An Immunomagnetic separation method for the isolation of *Escherichia coli* O157 from culture

E. coli O157-IMS "SEIKEN"

For 100 tests

INTENDED USE

E. coli O157-IMS "SEIKEN" is an immunomagnetic separation (IMS) method for the concentration of *Escherichia coli* O157 from enrichment cultures prepared from food, environmental and clinical specimens for more efficient and rapid isolation rates.

SUMMARY AND EXPLANATION

Enterohemorrhagic *Escherichia coli* (EHEC) and in particular the O157 serotype are a class of diarrheagenic *E. coli* characterized by their ability to produce toxins highly cytopathic for Vero cells and which closely resemble the Shiga toxin of *Shigella dysenteriae* type 1. The toxins have thus been variously referred to as both verocytotoxins (VTs) and Shiga-like toxins (SLTs). As EHEC typically reside in the intestinal tract of both farm and wild animals, infections typically result from the ingestion of undercooked meat, unpasteurized milk, contaminated vegetables, water, etc., as well as through person-to-person contact and with animal feces, such as during visits to farms and petting zoos.

E. coli O157:H7 is by far the most important serotype and has caused several large, well-publicized outbreaks in the US, Canada, Scotland and Japan, resulting in many cases of hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) and in some cases, death, especially in young children and the elderly. While *E. coli* O157 is typically identified by its sorbitol-negative phenotype, ie, colorless colonies on sorbitol MacConkey agar (SMAC), unlike most serotypes of *E. coli*, which are sorbitol positive and thus form pink colonies afterovernight growth, it is not uncommon that competing microbial flora make it difficult to detect *E. coli* O157 in foods and clinical specimens after direct SMAC plating, especially when inital levels are low and/or the cells are heat or acid-stressed, etc. Accordingly, immunomagnetic separation (IMS) technology employing specific antibodies to the O157 LPS molecule have emerged as a useful method to increase isolation and detection rates, especially in combination with enrichment culture methods. *E. coli* O157-IMS "SEIKEN" was developed expressly for this purpose and use a highly specific monoclonal antibody to the O157 LPS molecule.

PRINCIPLE

The magnetic beads in this kit are sensitized with a monoclonal antibody specific to the *E. coli* O157 LPS antigen and will, when mixed with an enrichment culture containing *E. coli* O157, react to form a beads-bacterial complex which can subsequently be concentrated by application of a magnetic force.

REAGENT

2.5 ml x 1 vial

A suspension of magnetic particles sensitized with a monoclonal antibody specific against the *E. coli* O157 LPS molecule. Contains sodium azide as a preservative (0.08 w/v %).

WARNING AND PRECAUTION

This product is for in vitro diagnostic use only in properly accredited testing laboratories. As the reagent is prepared from biologic materials and specimens intended for use with this kit are potentially infectious, proper handling and disposal methods should be established and only personnel adequately trained in such methods should be permitted to perform the procedures. Specimens should be handled at a proper biosafety level according to local regulations.

SHELF LIFE AND STORAGE

The reagent is stable at 2 - 10℃ until the expiry date written on the label. The reagent should not be frozen or otherwise exposed to extreme temperatures.

SPECIMEN HANDLING

In general, standard microbiological procedures should be followed.

TEST PROCEDURE

1. Materials and reagents necessary for the test

1) *E. coli* O157-IMS

The following materials are required separately:

2) Sterile Tryptic Soy Broth (TSB) recommended, but sterile PBS or saline are acceptable
3) Magnetic rack, pipette, micropipette, tips, 1.5 ml centrifuge tube

2. Reagent preparation

Reagent is ready for use.

3. Sample preparation

Culture food, environmental specimen, stools, etc. according to officially accepted or recommended methods.

PROCEDURE

1) Add 1 ml of culture into the microcentrifuge tube.
2) Add one drop of IMS (25 ul) reagent into the tube.
3) Cap the tube and mix well by gently inverting the tube a few times.
4) Leave at room temperature for 30 minutes, mixing at 10 minute intervals.
5) Place the tube in the magnetic rack for five minutes to collect the magnetic beads. After approximately 2, 3 and 4 minutes, gently tilt the rack to its side and back to the original upright position a few times to allow the beads to collect more efficiently at one point of the tube.
6) Carefully remove the supernatant with a micropipette without disturbing the magnetic beads, ie, while the microcentrifuge tube still in the magnetic rack.
7) Remove the tube from the magnetic rack and add 1 ml of sterile TSB (or PBS or sterile saline) and resuspend the beads.
8) Repeat steps 5 - 6 to wash the beads.
9) Add 0.1 ml of sterile TSB (or PBS, etc.) and resuspend the beads and plate on appropriate solid agar media (TC-SMAC, etc.).

NOTES FOR HANDLING

1. General precautions

1) After enrichment culture of specimen, potentially infectious *E. coli* O157 may be present in large numbers so appropriate care should be taken when performing the IMS procedure. In particular, care should be taken to prevent aerosol formation when opening the microcentrifuge tube during the IMS procedures, ie, by surrounding the cap with an alcohol-soaked cottonball, performing the procedure in biosafety hood, etc., and it should be kept in mind that there are reports of EHEC infection to laboratory personnel while handling specimens.
2) The sensitive detection and isolation of *E. coli* O157 depends on their ability to grow during the culture enrichment step. Recovery will be reduced if the cells have been stressed by heat, acid treatment, etc. as well as if their starting cell numbers relative to contaminating microbial flora are low.

   Note: In general, two culture conditions are recommended before performing the IMS step: a 6 hour non-selective and an 18 hour selective culture enrichment.

2. Cautions in use and handling

1) Mix the reagent well to form a homogenous solution before use by gently swirling the contents of the vial and/or by inverting the vial a few times.
2) Do not freeze the reagent.
3) Allow the reagent to come to room temperature by placing on the bench top for 30 minutes before use.
4) Do not mix or interchange reagents from different lots.
5) Use only according to the concentration method undicated in this insert.
6) Do not use the reagent vial for other purposes.
7) Do not use the reagent for purposes other than described in this insert.

3. Cautions for waste handling

1) This reagent contains 0.08 w/v% sodium azide. As sodium azide may produce explosive heavy metal azides by reaction with lead or copper piping, etc., the reagent should by disposed of flushing with copious amounts of water.
2) Materials and equipment used in this test should be sterilized by one of the following methods and disposed of according to local waste disposal laws and regulations:
   I. Soaking in 0.08% sodium hypochlorite solution (chloride content of about 1000 ppm) for more than one hour.
   II. Soaking in 2% glutaraldehyde soultion (final concentration) for more than 1 hour
   III. Autoclaving at 121℃ for more than 20 minutes.

SELECTED REFERENCES

1. **Wasteson, Y. and V. Naess.** 1998. Detection of *E. coli* O157 from the Environment, Food and Faeces; Immunomagnetic separation and practical lab tips. Concerted Action CT98-3935, Verocytotoxigenic *E. coli* in Europe, 1. Methods for Verocytotoxigenic *E. coli.* p 64 - 66.
   Conclusions:
   I. When using a 6 hr pre-enrichment method prewarm broth to 37℃ before culture
   II. TSB was recommended as the enrichment broth for dairy products
   III. Enriched cultures from foods high in lipid content may need to be diluted 1:2 before performing IMS
   IV. It is important to thoroughly spread the IMS beads-bacteria complex when plating

2. **Tilburg, J. H. C., O. Creemers, H. van der Zee, A. Heeuvelink and E. de Boer.** 2000. Comparison of methods for the detection and Isolation of E. coli O157 from foods. VTEC2000, Kyoto, Japan, Poster session.
   Conclusions:
   I. mTSB + n (6hr, 37℃ or 41℃) culture followed by IMS and plating on CT-SMAC or CHROMagar O157 supplemented with cefixime and tellurite recommended
   II. Indicates above as ISO 16654 method (proposal), including recommendation for second isolation method

3. **Ogden, I. D., N. Hepburn and M. MacRae.** 2000. Optimising immunomagnetic separtion tech niques for detecting low numbers of *E. coli* O157 in foods. VTEC2000, Kyoto, Japan, Poster session.
   Conclusions:
   I. Proposes enrichment in BPW-V (6hr, 42℃) and plating on both CT-SMAC and CHROMagar O157

4. **Karch, H., C. Janetzki-Mittmann, S. Aleksic, and M. Datz.** 1996. Isolation of enterohemorrhagic *E. coli* O157 strains from patients with hemolytic-uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. J. Clin. Microbiol. **34**:516-519.