476
Escherichia coli serotype 0111:K58 (B4) - Spike	33780	0.461
Escherichia coli serotype 0111:NM - Spike	43887	0.499
Escherichia coli serotype 0124.NM - Spike	43893	0.435
Escherichia coli serotype 0157.H7 - Spike	43890	0.436
Salmonella typhimurium - Spike	SA972229	0.454
Shigella sonneii - Spike	25931	0.472

XIV. 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. Beumer, R.R., Cruysen, J.J., Birtantie, I.R. (1988) The occurrence of Campylobacter jejuni in raw cow's milk. J. Appl. Bacteriol 65: 93-96.
3. 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.
4. Rees, J.H., Soudain, S.E., et.al. 1995. Campylobacter jejuni infection and Guillain-Barre syndrome. N. Engl. J. Med. 333: 1374-1379.
5. Steinbruckner, B., Harter, G., Pelz, K., Kist, M. (1999) Routine identification of Campulobacter jejuni and Campylobacter coli from human stool samples. FEMS Microbiol. Lett 179: 227-232.
6. Bolton, FJ and Robertson, L. 1982. A Selective medium for isolating Campylobacter jejuni/coli. J Clin Pathol Vol 35, pp. 462-467.

XV. SYMBOLS

	Storage Temperature
LOT	Lot Code
	Expiration
	Manufacturer
EC REP	Authorized Representative
	Caution, see instructions
IVD	For in vitro diagnostic use
REF	Catalog No.

XVI. ORDERING INFORMATION

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IVD

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EC REP

according to IVDD 98/79/ EC
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