

HPA STANDARD METHOD

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

F 17

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

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

Issue no: 3 Issue date: 12.05.08 Issued by Standards Unit, Evaluations and Standards Laboratory in conjunction with the  
Regional Food, Water and Environmental Coordinators Forum Page 1 of 14

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>F 17</b>
<b>Controlled document title</b>	<b>Detection of <i>Escherichia coli</i> O157 by Automated Immunomagnetic Bead Separation</b>

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
6/ 12.05.08	2.4	3	1	<b>Front page</b>	Centre for Infections
				<b>Title</b>	“Automated” inserted
				<b>Background</b>	Information added about properties of variant strains; better distinction made between serotype O157 and VTEC strains
				<b>Principle</b>	Distinction made between O157 and VTEC strains
				<b>Definitions</b>	Definition of VTEC O157 strains inserted
				<b>Safety precautions</b>	Reference to IMS changed to AIMS; instructions when to use safety glasses changed
				<b>Equipment</b>	Manual IMS equipment removed, automated IMS equipment inserted. Only closure bags specified to contain the enrichment. Only pastettes or aerosol resistant tips with pipettor specified
				<b>Culture media and reagents</b>	Peptone replaced by “enzymatic digest of casein” throughout Tryptone soya broth replaced by individual components Strength of potassium tellurite corrected in CT-SMAC

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				<p><b>Sample processing</b></p> <p>6.1 Instructions added for swabs. Instructions included to double contain the enrichment broth</p> <p>6.3 IMS (manual) procedure replaced by AIMS (automated) procedure</p> <p>6.4 Information added about variant O157 strains</p> <p>6.5.2 Note: information on variant strains given</p> <p><b>Flow chart</b></p> <p>Amended in line with 6.3</p> <p><b>References</b></p> <p>7 &amp; 12 added</p>
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# DETECTION OF *ESCHERICHIA COLI* O157 BY AUTOMATED IMMUNOMAGNETIC BEAD SEPARATION

## SCOPE OF DOCUMENT

The method described is applicable to the detection of *Escherichia coli* O157 in all food types and environmental samples.

## BACKGROUND

*Escherichia coli* O157 that produces vero-cytotoxin is an important foodborne pathogen causing symptoms ranging from mild diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome.

Many outbreaks and sporadic cases have been associated with consumption of undercooked beef or dairy products such as untreated milk. However, infections due to *E. coli* O157 are increasing and a wide range of foods have been associated with outbreaks. These include cooked meat products, vegetables, salad vegetables, coleslaw, and acid based foods such as mayonnaise, apple cider, yoghurt and fermented sausages. Microbiological guidelines for ready-to-eat foods<sup>2</sup> recommend the absence of *E. coli* O157 in 25 g. Specific tests for *E. coli* O157 are usually carried out on selected foods eg foods implicated in outbreaks and those submitted in surveys.

Unlike most other strains of *E. coli*, the majority of O157 strains do not ferment sorbitol within 24 hours and are  $\beta$  glucuronidase negative. However variant strains of *E. coli* O157 have been reported that are sorbitol and  $\beta$  glucuronidase positive. Detection of *E. coli* O157 in foods has proved difficult but the methods described in this document have successfully isolated the organism from naturally contaminated and artificially inoculated foods. Vero-cytotoxin producing strains of *E. coli* O157 (VTEC) cause potentially serious illness in humans; they have a low infective dose and laboratory acquired infections have been reported. Therefore isolation and identification of these organisms must be carried out by trained laboratory personnel in properly equipped laboratories, under the control of a qualified microbiologist (see section 3 – Safety Considerations).

The method has been derived from the work of two groups<sup>3-5</sup> and involves analysis for the presence of *E. coli* O157 in 25 g of sample. It is very similar to BS EN ISO 16654:2001<sup>6</sup> but differs in that immunomagnetic separation is automated (for safety reasons as well as efficiency) and only one isolation agar is specified.

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# 1 PRINCIPLE

The detection of *E. coli* O157 involves selective enrichment with isolation from the enrichment culture by automated immunomagnetic separation (AIMS) followed by subculture to a selective agar. The selective agar cultures are examined for characteristic colonies. Confirmation of *E. coli* O157 presence is done using serological and biochemical tests. The potential to produce vero-cytotoxin is determined by the detection of vero-cytotoxin (VT) genes. Refer to the Centre for Infections, Health Protection Agency, Colindale for demonstrations of vero-cytotoxin (VT) gene presence.

# 2 DEFINITIONS

## ***Escherichia coli* O157**

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

## **VTEC O157**

VTEC O157 refers specifically to strains of *E. coli* serogroup O157 capable of vero-cytotoxin production.

## **Detection of *E. coli* O157**

Determination of the presence or absence of these micro-organisms in 25 g or other specified weights of food samples when tests are carried out in accordance with this method.

# 3 SAFETY CONSIDERATIONS<sup>7-11</sup>

Normal microbiology laboratory precautions apply. In addition, vero-cytotoxin producing *E. coli* have been reclassified from **Hazard Group 2** organisms to **Hazard Group 3** organisms. These organisms must be handled under the same conditions as *Salmonella* Typhi and *Shigella dysenteriae* type 1.

The current advice for routine examination of food samples is unchanged. The normal containment level conditions required by the Advisory Committee on Dangerous Pathogens are considered to be adequate. However, if it is known that *E. coli* O157 or other potential VTEC strains are present, or where work with such strains is necessary, then the work must be performed using containment conditions required for **Hazard Group 3** agents. Each laboratory will need to undertake a risk assessment to identify high and low risk samples and introduce a protocol for dealing with them.

Disposable gloves must be worn when performing the AIMS procedure and changed between each sample. Safety glasses should also be worn if the AIMS procedure is performed on the open bench. Any enrichment broths that give presumptive results at 6 hours must be transferred to a containment level 3 (CL3) laboratory and further AIMS performed at this level.

Likewise, cultures producing a positive or equivocal result and scanty or mixed cultures requiring subculture for purification must be transferred to a CL3 laboratory.

All confirmatory tests must be performed in a CL3 laboratory. When a positive sample is identified the food sample, enrichment broths and any other material that has come into direct contact with the sample must be taken to a CL3 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 treated in the same manner as containment level 3 waste.

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## 4 EQUIPMENT

Usual laboratory equipment and in addition:

- Top pan balance capable of weighing to 0.1 g
- Gravimetric diluter (optional)
- Stomacher
- Incubators: 37°C ± 1°C  
41.5°C ± 1°C
- Vortex mixer
- Bead Retriever™ (Dyna®)
- Bead Retriever™ tube strips and tip combs
- Stomacher bags 185 mm x 310 mm (complete with mesh insert)
- Disposable bags with closure / container
- Other secondary containers for enclosing the loaded closure bags
- Sterile graduated single use fine tipped pastettes or single use long bodied pastettes if disposable bags are used
- Variable pipettor and aerosol resistant tips capable of delivering 10 - 100 µL
- Plastic disposable loops

## 5 CULTURE MEDIA AND REAGENTS

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

### *Modified tryptone soya broth*

Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Glucose	2.5 g
Bile salts No. 3	1.5 g
Dipotassium hydrogen orthophosphate	4.0 g
Novobiocin	20 mg
Water	1 L

pH 7.4 ± 0.2 at 25°C

### *Cefixime tellurite sorbitol MacConkey agar*

Enzymatic digest of casein	20.0 g
Sorbitol	10.0 g
Bile salts No. 3	1.5 g
Sodium chloride	5.0 g
Neutral red	30mg
Crystal violet	1 mg
Cefixime	0.05 mg
Potassium tellurite	2.5 mg
Agar	15.0 g
Water	1 L

pH 7.1 ± 0.2 at 25°C

### *MacConkey agar (or equivalent)*

Bile salts	5.0 g
Enzymatic digest of casein	20.0 g

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Lactose	10.0 g
Sodium chloride	5.0 g
Neutral red	50 mg
Agar	12.0 g
Water	1 L
pH 7.4 ± 0.2 at 25°C	

*Nutrient agar (or equivalent)*

Meat extract	10.0 g
Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Agar	15.0 g
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	0.2 g
Disodium hydrogen phosphate	1.15 g
Potassium dihydrogen phosphate	0.2 g
Tween 20	0.5mL
Water	1 L
pH 7.4 ± 0.2 at 25°C	

*Saline solution*

Sodium chloride	8.5 g
Water	1 L

*Para - magnetic beads coated with antibody to E. coli O157 antigen (Dynabeads or equivalent)*

*Latex agglutination kit*

*Validated in house biochemical test media (optional)*

*Commercial biochemical test kits (optional)*

## 6 SAMPLE PROCESSING

### 6.1 SAMPLE PREPARATION

Using a fresh pair of sterile gloves for each sample weigh out 25 g of sample into a stomacher bag (with filter for high fat foods) and carefully add 225 mL of prewarmed (room temperature) modified tryptone soya broth (MTSB). If a limited amount of sample or more than 25 g is examined then add sufficient MTSB to give a 1 in 10 dilution. Homogenise for approximately one minute using a stomacher. Transfer the homogenate to a closure bag or container, close the bag securely then place in a further container capable of closure (such as a further closure bag, lidded box or screw topped container).

For swab samples, ensure that the swab is completely immersed with MTSB, such that an approximate 1/10 dilution is achieved. Vortex or stomach to bring the organisms into suspension. Double contain for enrichment in a similar way to that described above.

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## 6.2 ENRICHMENT

Place the enrichment broth in an incubator at 41.5°C for 6.5 ± 0.5 hours for samples suspected of having a high background flora or 22 ± 2 hours for other samples.

As guidance, routine heat processed samples may be subjected to AIMS after 22 ± 2 hours only. Raw products and other matrices likely to contain high levels of background Gram negative flora should be examined after 6.5 ± 0.5 hours and after 22 ± 2 hours. Samples that are the subject of a public health investigation should always be examined after 6 and 22 ± 2 hours.

## 6.3 ISOLATION BY AUTOMATED IMMUNOMAGNETIC BEAD SEPARATION AIMS PROCEDURE

The enrichment broth cultures are subjected to AIMS after 6.5 ± 0.5 hours and/or 22 ± 2 hours using the Bead Retriever™.

Follow the instructions contained in the Health Protection Agency Guidance Note QSOP 45<sup>11</sup> for operation of the Bead Retriever™.

- (a) Re-suspend the paramagnetic beads by gentle vortex mixing to ensure that the pellet at the bottom of the vial is completely suspended
- (b) Prior to removal of enriched samples from the incubator, place the required number of tube strips into the sample tray. To each tube strip transfer 10 µL of resuspended para-magnetic beads of anti *E. coli* O157 to tubes 1 and 2 using a variable pipettor and aerosol resistant disposable tip. Then transfer 0.5 mL of PBST to tubes 1 and 2, 1.0 mL of PBST to tubes 3 and 4 and 100 µL of PBST to tube 5 of the tube strip. This prepared sample tray is designated tray A
- (c) Place a sample tray without tubes (tray B) in a Class II cabinet or on the bench at least 1 metre away from tray A
- (d) Transfer the first tube strip for inoculation from tray A to tray B
- (e) Place the appropriate enriched sample in the cabinet or next to tray B if working on the bench (1 metre away from tray A). Put on a pair of disposable gloves and open the sample container. Transfer 0.5 mL of the enrichment culture to tubes 1 and 2 of the tube strip using a fine tipped graduated pastette. Close the sample container and remove the enriched sample from the vicinity of tray B. Transfer the tube strip back to tray A
- (f) Repeat the process described in step (e) for each sample using a separate pair of gloves for each sample
- (g) When all the tube strips have been inoculated slide tray A into the Bead Retriever™
- (h) When the tip combs have been inserted and the sample tray (A) is in place, select the appropriate assay and press the start key to initiate the run
- (i) On completion of the assay (approximately 23 min) subculture the contents of tube 5 (100 µl) in the same way as for tube inoculation. Transfer the first tube strip from tray A to tray B. Place a labelled plate of cefixime tellurite sorbitol MacConkey agar (CT-SMAC) in the cabinet or next to tray B if working on the open bench. Ensure that all para-magnetic beads have been re-suspended. Transfer the contents of tube 5 using a single use graduated fine pastette to the CT-SMAC plate, then spread the plate in order to obtain single (discrete) colonies. Discard the tube strip as for contaminated waste. Repeat this process for each tube strip
- (j) Place the CT- SMAC plates in an incubator at 37°C for 22 ± 2 hours

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- (k) Allow the tip combs to dry, then remove from the apparatus and discard as for contaminated waste

## 6.4 RECOGNITION OF COLONIES

Examine the selective agar plates for colonies characteristic of presumptive *E. coli* O157. On CT-SMAC the majority of these strains appear as colourless non sorbitol fermenting colonies. The variant sorbitol fermenting strains of *E. coli* O157 (see above) will produce pink/red colonies, although growth may be poor on CT-SMAC. Clinical details of infections that have been linked to the food source should therefore be considered before disregarding these colonies. All plates with suspect colonies (non-sorbitol fermenters) should be moved immediately to CL3 for all confirmatory tests.

## 6.5 CONFIRMATORY TESTS

Subculture 5 presumptive colonies from the CT-SMAC to MacConkey agar (MA) and nutrient agar (NA) and place in an incubator at 37°C ± 1°C for 16 - 24 hours. If there is a pure growth of lactose fermenting colonies use the growth from the NA plate for slide agglutination and biochemical testing. If different colony types are present on MA subculture then slide agglutination tests must be performed on each type.

**Note:** Confirmatory tests must be performed on presumptive isolates from sub-cultures after both 6.5 ± 0.5 hours and 22 ± 2 hours.

### 6.5.1 SEROLOGICAL CONFIRMATION

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.

**Safety note:** Plastic loops may produce aerosols. It is recommended that rigid sticks are used for emulsifying colonies.

**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.

### 6.5.2 BIOCHEMICAL CONFIRMATION

Identify latex agglutination positive isolates using a validated biochemical method.

**Note:** Strains of presumptive *E. coli* O157 are generally β glucuronidase negative and often produce other biochemical reactions that are atypical for *E. coli* eg urease positive. Biochemical reactions must be interpreted with care and reactions that are atypical for *E. coli*, should be recorded. An isolate producing an acceptable biochemical profile for *E. coli* and a positive somatic O157 slide agglutination reaction should be reported as *E. coli* O157 and sent to the Laboratory of Enteric Pathogens, Centre for Infections, HPA, for confirmation and vero-cytotoxin (VT) gene detection.

#### *Control organisms*

Positive control: *E. coli* O157 NCTC 12900 (non toxigenic)

Negative control: *E. coli* NCTC 9001

**Note:** In order to reduce the risk of cross contamination with the control strain it is preferable not to perform Internal quality control (IQC) tests with each batch of tests. It is better to perform IQC at a different location or at a different time.

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## 7 REPORTING OF RESULTS

When the investigations are complete, report either as:

*E. coli* O157 not detected in 25 g or in swab  
or  
*E. coli* O157 detected in 25 g or in swab

For food samples, report the actual weight of sample examined, for example 10 g, 25 g, 100 g.

Following results from the reference laboratory it will be necessary to issue a further report indicating whether the *E. coli* O157 strain is a vero-cytotoxin (VT) producing or non-VT producing strain.

## 8 RETENTION OF CULTURES

All presumptive isolates of *E. coli* O157 must be retained on two nutrient agar slopes labelled with: laboratory accession number, identity of isolate and the date slope prepared.

One slope should be sent to the Reference Laboratory and one slope archived in the CL3 laboratory.

## 9 REFERENCE FACILITIES

All isolates of *E. coli* O157 must be referred to the Laboratory of Enteric Pathogens, Centre for Infections, HPA, Colindale for confirmation of identity, O and H antigen determination, phage typing and demonstration of vero-cytotoxin (VT) gene presence. Non toxigenic strains may also be isolated but these are not known to be of public health significance.

A request form for referral to reference facilities can be accessed at the following link <http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370> .

## 10 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Food and Dairy Working Group for National Standard Methods ([http://www.hpa-standardmethods.org.uk/wg\\_food\\_dairy\\_group.asp](http://www.hpa-standardmethods.org.uk/wg_food_dairy_group.asp)). The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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# APPENDIX: FLOWCHART SHOWING THE PROCESS FOR THE DETECTION OF *ESCHERICHIA COLI* O157 BY AUTOMATED IMMUNOMAGNETIC BEAD SEPARATION

Prepare a 10<sup>-1</sup> dilution by weighing 25 g of sample into 225 mL of modified tryptone soya broth (MTSB)



Homogenise by stomaching



Incubate at 41.5°C for 6.5 ± 0.5 hours and/or 22 ± 2 hours



Concentrate *E. coli* O157 cells by AIMS



Subculture 100 µL to CT –SMAC



Incubate CT-SMAC plates at 37°C for 16 - 24 hours



Subculture positive colonies to nutrient agar and MacConkey agar



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



Refer isolates to the Laboratory of Enteric Pathogens

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

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