Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

CE

AccuDiag™ E. Coli Verotoxin (Fecal) ELISA Kit

REF 8303-3

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

E. Coli Verotoxin 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	99%
Sensitivity	100%
Total Time	100 minutes
Shelf Life	12 Months

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

**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* Verotoxin Detection ELISA is an *in vitro* procedure for the qualitative determination of verotoxin in feces. It is a double antibody (sandwich) ELISA using anti-verotoxin antibodies to capture the antigen from the stool supernatant. A second anti-verotoxin monoclonal antibody cocktail is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color following the addition of the chromogen indicates the presence of verotoxin being bound by the anti-verotoxin antibodies.

SIGNIFICANCE AND SUMMARY

The verotoxin (VT), or shiga-like toxin family is a group of closely related toxins produced by certain strains of Escherichia coli.[4] These strains are a significant cause of human hemorrhagic colitic (HC) and hemolytic uremic syndrome (HUS).[1,3,7-8,12] In addition, they are both water borne and food borne and may also be transmitted from person-to-person by the oral-fecal route.[2] In adults, illness caused by verotoxin may last several days, while in children and the elderly, the illness can be fatal.[2,11]

Infections occur as sporadic illnesses or outbreaks, and are distributed throughout North and South America, Europe, and Asia.[4,11] The peak incidence of infection occurs during summer months.[4]
In the past, lengthy and cumbersome diagnostic procedures have been utilized for the detection of verotoxin.[3-5,8,10] Currently, simple to perform enzyme-linked immunosorbent assays (ELISA) are being used as an alternative to these longer tedious methods.[5-6,9-10] These antigen-detection assays provide a cost effective tool for the management of diarrheal disease with an accurate and rapid test.[6,9-10]

ASSAY PRINCIPLE

During the first incubation, verotoxin present in the stool supernatant is captured by antibodies attached to the wells. The second incubation adds an additional anti-verotoxin antibody cocktail that "sandwiches" the antigen. The next incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

REAGENTS

Materials provided with the test kit

Item	Description
Test Strips	Microwells rabbit anti-verotoxin (VT1 &VT2) antibodies- 96 test wells in a test strip holder.
Reagent 1	One (1) bottle containing 11 ml of monoclonal anti-verotoxin antibodies in a buffer with Thimerosal.
Reagent 2	One (1) bottle containing 11 ml of an anti-mouse antibody conjugated to horseradish peroxidase in a buffer with Thimerosal.
Positive Control	One (1) vial containing 2 ml of a verotoxin positive antigen in buffer.
Negative Control	One (1) vial containing 2 ml of a buffer.
Chromogen	One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.

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Materials Required But Not Provided
Pipettes
Squeeze bottle for washing strips
Reagent grade (DI) water
Graduated cylinder

Suggested Materials
ELISA reader capable of reading bichromatically at 450/620-650 nm

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.

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/Dilution Buffer
Remove cap and add contents of one bottle to 475 ml DI water. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle).

SPECIMEN COLLECTION AND PREPARATION

Collection of Stool (Feces)
Stools should be collected in clean containers.
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. Freezing the specimens does not adversely affect the test; however, avoid repeated freeze/thawing.
All dilutions must be made with the diluted wash buffer.

Preparation of Sample
Fresh/Frozen stools
Thaw frozen stools. Prepare a 1:4 dilution of stool by adding 1 gram (approximately the size of a pea) to 3 ml of diluted wash buffer. Mix well and allow the heavy particulates to settle.
For diarrheal stools, a lower dilution (1:2) may be used.

ASSAY PROCEDURE

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the stool supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash.*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 30 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 30 minutes, then wash. **Rinse each well three times with DI water.**
9. Add 2 drops of Chromogen to each well.
10. Incubate at room temperature for 10 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

*Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps. Controls must be included each time the kit is run.

RESULTS

Interpretation of Results - Visual
Reactive: Any sample well that has distinct and substantial yellow color.
Non-reactive: Any sample well that does not have distinct yellow color.

NOTE: The negative control, as well as some samples, may show some slight color.

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

Reactive: Absorbance reading of 0.15 and above indicates the sample contains verotoxin antigen.
Non-reactive: Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of verotoxin antigen.

LIMITATIONS OF THE ASSAY

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

EXPECTED RESULTS

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

QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

PERFORMANCE CHARACTERISTICS

Study #1 – vs. SMAC

N=110

		SMAC	
		+	-
DAI	+	12	2
	-	2	94

Sensitivity – 12/14 = 86%
Specificity – 94/96 = 98%

Study #2 – vs. another ELISA

N = 110

		Other ELISA	
		+	-
DAI	+	12	1
	-	0	97

Sensitivity – 12/12= 100%
Specificity – 97/98 = 99%

TROUBLESHOOTING

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

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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REF 8303-3	**AccuDiag™ - E. Coli Verotoxin (Fecal) ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu**
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