*E. coli O*157
ELISA [Enzyme-Linked ImmunoSorbent Assay]

REF **7066**

Qualitative assay for the determination of
Escherichia coli O157 antigen in human stool samples

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BIOMERICA

I. INTENDED USE

The Biomerica E. *coli* O157 ELISA is intended for the qualitative detection of *E. coli* O157 antigen in human fecal specimens. The assay is designed as a screening tool to allow rapid determination of the presence of *E. coli* O157 bacteria without prior culturing of the stool specimen. All samples which are positive in the ELISA test should be cultured and serotyped to confirm the presence of O157 and its H antigen type.
This assay is intended for *IN VITRO* DIAGNOSTIC USE ONLY.

II. SUMMARY AND EXPLANATION

Escherichia coli are a large and diverse group of gram-negative bacteria found in normal human flora. While most strains are harmless, certain strains produce a toxin known as Shiga toxin, and these strains are called Shiga toxin-producing *E. coli*, or STEC, for short. The most common STEC is *E. coli* O157:H7, which has been implicated in a wide spectrum of human diseases, including bloody and non-bloody diarrhea, hemolytic uremic syndrome (HUS), kidney failure, and hemorrhagic colitis (HC). [1,2,3,4]

The symptoms of STEC infections vary considerably from case-to-case, but often include stomach cramps, diarrhea, vomiting, and mild fever. About 5-10% of people diagnosed with STEC infection develop HUS, which is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure, which can cause permanent damage or death. HC typically presents with abdominal cramps and watery diarrhea followed by a hemorrhagic discharge resembling lower gastrointestinal bleeding.[1,3,5,6]

STEC infections start when bacteria are ingested through consumption of contaminated food or water, unpasteurized milk, contact with cattle, or contact with the feces of infected animals or humans.[7,8,9,10,11] People have become infected by swallowing lake water while swimming, eating undercooked beef, touching the environment in petting zoos, or by eating foods prepared by people who did not wash their hands properly after using the toilet. Because there are so many possible causes, most people never learn what the source of the infection was. However, about 20% of cases are associated with a recognized outbreak, and the health department may determine the source.

Typically detection of *E. coli* O157 has been accomplished by culturing on sorbitol-MacConkey medium or broth amplification, followed by confirmatory testing using typing antiserum or latex assays. However, current latex assays and some typing antisera have shown cross reactions with non-*E. coli* O157 colonies.[12,13,14] In addition, the culture methods usually take 24-48 hours and require a skilled microbiologist in identifying colonies.

It has been recommended by the Council of State and Territorial Epidemiologists that clinical laboratories screen at least all bloody stools for this pathogen.[11] The American Gastroenterological Association Foundation (AGAF) has also recommended that all stool specimens should be routinely tested for *E. coli* O157:H7.[15] The Biomerica *E. coli* O157 ELISA provides a rapid and convenient screening option to identify positive specimens for culture and serotyping.

III. PRINCIPLE OF THE TEST

The Biomerica *E. coli* test is an enzyme immunoassay which detects *E. coli* antigen in stool samples. An antibody to *E. coli* antigen has been immobilized on breakaway microwells. Diluted patient specimens are added to the microwells along with horseradish peroxidase-conjugated antibody to *E. coli* antigen. If *E. coli* antigen is present in the sample, it 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 rabbit anti-*E. coli* polyclonal antibodies in a test strip holder.	**PLA**
Enzyme Conjugate	One (1) bottle containing 11 ml of polyclonal anti-*E. coli* O157 antibody conjugated to horse radish peroxidase in a buffer with red dye and Thimerosal.	**CONJ ENZ**
Positive Control	One (1) vial containing 2 ml of inactivated *E. coli* O157 cells in a buffered base.	**CTRL +**
Negative Control	One (1) vial containing 2 ml of dilution buffer.	**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 surfactant with Thimerosal.	**BUF WASH 20X**
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	**STOP**

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader (Optional)
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Micropipette 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.
- Graduated Cylinder
- 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 desiccant 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 spills.
- Stop solution is a 5% solution of phosphoric acid in water. In case of contact with skin or mucous membranes, flush with water immediately.
- If formalin-preserved samples are used, a fresh sample must also be collected so culturing may be done if the sample is reactive in the assay.

VI. SAMPLE COLLECTION

Since the organism may only be shed for a period of a few days (studies have shown that the level of detectable organisms by culture drop dramatically after day 6), the sample should be taken as soon as possible. It is recommended that the clinician check with their state health department or the Centers for Disease Control and Prevention to determine which specimens should be tested and whether the results are reportable.

Collection of Stool (Feces)

Stool samples may be used as fresh, frozen, in Cary Blair media, or in preservation media of 10% buffered formalin. Formalin samples should be collected as per the manufacturer's instructions (approximately a 1:4 dilution in the container). Unpreserved samples should be kept at 4 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20°C until used.

Samples in Cary Blair must be frozen once before using. This will break up the agar matrix and free up the antigen.

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

Fresh/Frozen Stools

Thaw frozen stools. Prepare a slurry of stool by adding approximately **0.1 g (100 mg)**, about the size of a small pea (~4 mm diameter), of fecal sample using an applicator stick to **300 µl** of diluted wash buffer. Mix well (vortex) and allow the heavy particulates to settle.

For Watery Unpreserved Specimens

Mix contents then add **100 µl** of sample to **300 µl** of diluted wash buffer. Mix thoroughly before using. Make the same dilution of the sample regardless of it consistency.

Cary Blair Transport Stools

Freeze sample or aliquot of sample. Thaw and mix contents. Add **100 µl** of sample to **300 µl** of diluted wash buffer. Mix thoroughly before using.

Formalin Preserved Stools

No further preparation of the sample is needed. However, remember that confirmation of the result by culturing cannot be performed on formalin preserved samples.

All dilutions must be made with 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** of the negative control to well #1.
3. Using a micropipette, add **100 µl** 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 two more times for a total of three 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 three times.

8. Add **100 µl (or 2 drops)** of Enzyme Conjugate (red solution) 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 three 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 *E. coli* O157 antigen.

Negative: Absorbance reading less than 0.15 O.D. units indicates the sample does not contain detectable levels of *E. coli* O157 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 read at greater than 1.0 O.D. when read at a dual wavelength of 450/620-650 nm.
- The positive control included in the kit is a high reactive control. The laboratory may also want to include an in-house positive control that is closer to the cut-off value.

XII. LIMITATIONS OF PROCEDURE

- Do not 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.
- Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. This assay is designed to be a rapid presumptive screen for the presence of *E. coli* O157 regardless of H (flagellar antigen) or verotoxin production.
- A negative result can occur from an antigen level lower than the detection limits of this assay. Seeding studies have shown this assay to have a minimum number of organisms (MOI) level of 3,000 to 11,000 CFU/ml depending upon the strain of *E. coli* O157 present.
- Persons infected with this organism may only shed this detectable level in the first 3-6 days after onslaught of symptoms. A positive result in this ELISA indicates that the O157 LPS antigen has been detected in the sample. This LPS antigen is found on *E. coli* O157 organisms regardless of their H antigen status and verotoxin production. This LPS antigen is also found on *Salmonella urbana* (030). Thus, a positive sample in this ELISA should be cultured and serotyped to determine the exact genus, species and serotype present. A verotoxin cell assay using the isolate will help determine if the organism is producing verotoxins.
- Although actual stool specimens have shown no loss of reactivity when stored at -20ºC for 2 years, the effects of prolonged storage could potentially affect the assay.
- Performance of this assay on patients that have received antibiotic treatment prior to sample collection has not been fully investigated. Some antibiotics have the potential to destroy the cell wall component that is detected in this assay.

XIII. PERFORMANCE CHARACTERISTICS

Clinical Evaluation

A total of 101 stool specimens were tested on the Biomerica E. coli O157 ELISA. 72 of the 101 samples tested were confirmed negative by culture using SMAC medium. 29 of the 101 samples tested were confirmed positive by culture using SMAC medium. The clinical profile for the Biomerica *E. coli* O157 ELISA is shown below.

		Culture		
		POS	NEG	TOTAL
BIOMERICA *E.coli 0157* ELISA	POS	29	0	29
	NEG	0	72	72
	TOTAL	29	72	101

Accuracy	100%
Sensitivity	100%
Specificity	100%

XIV. REFERENCES

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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

Contact: 
BIOMERICA, INC.
17571 Von Karman Avenue
Irvine, CA 92614 USA

Toll Free (800) 854-3002 Within the U.S.
Phone: (949) 645-2111
FAX: (949) 553-1231
E-Mail: bmra@biomerica.com

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IVD

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MDSS GmbH
Schiffgraben 41
D-30175 Hannover, Germany