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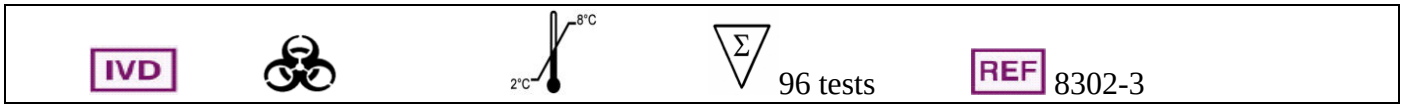

E.Coli O157

REF **8302-3**

Test	**E.coli O157 ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**ELISA - Sandwich; Antibody Coated Plate**
Detection Range	**Qualitative Positive; Negative control**
Sample	**1 gm stool sample**
Specificity	**100%**
Sensitivity	**92%**
Total Time	**~ 100 min**
Shelf Life	**24 Months**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended Use

The DAI *E. coli* O157 Antigen Detection ELISA is an *in vitro* procedure for the qualitative determination of *Escherichia coli* O157 antigen in feces. The assay is designed as a screening tool to allow rapid presumptive determination of the presence of *E. coli* O157 bacteria without prior culturing of the stool specimen. All positive samples in the ELISA should then be cultured and serotyped to confirm the presence of O157 and its H antigen type.

Summary

E. coli O157, predominately O157:H7, has been implicated as the causative organism of hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC). HUS syndrome is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure while HC typically presents with abdominal cramps and watery diarrhea followed by a hemorrhagic discharge resembling lower gastrointestinal bleeding.[1,8,11,12,14]

Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antiserum have shown cross reactions with non-*E. coli* O157 colonies. Furthermore, not all *E. coli* O157 strains associated with HUS are nonsorbitol fermentors.[2,3,5,7]

Both syndromes have been associated with *E. coli* O157 and O157:H7 but are not limited to these organisms. These syndromes appear to be more closely linked to any organism that produces Shiga-like toxins.[9,10,13]

The *E. coli* O157 outbreaks are believed to be passed by the fecal oral route and has been isolated in an increasing number of food related poisonings involving undercooked beef and dairy products.[4,6]

The Council of State and Territorial Epidemiologists recommend that clinical laboratories screen at least all bloody stools for this pathogen.[17] The American Gastroenterological Association Foundation (AGAF) recommended in July 1994 that all stool specimens should be routinely tested for *E. coli* O157:H7.[15] 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.[15]

Principal of Procedure

This assay is a double antibody (sandwich) ELISA using an anti-*E.coli* O157 antibody to capture the antigen from the stool supernatant. A second antibody, conjugated to peroxidase (HRP), is then added which binds to the complex. This reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *E. coli* O157 antigens being bound by the anti-*E. coli* O157 antibodies.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing rabbit anti-*E. coli* O157 polyclonal antibodies – 96 test in a test strip holer.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of goat anti-*E. coli* O157 polyclonal antibody conjugated to peroxidase with red dye and Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of *E. coli* O157 cells in a buffered base.	CONTROL +
Negative Control	One (1) vial containing 2 ml of dilution buffer.	CONTROL −
Chromogen	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.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

Materials Provided

E. coli O157 Antigen Detection ELISA Kit

Materials Required But Not Provided

Transfer Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Reagent grade (DI) water
Graduated cylinder

Suggested Equipment

ELISA Reader with a 450 and 620-650 nm filters.

Warnings and 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.

Controls and some reagents contain Thimerosal as a preservative.
Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
Do not mix components from different kit lots.
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.

Storage Conditions

Reagents, strips and bottled components: Store between 2 – 8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature. The diluted wash buffer should be used within one year.

Preparation

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate 20X to a squeeze bottle containing 475 ml of DI water. Swirl to mix. pH should be in the range of 7.0 to 7.4. Squeeze bottle should have a narrow tip to optimize washings.

Test Samples

Although the majority (estimated at 90%) of *E. coli* O157 infections are associated with at least one episode of bloody diarrhea, any abnormal specimen may contain *E. coli* O157. [16]

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.[1,14] 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.
All dilutions must be made with diluted wash buffer.

Preparation of sample:

Fresh/Frozen Stools

Thaw frozen stools. Prepare a slurry of stool by adding approximately 1 gram of stool to 3 ml of buffer. Mix well (vortex) and allow the heavy particulates to settle. Make the same dilution of the sample regardless of its consistency.

Cary Blair Transport Stools

Freeze sample or aliquot of sample. Thaw and make a dilution as with fresh/frozen stools (see above).

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.

Test Procedure

All procedures are performed at room temperature (15 to 25 °C).

1. Break off the required number of wells needed (number of samples plus 2) and place in strip holder.
2. Add 2 drops (100 µl) of the negative control to well #1 and 2 drops (100 µl) of the positive control to well #2 (use both as undiluted).
3. Add 2 drops (100 µl) of the test stool supernatant to the appropriate well.
4. Incubate for 30 minutes, then wash.*
5. Add 2 drops of Enzyme Conjugate (red solution) to each well.
6. Incubate for 30 minutes, then **wash three times with DI water**.
7. Add 2 drops of Chromogen to each well.
8. Incubate for 10 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 within 1 hour. Zero the ELISA reader on air.

* Each washing consists of using the diluted wash buffer to fill to overflowing in each well, shaking out the contents and refilling the wells for a total of 3 to 5 times. Stools with sticky particulate matter and/or mucous may require more thorough washing than other stools. The potential exists for false positive results if the stool is not thoroughly washed from the well before addition of subsequent reagents.

Only one set of controls is required per run.

Interpretation of Results - Visual

Reactive: Any sample well that has significant and obvious yellow color. A slight tinge of yellow color in the well is not sufficient color development to call the sample reactive.
Non-reactive: 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 reactive result. Please refer to the visual interpretation card if needed.

Interpretation of Results - OD Readings

Reactive: OD readings of 0.15 and above.
Non-reactive: OD readings of less than 0.15.
Equivocal Zone: OD readings in the range of 0.15 to 0.30. Samples with readings in this range should be repeated. Alternately, a second sample may be taken or a different methodology used (such as culture).

Test Limitations

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.[1,14] 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 must 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 -20º C 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.

Performance of this assay on formalin preserved samples has not been fully investigated. The positive control will fail if the stability of the kit is compromised.

Expected Results

Normal healthy individuals should be free of *E. coli* O157 and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of antigen. This antigen shedding may only occur for only a few days (3-6 days is typical) after start of symptoms.[1,14]

Quality Control

The Positive and Negative Controls must be run each time the assay is performed. 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.
For a valid run, the Negative Control must be within the range of 0.00 to 0.14 OD units and the Positive Control greater than 1.0 OD units. If either Control is out of range, do not use the kit.

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.15 OD units. Should the value fall below this limit, the kit should not be used.

Performance Characteristics

Study #1 – vs. SMAC
N=174

		SMAC	
		+	-
DAI	+	11	0
	-	1	162

Sensitivity – 11/12 = 92%
Specificity – 162/162 = 100%

Troubleshooting

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

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