invitrogen™

Cat. no. 710.11

Rev. no. 002

Dynabeads® EPEC/VTEC O103

For rapid selective concentration of *Escherichia coli* serotype O103 using the BeadRetriever™

For research use only.

INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* serotypes other than O157 VTEC are emerging as important human pathogens although their disease-causing abilities as enteropathogenic *E. coli* (EPEC) in animals have been recognised long ago. Illnesses caused by non-O157 VTEC infections can range from self-limiting watery diarrhoea, bloody diarrhoea or haemorrhagic colitis (HC) to life-threatening manifestations such as haemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP). Non-O157 VTEC infections may be associated with consumption of animal products, although knowledge of their incidence throughout the entire food chain is limited.

In general, the detection of non-O157 VTEC is not widely practised in most microbiological laboratories worldwide. Few laboratories are able to detect non-O157 strains. This is primarily because many non-O157 VTEC strains lack the phenotypic characteristics of O157 VTEC, such as delayed fermentation of sorbitol and the haemolytic activity on haemolysin agar, and therefore cannot be identified on the routinely used modified sorbitol-MacConkey agar, CT-SMAC. Some strains of *E. coli* O103 exhibit increased susceptibility to cefixime and tellurite in CT-SMAC and do not seem to grow on this medium. However, Immunomagnetic Separation (IMS) using Dynabeads EPEC/VTEC O103 represents a physically selective concentration procedure needed to improve the isolation and detection of the organisms from diverse sample matrices. The performance of this product is improved significantly by using the BeadRetriever; the automated IMS instrument that removes all the major problems associated with manual IMS and assures the safety of test performers.

PRINCIPLE

Dynabeads EPEC/VTEC O103 is designed for rapid, selective concentration of *E. coli* serotype O103 directly from a pre-enriched sample aliquot using the BeadRetriever™. Dynabeads, wash buffers and samples are loaded into the tube-strips provided. All incubations and washing steps are carried out automatically in the instrument. During the incubation process the antibodies coated onto the beads will specifically bind the target bacteria. Washing of the beads is achieved by transferring the bead-bacteria complexes through a series of tubes before resuspending into the final tube for further processing to detect and/or isolate the target organisms.

PRODUCT DESCRIPTION

Dynabeads EPEC/VTEC O103 are uniform, superparamagnetic, polystyrene microscopic beads with purified antibodies against *E. coli* O103 covalently bound to the surface. The beads are supplied in a suspension of phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide (NaN_3).

INTENDED USER

Any laboratory skilled in using conventional microbiological techniques, equipped and/or certified to do pathogen testing on food, feed and environmental samples, may use Dynabeads EPEC/VTEC O103.

The user must be skilled in using conventional microbiological techniques and in interpreting results.

SAMPLE MATRIX

Any food, water, feed and environmental samples that has been pre-enriched for 24 hours in Buffered Peptone Water (BPW) at 42°C can be used for automated IMS (AIMS) with Dynabeads EPEC/VTEC O103.

Environmental samples include swab streaks of surfaces and containers and faecal material of animal or human origin. A water sample is defined as any source water for potable supply. Food is defined as material intended for use in human consumption and feed is defined as material used for animal consumption.

ANALYTE

Dynabeads EPEC/VTEC O103 specifically reacts with all strains of *E. coli* O103 serotypes of both human and animal origin.

INTERPRETATION CRITERIA

Since strains of E. coli O103 possess no distinguishing diagnostic feature like sorbitol negativity of *E. coli* O157, no plating medium is particularly recommended except that the medium must be rich enough to allow profuse growth. Modified sorbitol MacConkey agar (CT-SMAC) used of *E. coli* O157 must never be used since some strains of *E. coli* O103 seem to be inhibited on this medium. However, bead-bacteria complexes may be plated onto washed sheep blood agar (WSBA Beutin et al, 1989 J. Clin. Micro 27:2559-2564). Blood agar based on bovine or equine blood with sodium citrate as anticoagulant can also be used. Any one of the following plating media may be used in addition to blood agar, CHROMagar® (Invitrogen Dynal), MacConkey agar, modified Haemorharragic colitis agar (mHC), Eosin Methylene Blue (EMB), Chromocult® Coliform Agar (Merck). One should follow the recommended swab-streak technique when plating the bead-bacteria complexes as this will result in a better-isolated colony formation on the culture media (Figure 1). Colonies of presumptive E. coli O103 would show the same morphology as any generic E. coli on blood agar or any of the above plating media. However, these colonies should be serologically confirmed with the agglutination sera recommended for use with the kit and/or by performing other standard differential biochemical tests if necessary.

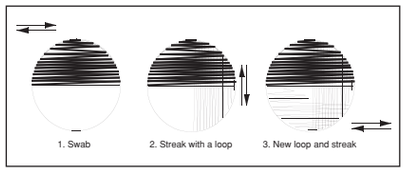

Figure 1. Swab-streak plating technique

FACTORS AFFECTING PRODUCT PERFORMANCE

The performance of Dynabeads EPEC-VTEC O103 is fully optimised to give consistent reproducible results when used in the BeadRetriever for AIMS instead of manual IMS. For manual IMS, this performance is solely dependent on the extent to which particles are recovered from different sample matrices. Failure to recover the Dynabeads-bacteria complexes from the sample could result in failure to detect the presence of *E. coli* O103. If Dynabeads-bacteria complexes are accidentally aspirated from the sample tube, immediately dispense back into the tube and dilute with wash buffer, repeat step 5 in section C before aspirating again. The entire IMS procedure is performed on a bench top at room temperature ranging from 18-28°C.

APPLICATION NOTE

To avoid cross-contamination of the prepared tubes it is recommended that sample transfer into the tubes is performed in a designated area at least one metre from the prepared tubes (see section B). Tube-strips for the BeadRetriever are designed to fit into the rack in one direction only. Tip combs and tube tray should be inserted as instructed until a click sound is heard. At the end of the processing of a sample, remove the sample tray first before removing the tip combs. It is recommended that the tip combs remain for at least 10 minutes after the assay has been completed to allow for air-drying, before removal.

For manual IMS, take care not to aspirate the Dynabeads from the sample tube when discarding the supernatant as this will result in lack of recovery of *E. coli* O103. If aspiration becomes difficult, leave some of the supernatant in the tube and dilute with wash-buffer as this will break the fat content which causes the Dynabeads to slide down the tube wall. In extremely fatty, viscous or particulate samples, a two-fold sample dilution using the described wash buffer must be made prior to IMS to ensure maximum particle recovery. It is important that filtered pipette tips are used to transfer samples into the test tubes for both manual and automated IMS.

Additional Materials Required

- Micropipette (10 - 100µl)
- 1 ml Dispenser Pipette
- BPW (available from most media manufacturers)
- Stomacher and stomacher bag with filter.
- Test tubes, glassware, loops, swabs and pipettes.
- Washing buffer (PBS tween): 0.15M NaCl, 0.01M Sodium Phosphate buffer, pH 7.4, with 0.05 % Tween-20. (Autoclavable at 121°C for 15 minutes, can be stored under refrigeration). Available in powder form from Sigma Product No. P3563
- CHROMagar® O157 (available from Invitrogen Dynal Product No. 740.02)
- Modified Haemorrhagic Colitis medium (for isolating haemorrhagic colitis strains of *E. coli*) may be prepared from (g/L), tryptone - 20; bile salts #3 - 1.12; sodium chloride - 5; sorbitol - 20; bromocresol purple - 0.015; distilled water and Bacto agar-15.
- EPEC/VTEC O111 Antiserum, purchased as Colony Verification Kit from Statens Serum Institut, Artellerivej 5, 2300 Copenhagen S, Denmark. Tel: +45 326 88 378, fax:+45 326 88 179 or e-mail: microbiology@ssi.dk.

All reagents should be of analytical grade.

INSTRUCTIONS FOR USE

A) SAMPLE PREPARATION

Food samples

1. Weigh 25 g of food sample and place into a filter homogeniser[1] bag.
2. Add 225 ml of Buffered Peptone Water (BPW).
3. Incubate at 42°C for 24 hours
4. Mix the pre-enriched sample thoroughly by homogenising once more.
5. Using a sterile pipette, transfer a 2 x 0.5 or 1 ml aliquot of the filtered suspension to be tested to the assay tubes. (See section B and C).

Human stools, bovine faeces and environmental swab samples

Whole stool specimens must be refrigerated as soon as possible after collection and examined within 1-2 hours of collection. If they cannot be examined within 1-2 hours, whole stools or a swab of the stool or rectal swabs should be placed in a transport medium (e.g. Stuart's, Cary Blair etc) and refrigerated until examination within 2-3 days. If samples will be held longer than 3 days before examination, they should be frozen at -70°C. Specimens in transport medium should not be left at ambient temperature.

1. Transfer 1 ml of human liquid stool sample into 10 ml of BPW.
2. For solid human stool samples and bovine faeces, prepare a 10% suspension and transfer 1 ml into 10 ml BPW.
3. Human rectal and environmental swab samples should be transferred into 10 ml of BPW.
4. Human stool, bovine faeces and environmental samples must be pre-enriched for 24 hours at 42°C.

Water samples

1. Filter 1 litre of water according to standard local procedures.
2. Use flat-ended forceps to remove the filter and transfer directly into a wide-mouthed bottle.
3. Add 90 ml of BPW to the contents of the bottle and shake vigorously to dislodge bacteria from the membrane surface.
4. Incubate at 42°C for 24 hours.
5. The use of a filter aid is recommended for samples that are too turbid for membrane filtration.

B) AUTOMATED IMS

All reagents and samples must be aseptically dispensed sequentially into the strips of tubes, after they are fitted into the rack. Users must read the user instructions provided with Dynabeads EPEC/VTEC O103 before use as follows:

1. Resuspend Dynabeads EPEC/VTEC O26 product until the pellet in the bottom disappears by using a vortex machine and aseptically add 10 µl into sample tubes 1 and 2.
2. Aseptically add 500 µl of wash buffer to sample tubes 1 and 2.
3. Aseptically add 1 ml of wash buffer to tubes 3 and 4 within the strip.
4. Aseptically add 150 µl of wash buffer to tube 5.
5. Remove the desired tube from rack A and place in rack B (one metre away). Add 500 µl of a test sample to tubes 1 and 2 and transfer the inoculated tube to rack A. Repeat for the remaining samples.
6. Aseptically insert the sterile protective tip combs into the instrument.
7. Insert the rack containing filled tubes into the instrument locking in place.
8. Check that everything is properly aligned and close the instrument door.
9. Select the EPEC/VTEC program sequence by scrolling with the arrow key and press the START button.
10. While the instrument is in operation, the door must be kept closed. Each processing step and the total time remaining can be followed on the LC display.
11. At the end of the program run, remove the tube rack from the instrument and plate the bead-bacteria complexes from the 5th tube onto the appropriate plating media as recommended in section D.
12. Remove the tip combs and discard into a biohazard waste container together with the tube strips.

C) MANUAL IMS

NOTE: To avoid cross-contamination and for safety reasons, it is strongly recommended that immunomagnetic separation should be performed using the BeadRetriever. In the absence of the BeadRetriever, strict adherence to good laboratory practice and the following instructions are a prerequisite to obtaining valid results.

1. Remove the magnetic plate and load the necessary number of 1.5ml microcentrifuge tubes into the Dynal® MPC™-S.
2. Resuspend Dynabeads EPEC-VTEC O103 until the pellet in the bottom disappears by using a vortex machine. Pipette 20 µl of Dynabeads EPEC-VTEC O103 and dispense into each tube.
3. Add 1ml of the pre-enriched sample aliquot from section A and close the tube. Change to a new pipette for each new sample.
4. Invert the Dynal MPC-S rack a few times. Incubate at room temperature for 10 minutes with gentle continuous agitation to prevent the

Ref 1 A filter homogeniser bag removes particulate material and fatty substances, which are inhibitory to IMS. For certain foods, for example, meat with bones or dry pasta, a blender is preferred prior to using a homogeniser bag to avoid the risk of perforation. After blending, the contents should be transferred into a filter homogeniser bag or a wide scew cap bottle.

beads from settling (e.g. in a Dynal MX-4 sample mixer).

5. Insert the magnetic plate into the Dynal MPC-S. Invert the rack several times to concentrate the beads into a pellet on the side of the tube. Allow 3 minutes for proper recovery.
6. Open the tube cap using the tube opener provided and carefully aspirate and discard the sample supernatant as well as any remaining liquid in the tube's cap.
7. Remove the magnetic plate from the Dynal MPC-S.
8. Add 1 ml of wash buffer (PBS-Tween). Do not touch the tube with the pipette since this can cross-contaminate the samples as well as the wash buffer. Close the cap and invert the Dynal MPC-S a few times to resuspend the beads.
9. Repeat steps 5 - 8.
10. Repeat steps 5 - 7.
11. Resuspend the Dynabeads-bacteria complex in 100µl of wash buffer (PBS tween). Mix briefly using a vortex mixer.

The concentrated bacteria on the beads are now ready for use in the detection step D.

D) ISOLATION OF *E. COLI* O103

After either manual or automated IMS, either transfer all the resuspended bead-bacteria complex onto blood agar. Alternatively transfer one half of the bead-bacteria complex onto blood agar with the remaining half onto any one of the following plating media: CHROMagar O157 (Invitrogen Dynal) MacConkey agar, modified Haemorharragic Colitis agar (mHC), Eosin Methylene Blue (EMB), Chromocult Coliform Agar (Merck).

1. Spread the bead-bacteria complexes over one half of the plate with a sterile swab. This ensures the break-up of the bead-bacteria complexes. Dilute further by streaking with a loop. Always carry the loop back into the previously streaked quadrant several times to ensure that the beads reach a fresh unstreaked quadrant.
2. Incubate the plates at 35-37°C for 18-24 hours.
3. Proceed to section E.

E) PRESUMPTIVE IDENTIFICATION AND CONFIRMATION

1. Add 10µl of physiological saline onto a glass slide placed on a dark background. Two or three tests may be performed on one slide.
2. Transfer a sweep of mixed growth from the first half of the blood agar plate onto the slide and make a smooth milky suspension.
3. Observe for auto-agglutination.
4. In the absence of any auto-agglutination, add 10 µl of the OK O103 antiserum provided in the Colony Verification Kit to the suspension and mix well. Observe for agglutination by tilting the slide for 10-30 seconds.
5. If auto-agglutination occurs, test one to five distinct individual colonies (see 7).
6. A visible agglutination reaction within 30 seconds is a strong indication of a presumptive positive sample.
7. When testing the sweep of mixed growth (see 1), confirm the initial presumptive result by testing one to five distinct individual colonies from the blood agar plate in a similar manner using the OK O103 antiserum. If no distinct colonies could be picked, plate further for purity from the other plating media onto blood agar and proceed as described above.
8. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).
9. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogenous milky turbidity. A late or weak agglutination should be considered negative.
10. Plate the agglutination positive colonies further for purity and confirm them by standard biochemical, serological and DNA tests (e.g. PCR).

FALSE NEGATIVE/POSITIVE RATES

Dynabeads EPEC/VTEC O103 might record a false negative rate ranging between 2-10% depending on the inoculum level, background flora and sample matrix. In the same sample without IMS, this false negative rate is significantly increased and is often more than 25%. Hence Dynabeads EPEC/VTEC O103 will consistently decrease the false negative rate by more than 15%. False positives do not occur since the possibility to verify presumptive colonies is always applicable. However the efficacy of the methods employed depends on the user's aptitude in following good laboratory practices and avoiding cross-contamination of samples.

SPECIFICITY AND SENSITIVITY

Following the described protocol for use with Dynabeads EPEC/VTEC O103 will determine the presence or absence of one viable *E. coli* O103 in the sample sizes described if this one cell is able to replicate and is not competed out by resident background flora. Dynabeads EPEC/VTEC O103 will bind both motile and non-motile strains of *E. coli* O103. The binding is independent of the ability to produce either Shiga toxins 1 or 2, or both. Antigenically similar organisms, for example *Escherichia hermanii*, Salmonella O group N, or *Proteus spp.*, can cross-react and bind to a limited extent. In addition, extremely "sticky" organisms like, *Pseudomonas spp.* or *Serratia liquifaciens* could bind non-specifically. However, the presence of high numbers of competitive background flora in the sample will not affect the specific binding of target organisms to the beads. Routinely, immunomagnetically selected and concentrated *E. coli* O103 are detectable on any enteric plating media from pre-enriched sample aliquots containing as low as 100 target cells against high numbers of background flora of 10^6 organisms or more per ml.

ACCURACY AND PRECISION

The accuracy of the method is not measurable since IMS is a qualitative and not a quantitative technique. Several bacteria may be bound to the Dynabead, but only give rise to one colony-forming unit on the culture media. The precision depends on the extent to which particles are recovered from different sample matrices.

REFERENCES

1. Zoonotic Non-O157 Shiga Toxin Producing *Escherichia coli* (STEC): Report of a WHO Scientific Working Group Meeting, Berlin, Germany, 1998. Department of Communicable Disease Surveillance and Response, World Health Organisation
2. Concerted Action CT98-3935: Verocytotoxigenic *E. coli* in Europe. 1. Methods for Verocytotoxigenic *E. coli.* http://www.research.teagasc.ie/vteceurope/methtech.html

GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

STORAGE/STABILITY

Dynabeads EPEC/VTEC products are stable when stored at 2-8°C, until the expiration date stated on the label.

PRECAUTIONS

Resuspend Dynabeads well before use to obtain a homogeneous dispersion of beads in solution. Precautions should be taken to prevent bacterial contamination of opened vials. Preservatives should be carefully removed before use by washing. All material that is used and contaminated must be autoclaved and properly disposed of according to local regulations.

WARNINGS AND LIMITATIONS

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated.

Preservatives such as sodium azide are toxic if ingested. **Avoid pipetting by mouth!**

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build-up.

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