SafePath™
LABORATORIES, LLC.

E. coli O157 Microwell ELISA

For the rapid detection of *E. coli* O157 in food samples

Directions For Use

For Laboratory Use Only

"When given the choice...
take the SafePath"

Catalog Code: ECM-96 (96 Test)

Supplying the World with Quality Diagnostics for
Food Safety & Animal Health

Intended Use

The SafePath™ *E. coli* O157 assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of *E. coli* O157 antigen.

Summary

Strains of *Escherichia coli* that produce Shiga-like toxins (SLTs), also known as Verocytotoxins (VTs), are an important cause of human disease. Clinical manifestations of infection include diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Although Shiga-like toxin producing *E. coli* (SLTEC) belong to many O serogroups, serotypes O157:H7 and O157:H are the predominant SLTEC associated with HC and HUS. (Riley et al, 1983; Karmali 1989; Griffin and Tauxe 1991).

Outbreaks of HC and HUS due to *E. coli* O157 have been linked to consumption of undercooked ground beef (Riley et al, 1983; Wells et al 1983; Padhye and Doyle, 1992). Prevalence studies indicate that up to 3.7% of retail meats may contain *E. coli* O157 (Doyle and Schoeni 1987). Although this may seem low compared to some other foodborne pathogens, the seriousness of disease caused by *E. coli* O157 has made this pathogen a major food safety concern.

Detection of *E. coli* O157:H7 in meats by cultural methods is time consuming, requiring several days to obtain presumptive positive results. Typically, meat samples are cultured in primary enrichment broth which is plated onto sorbitol-MacConkey (SMAC) agar. Non-sorbitol fermenting colonies are then subcultured for identification by serotyping or related methods. As some *E. coli* O157 strains associated with HC and HUS ferment sorbitol (Karch et al 1993), this approach may give false negative results.

The SafePath™ *E. coli* O157 ELISA is a rapid and reliable test which significantly reduces the time required to screen foods for the presence of *E. coli* O157. Primary enrichment cultures grown for 8-16 hours can be tested in less than one hour, allowing ELISA-negative product to be released within 24 hours. Enrichment broths presumptively positive for *E. coli* O157 on the basis of positive ELISA tests can be cultured further for confirmation by standard methods.

Principle of the Test

The SafePath™ *E. coli* O157 ELISA is a double antibody (sandwich) ELISA utilizing specific anti-*E. coli* O157 antibodies coated to microwells. After addition of the sample and the enzyme conjugate, a positive reaction (indicating the presence of *E. coli* O157 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

- Microwell test strips containing anti-*E. coli* O157 polyclonal antibodies: 96 Test Wells
- Test strip holder: One (1)
- Enzyme Conjugate: One (1) bottle containing 11 ml of peroxidase conjugated anti-*E. coli* O157 polyclonal antibody with red dye and a Preservative.
- Positive control: One (1) vial containing 1 ml of killed *E. coli* O157 cells in a buffered base.
- Negative control: One (1) vial containing 1 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 surfactant with preservative.
- Stop solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Additional Materials Required:

- Stomacher (Tekmar stomacher lab-blender 400) or blender
- Shaking or similar incubator
- Microelisa plate reader capable of reading bichromatically at 450/650 nm (optional)
- Incubator, 37 °C
- Pipetter, 100 µl
- Disposable micropipette tips

Microbiological media and antibiotics for preparation of necessary enrichment broths and plating media:

Novobiocin (Sigma N1628)
Modified EC broth (BBL #11187 or Difco #0314-01-0)

- Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
- Data record sheets
- Disinfecting Solution

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.

Storage Conditions

Reagents, strips and bottled components: Store between 2 – 7 °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.

Medium Preparation

Modified EC broth with Novobiocin (mEC+n)

1. Combine the following components with 1 liter of distilled water (if prepared media is not used):

Tryptone	20.0 g	
Lactose	5.0 g	
K_2HPO_4	4.0 g	
KH_2PO_4	1.5 g	
NaCl	5.0 g	
Bile Salt #3	1.5 g	pH adjusted to 6.9 ± 0.1

2. Autoclave at 121°C for 15 minutes.
3. Allow to cool to room temperature and add 1 ml of a filter sterilized aqueous solution of 20 mg/ml Novobiocin. (for 225 ml add 0.225 ml of Novobiocin solution.) Final concentration should be 20 µg/ml.

Sample Preparation

1. Add 225 ml of mEC+n to 25 g food product in a sterile stomacher bag or blender jar.
2. Stomach or blend sample and broth for 2 minutes.
3. Transfer stomacher bag to shaker at 37 °C or alternatively transfer contents of blender jar to a sterile flask and attach to shaker.
4. Incubate stomacher bags or flasks at 37 °C with shaking (120 rpm) for 18 hours.
5. Remove a 1 ml aliquot from each sample and place in a separate clean screw top test tube. This is the sample that will be used in the assay.

Test Procedure

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 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 30 minutes, then wash*. **Rinse wells one time with DI water**. Slap out excess fluid against an absorbent towel.
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.

* 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 to overflowing in each well, shaking out the contents and refilling the wells for a total of 3 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

Positive: OD readings of 0.20 and above.
Negative: OD readings of less than 0.20.

Test Limitations

Seeding studies have shown this assay to have a limit of detection of approximately 1,000 CFU/ml, depending on the strain of *E. coli* O157 tested.
Enrichment is required for 16-18 hours for optimum growth of the *E. coli* O157. Meats inoculated with less than 1 CFU/g of *E. coli* O157 were consistently positive in the ELISA after 18 hours of enrichment.

The following organisms were tested in seeding studies for reactivity in this assay. The following results were obtained:

E. coli O157:NM (+)	*Aeromonas hydrophila* (-)
E. coli O157:H19 (+)	*Brucella abortus* (phenol) (-)
E. coli O157:H12 (+)	*Citrobacter freundii* (-)
E. coli 026:H11 (-)	*Enterobacter cloacae* (-)
E. coli 055 (-)	*Hafnia alvei* (-)
E. coli 088:H49 (-)	*Pseudomonas aeruginosa* (-)
E. coli 091:21 (-)	*Salmonella urbana* (030) (wk)
E. coli 0111:NM (-)	*S. typhimurium* (-)
E. coli 0163:NM (-)	*Xanthomonas maltophilia* (-)
E. hermanii (-)	*Yersinia enterocolitica* 09 (-)

Salmonella urbana gave a positive result in this ELISA. This organism is rarely found in cattle.
Positive results were obtained in the ELISA, as expected, for non-Verotoxin producing *E.coli* O157 strains.
No interfering substances which can give erroneous results have been identified. However, media other than those listed must be qualified before use in this ELISA.

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 0.12 ODs and the Positive Control greater than 1.0 OD units. If either Control is out of range, do not use the kit and contact SafePath Laboratories Technical Service at (760) 929-7744 or info@safepath.com.

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

References

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