

NATIONAL STANDARD METHOD

**DETECTION OF  
*ESCHERICHIA COLI* O157  
BY AUTOMATED  
IMMUNOMAGNETIC  
SEPARATION**

**W 16**

Issued by Standards Unit, Evaluations and Standards Laboratory  
**Centre for Infections**

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### DETECTION OF *ESCHERICHIA COLI* O157 BY AUTOMATED IMMUNOMAGNETIC SEPARATION

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# AMENDMENT PROCEDURE

Controlled document reference	W 16
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
7/ 22.03.06	3.1	4	1	<b>Title</b>	Amended
			5	<b>Scope and Background</b>	Updated
			6	<b>Principle</b>	Amended to include reference to para-magnetic beads
			6	<b>Safety Considerations</b>	Updated
			7	<b>Equipment</b>	Amended
			8	<b>Culture media and Reagents</b>	Amended
			9	<b>Sample Processing</b>	Amended to include the procedure for the automated immunomagnetic separation technique.
			12	<b>Reference facilities</b>	Updated

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# STANDARD OPERATING PROCEDURE FOR THE DETECTION OF *ESCHERICHIA COLI* O157 BY AUTOMATED IMMUNOMAGNETIC SEPARATION

## INTRODUCTION

### Scope

The method described is applicable to the detection of *Escherichia coli* O157 in water by the automated immunomagnetic separation technique. The method is based on ISO 16654:2001<sup>2</sup> and the manual method described in the Microbiology of Drinking Water 2002 document<sup>3</sup>.

### Background

*E. coli* O157:H7 is an important enteric pathogen causing symptoms ranging from mild diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome. Outbreaks have been associated with consumption of undercooked beef, milk and other dairy products, contact with the farm environment and person to person contact<sup>4</sup>. In addition to this a number of outbreaks have been attributed to contaminated water<sup>5,6</sup>. Infections due to *E. coli* O157 are increasing in number and further sources and reservoirs of this organism may be revealed.

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## 1.0 DEFINITIONS

### *Escherichia coli* O157

Micro-organisms which form typical colonies on solid selective media and which display the serological and biochemical characteristics described when tests are carried out in accordance with this method.

### Detection of *E. coli* O157

Determination of the presence or absence of these micro-organisms in 1 litre or other specified volumes of water samples when tests are carried out in accordance with this method.

## 2.0 PRINCIPLE

The detection of *E. coli* O157 in water involves concentration of organisms by passage through a membrane filter(s) or by the use of filter aid<sup>7</sup>. The membrane filter(s) or pad(s) containing filter aid are incubated in a selective liquid enrichment medium followed by Automated Immunomagnetic Separation (AIMS) with para-magnetic beads coated with antibodies to *E. coli* O157 antigen. The bound analyte (*E. coli* O157 specifically attached to the Dynabeads) is sub-cultured onto a selective agar. The selective agar cultures are examined for characteristic colonies. Confirmation as *E. coli* O157 is by serological and biochemical tests.

## 3.0 SAFETY CONSIDERATIONS<sup>8-17</sup>

Normal microbiology laboratory precautions apply.

*E. coli* O157 has been classified as a **Hazard Group 3** organism. Presumptive isolates and waters for which the epidemiology strongly suggests *E. coli* O157 will be present must be handled under **Containment Level (CL) 3** conditions (as for *Salmonella* Typhi). Local procedures for using and handing over to the CL 3 laboratory must be followed.

**NOTE: THE FOLLOWING PROCEDURE MUST BE ADHERED TO AT ALL TIMES WHEN SETTING UP AIMS SAMPLES.**

- Steps to prevent cross contamination of samples should be made as described in QSOP 45 - Installation, operation, maintenance and performance testing of the Dynal BeadRetriever™
- Once tests are set up MTSB broths must be retained **securely** in the designated fridge or cold room until tests are completed
- Control samples or spikes must NOT be processed at the same time as the test samples

**Note:** If a laboratory does not have the BeadRetriever™ equipment available then the sample should be sent to a laboratory which has this equipment. They should **not** perform the manual immunomagnetic separation method.

### 3.1 Sample collection

N/A

### 3.2 Sample transport and storage

Compliance with current postal and transport regulations is essential. Samples should be transported to the laboratory in a cooled cold box (2-8°C) as soon as practical with appropriate safety precautions and processed as soon as possible.

### 3.3 Sample processing

- Disposable gloves may be worn throughout the AIMS procedure subject to local risk assessments

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- Any samples for which the epidemiology suggests that a positive result is a likely outcome should be processed in a CL 3 laboratory
- Any enrichment broths that give presumptive positive results at 6 hours must be transferred to a CL 3 laboratory and further AIMS performed at this level of containment
- Cultures producing a positive or equivocal result and scanty or mixed cultures requiring sub-culture for purification must be transferred to a CL 3 laboratory
- All confirmatory tests must be performed in a CL 3 laboratory. When a positive sample is identified the water sample, enrichment broths and any other material that has come into direct contact with the sample must be taken to a CL 3 laboratory and disposed of appropriately
- Great care must be taken in the disposal and sterilisation of all test materials known to be contaminated with *E. coli* O157. These must be disposed of in the same manner as CL 3 waste including the filtrate in the reservoir following sample filtration

***The above guidance should be supplemented with local COSHH and risk assessments***

## 4.0 EQUIPMENT

Usual laboratory equipment and in addition:

- BeadRetriever™
- Membrane filtration manifold
- Filter funnels (graduated)
- Pyrex vacuum flask with protective jacket: large volume e.g 5 L
- Vacuum pump with moisture trap or protective filter, or alternative vacuum source
- Stainless steel flat tipped forceps
- Boiling waterbath (instrument steriliser)
- Vortex mixer
- Incubators: 37°C ±1°C, 41.5°C ±1°C
- Cellulose ester 0.45µm pore size gridded membrane filters
- Pipettes (sterile total delivery) 10mL and 1 mL graduated in 0.1 mL volumes (optional)
- Automatic pipettors and associated sterile pipette tips fitted with filters to prevent aerosol contamination of the pipette barrel and capable of delivering up to 10mL and 1 mL volumes (optional)
- Variable pipettors and sterile pipette tips fitted with filters to prevent aerosol contamination of the pipette barrel and capable of delivering 10-100 µL
- 10 µL plastic disposable loops
- Fine tipped graduated pastettes
- Plastic sterile tube strips and sterile tube tip combs

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## 5.0 CULTURE MEDIA AND REAGENTS

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

### *Modified tryptone soya broth*

Tryptone soya broth	30.0 g
Bile salts No. 3	1.5 g
Di-potassium hydrogen orthophosphate	1.5 g
Novobiocin	20 mg
Distilled or deionised water	1 L
pH 7.4 ± 0.2 at 25°C	

### *Cefixime tellurite sorbitol MacConkey agar*

Peptone	20.0 g
Sorbitol	10.0 g
Bile salts No. 3	1.5 g
Sodium chloride	5.0 g
Neutral red	30 mg
Crystal violet	1 mg
Cefixime	0.05 mg
Potassium tellurite	2.5 mg
Agar	15.0g
Distilled or deionised water	1 L

pH 7.1± 0.2 at 25°C

### *MacConkey agar (or equivalent)*

Bile salts	5.0 g
Peptone	20.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Neutral red	50 mg
Agar	12.0 g
Distilled or deionised water	1 L
pH 7.4 ± 0.2 at 25°C	

### *Nutrient agar (or equivalent)*

Meat extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled or deionised water	1 L
pH 7.5 ± 0.2 at 25°C	

### *Phosphate buffered saline pH 7.4 with 0.05% Tween 20 (PBST)*

Sodium chloride	8.0 g
Potassium chloride	20 mg
Disodium hydrogen phosphate	1.15 g
Potassium dihydrogen phosphate	0.2 g

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## 6.4 Automated Immunomagnetic separation technique (AIMS)

The enrichment cultures are subjected to AIMS after incubation for  $6.5 \pm 0.5$  hours if the water is likely to contain a high background of bacteria and/or  $22 \pm 2$  hours if the water is likely to be clean.

**Note:** For rapid results during outbreak situation perform AIMS after  $6.5 \pm 0.5$  hours and  $22 \pm 2$  hours.

### AUTOMATED IMS

The BeadRetriever™ should be located in close proximity to a class 1 safety cabinet in the CL2 or CL3 laboratory as appropriate (refer to National Standard Method QSOP 45 – Installation, operation, maintenance and performance testing of the Dynal BeadRetriever™).

- (a) Switch on the machine and select the required programme (EPEC / VTEC) using the arrow keys
- (b) Before preparation of the sample tray, sample tip combs must be inserted into the BeadRetriever™. This is done by holding the long overhang of the tip comb between the thumb and forefinger and sliding the tip comb into the tip comb slots as far as possible (User Manual Section 4, Figure 4.4). The apparatus will malfunction if the tip combs are not properly inserted as this causes misalignment with the magnetic rods. One tip comb with five tips is used for processing 5 or less samples at a time
- (c) One tube strip per sample is placed into the tube strip tray. Each tube strip is labelled with the appropriate sample identification number
- (d) Resuspend the Dynabeads anti-*E.coli* 0157 by vortexing. Pipette 10µL of the Dynabeads into sample tubes 1 and 2 of each tube strip. Pipette 0.5 mL of PBST into sample tubes 1 and 2, and 1mL of PBST into sample tubes 3 and 4 of each tube strip. Finally add 100µL of PBST to sample tube 5 of each tube strip

It is recommended that the above processing steps are carried out prior to removal of enriched samples from the incubator. This prepared sample tray is designated Tray A

- (e) Place a sample tray without tubes (designated Tray B) in a Class II cabinet or on the bench at least 1 metre away from Tray A
- (f) Transfer the first sample tube strip for inoculation from Tray A to Tray B
- (g) Place the selective enrichment culture container in the cabinet or on the designated bench area 1 metre away from Tray A, next to Tray B. Put on a fresh pair of disposable gloves and open the vessel. Transfer 0.5mL of sample to sample tube 1 and tube 2 of the tube strip using a 1mL sterile graduated disposable pastette
- (h) The sample vessel is closed and removed from the cabinet or bench and the inoculated sample tube is returned to Tray A. Discard gloves and put on a fresh pair
- (i) Transfer the next tube strip for inoculation to from Tray A to Tray B and inoculate as described above. This inoculation procedure should be repeated for each sample until all sample tubes have been inoculated
- (j) Slide the prepared strip tray into the machine. Close the top and front doors and press start
- (k) Each run takes 23 minutes. An audible alarm indicates when the AIMS procedure is complete
- (l) Remove the sample tube tray from the BeadRetriever™. Well 5 will contain all the beads in a volume of 100µL. Transfer the 100µL of para-magnetic beads/bacteria complex using a sterile pastette onto Cefixime tellurite sorbitol MacConkey agar

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(CT-SMAC). Ensure that all para-magnetic beads have been re-suspended prior to transfer. Plate out for discrete colonies. Place the CT-SMAC plates in an incubator at 37°C for 22 ± 2 hours

- (m) The sample tube strips and sample tip combs should be discarded carefully into a suitable leakproof container for autoclaving prior to disposal

**Note:** Great care must be taken when performing the AIMS procedure. Cross contamination may also occur when opening sample containers or during pipetting. AIMS or associated sample tray preparation activity should NOT be performed in the same laboratory area as sample preparation.

## 6.5 Recognition of colonies

After incubation examine the selective agar plates for colonies characteristic of *E. coli* O157. On CT-SMAC the majority of strains of *E. coli* O157 appear as colourless and pale pink non sorbitol fermenting colonies. It should be noted that sorbitol fermenting strains of *E. coli* O157 have been reported and these will produce pink/red colonies.

## 6.6 Confirmatory tests

All plates with suspect colonies (non-sorbitol fermenters) should be moved immediately to CL3 for all confirmatory tests.

Sub-culture 5 presumptive colonies from the CT-SMAC to MacConkey agar (MA) or Cysteine Lactose Electrolyte Deficient (CLED) Agar and nutrient agar (NA) or blood agar (BA) and place in an incubator at 37°C ± 1°C for 20 ± 4 hours. If there is a pure growth of lactose fermenting colonies use the growth from the NA or BA plate for slide agglutination and biochemical testing. If different colony types are present on MA or CLED sub-culture then perform slide agglutination tests on each type.

**Note:** Confirmatory tests must be performed on presumptive isolates from sub-cultures after both 6 hours and 22 hours.

Latex agglutination tests are commercially available and must be performed as described by the manufacturer. These kits must also be validated as suitable for the purpose before use.

**Note:** In some instances a rapid response may be required. Provisional recognition of *E. coli* O157 can be obtained by performing agglutination on discrete colonies obtained on CT-SMAC agar.

### *Biochemical confirmation*

Identify slide agglutination positive isolates using either validated in-house biochemical methods or an appropriate commercial biochemical test kit.

**Note:** Strains of *E. coli* O157 often produce biochemical reactions which are atypical for *E. coli* e.g. urease positive, β glucuronidase negative. Biochemical reactions must be interpreted with care and reactions which are atypical for *E. coli* should be recorded. An isolate producing an acceptable biochemical profile and a positive somatic O157 slide agglutination reaction should be reported as *E. coli* O157 (see section 9 – Reference facilities)

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## 7.0 REPORTING

Report the results using the procedure described in SOP: W 1 Section 9.

When the investigations are complete, report either as:

**'E.coli O157 not detected in 1 litre of water'**

or

**'E.coli O157 detected in 1 litre of water'**

In both instances, the amount of water examined must be reported. However, if 1 litre has not been examined the volume tested must be indicated on the report form. When results are received from the reference laboratory it will be necessary to issue a further report indicating if the *E. coli* O157 strain is Verocytotoxin (VT) producing or non-VT producing.

## 8.0 QUALITY CONTROL

### *Membrane filtration*

When the membrane filtration technique is used internal quality control procedures must be carried out at least once a month depending on the workload of the laboratory.

The internal quality controls should be carried out using suspensions of positive and negative control organisms known to contain less than 100 colony forming units in the volume filtered.

### *Positive control*

Prepare a dilute suspension of *E. coli* O157 NCTC 12900 (non-toxigenic)

### *Negative control*

Prepare a dilute suspension of *E. coli* NCTC 9001

### *Blank control*

Filter 1 L of sterile distilled water, peptone saline diluent or quarter strength Ringer's solution using the same funnel as that used for the positive control following sterilisation.

**Note:** Positive internal quality control (IQC) tests must not be performed with each batch of tests. This increases the chance of cross contamination with the control strain. It is better to perform IQC at a different location or at a different time eg: at the end of a run.

### *Confirmatory tests*

Carry out slide agglutination test in parallel with routine tests, as described previously using colonies from a working culture of *E. coli* O157 NCTC 12900. The latex controls must also be used. QC for latex kit should follow the manufacturer's instructions.

## 9.0 REFERENCE FACILITIES

All isolates of *E. coli* O157 including those obtained after AIMS and subculture at both 6 and 22 hours incubation must be referred to the Laboratory of Enteric Pathogens, Centre for Infections, Health Protection Agency, Colindale, 61 Colindale Avenue, London, NW9 5EQ, London for identification and confirmation, phage typing and demonstration of Verocytotoxin (VT) production. Non-toxigenic strains may also be isolated but these are not known to be of public health significance.

Five colony picks of each colony type should be sent for confirmation and typing. The packaging and transport must conform to category A material as set out in the HSE, Biological agents: Managing the risks in laboratories and healthcare premises<sup>18</sup> for ACDP Category 3 pathogens. The packaging should be clearly marked externally to identify that it contains an ACDP category 3 organisms and should only be opened in a Class I or III cabinet in a Category 3 laboratory.

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## Flowchart showing the detection of *Escherichia coli* O157

Transport to laboratory at 2°C – 8°C out of direct sunlight in a suitable container



Store at 2°C – 8°C in the dark and analyse as soon as is practicable on the day of collection, otherwise within 24 hours of collection



Mix sample well and filter 1L using a membrane or filter aid if necessary



Transfer filter and/or pad and filter aid to minimum of 90 mL modified tryptone soya broth



Incubate at 41.5°C for 6 hours and 22 hours



Concentrate *E. coli* O157 cells by AIMS



Sub-culture 100 µL to CT-SMAC



Incubate CT-SMAC plates at 37°C for 24 hours



Sub-culture positive colonies to nutrient agar and MacConkey agar



Identify *E. coli* O157 colonies using serological and biochemical tests



Refer isolates (5 colony picks) to the Laboratory of Enteric Pathogens

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