F8.4 *Modified phosphate buffered solution*

Sodium chloride	8 g
Potassium chloride	20 mg
Disodium hydrogen phosphate	1.15 g
Potassium dihydrogen phosphate	0.2 g
Polyoxyethylene-sorbitan monolaurate (for example, Tween 20)	0.5 ml
Distilled, deionised or similar grade water	1 litre

Dissolve the ingredients in the water and check that the pH is 7.4 ± 0.2. Sterilise the resulting solution by autoclaving at 121 °C for 15 minutes. After autoclaving, the pH of the solution should be checked to confirm a pH of 7.4 ± 0.2. Allow the solution to cool. This solution can be stored in the dark at room temperature and used within one month.

F8.5 *Filter-aid*[8]

Diatomaceous earth	1 g (approximately)
Distilled, deionised or similar grade water	15 ml

Weigh out appropriate amounts of filter-aid into suitable bottles and add the water. Sterilise by autoclaving at 121 °C for 15 minutes. Store in the dark at room temperature and use within 12 months.

F8.6 M*agnetic beads*

Para-magnetic beads coated with antibodies to *E. coli* O157 antigen[9] (for example, Dynabeads or equivalent).

F8.7 *Other media*

Standard and commercial formulations of other media and reagents used in this method include nutrient agar (NA), MacConkey agar (MA) and *E. coli* antisera and latex agglutination kits.

F9 Analytical procedure

F9.1 *Sample preparation.*

If present in drinking water, *E. coli* O157 are likely to be found in low numbers. Hence, a sample volume of at least 1000 ml should be examined. Smaller volumes may be more appropriate for polluted source waters.

F9.2 *Sample processing*

The sample is filtered using either a membrane filter or with filter aid and any residue added to medium. After incubation, a portion of the medium is then used for the detection of the organism.

F9.2.1 *Membrane filtration*

Filter an appropriate volume of sample, or diluted sample. If the sample is turbid, several membrane filters may be required.

Place the sterile or disinfected filtration apparatus in position and connect to a source of vacuum, with the stopcock turned off. Remove the funnel and, holding the edge of the membrane filter with sterile smooth-tipped forceps, place a sterile membrane filter, grid-side upwards, onto the porous disc of the filter base. Replace the sterile funnel securely on the filter base. Pour or pipette the required volume of sample into the funnel. Open the stopcock and apply a vacuum not exceeding 65 kPa (500 mm of mercury) and filter the sample slowly through the membrane filter. Close the stopcock as soon as the sample has been filtered so that as little air as possible is drawn through the membrane filter.

Remove the funnel and transfer the membrane filter carefully to 90 ml of modified tryptone soya broth or buffered peptone water. Whereas modified tryptone soya broth is suitable for polluted waters, buffered peptone water may be more appropriate for the recovery of stressed *E. coli* O157 from drinking waters and relatively unpolluted waters[10, 11].

When the funnel is removed it can be placed in a boiling water bath if it is to be re-used. Alternatively, pre-sterilised filter funnels can be used for each sample. If different volumes of the same sample are to be examined, the funnel may be re-used without boiling provided that the smallest volume, or highest dilution of sample, is filtered first. For different samples, take a fresh pre-sterilised funnel or remove a funnel from the boiling water bath, allow the funnel to cool and repeat the filtration process. If funnels are re-used, after filtration of each sample, disinfect the funnel by immersing it in boiling distilled, deionised or similar grade water for at least one minute. During the filtration of a series of samples, the filter base need not be sterilised unless it becomes contaminated or a membrane filter becomes damaged. Known polluted and non-polluted samples should be filtered using separate filtration equipment. Alternatively, polluted samples should only be processed after non-polluted samples. When funnels are not in use they should be covered with a sterile lid or a sterile Petri dish lid.

The time between the end of the filtration step and the beginning of the incubation stage should be as short as possible and no longer than 2 hours.

F9.2.2 *Filter-aid*

The usual membrane filtration apparatus may be used but with a sterile absorbent pad in place of a membrane filter to act as a supporting base for the filter-aid. An aliquot of filter-aid (15 ml) is added to the filter funnel and filtered to form an initial layer on the absorbent pad. The contents of a second aliquot of filter aid are then mixed with the sample, which is then filtered. For heavily polluted waters, additional aliquots of filter-aid may be required. When filtration is complete, remove the funnel carefully

and transfer the absorbent pad and filter-aid to modified tryptone soya broth. With the same medium, rinse any filter-aid adhering to the funnel into the culture vessel and make up to 90 ml.

F9.2.3 *Enrichment, immuno-magnetic separation and sub-culture to selective agar*

Thoroughly mix the modified tryptone soya broth or buffered peptone water from sections F9.2.1 or F9.2.2. Incubate the modified tryptone soya broth at 42 °C for 24 hours and the buffered peptone water at 37 °C for 24 hours[10, 11]. Enrichment broths should be subjected to IMS, firstly, after incubation for 6 - 7 hours, and then again at 24 hours.

Thoroughly mix the antibody-coated para-magnetic beads and transfer 20 µl of the suspension to a 1.5 ml Eppendorf tube, or suitable screw-capped tube. Add 1 ml of the thoroughly mixed incubated enrichment broth to the tube and mix again, gently, by inversion. Ensure that no air bubbles are trapped at the bottom of the tube. Place the tube onto a rotating mixer set at 30 revolutions per minute and gently mix for approximately 30 minutes. After mixing, place the tube into the magnetic particle concentrator with the associated magnetic strip in position. To concentrate the beads into a small pellet onto the side of the tube, gently invert the magnetic particle concentrator repeatedly for about 1 minute. With the magnetic strip in position, carefully open the tube and aspirate the liquid from the tube and any remaining liquid that might be inside the cap. Remove the magnetic strip from the magnetic particle concentrator and add 1 ml of modified phosphate buffered solution (F8.4) to the tube. Close the cap and gently invert to re-suspend the beads. Re-position the magnetic strip in the magnetic particle concentrator and concentrate the beads into a small pellet as before. Repeat the rinsing step with more modified phosphate buffered solution (F8.4). Re-suspend the beads in 50 µl of modified phosphate buffered solution (F8.4) and inoculate the beads onto cefixime tellurite sorbitol MacConkey agar, following manufacturer's instructions where provided, and incubate at 37 °C for 24 hours.

F9.3 *Reading of results*

After incubation, examine the cefixime tellurite sorbitol MacConkey agar Petri dishes for typical non-sorbitol-fermenting colonies that are smooth and circular, 1 - 3 mm in diameter and colourless to pale orange in colour, usually with dark centres. Strains of *E. coli* which ferment sorbitol are pink in colour.

F9.4 *Confirmation tests*

Inoculate typical colonies onto NA (and MA if isolate purity needs to be checked) and incubate at 37 °C for 24 hours. Isolates can then be subjected to serological identification using commercially available antisera or latex agglutination kits. Examine the slides for evidence of agglutination and carry out the tests with appropriate positive (non-verocytotoxin-producing strain of *E. coli* O157) and negative (non-O157 strain of *E. coli*) controls. Some isolates may require further identification by biochemical testing as some non-sorbitol-fermenting coliform bacteria (for example, *E. hermanii*) can cross react in the latex agglutination test. While chromogenic media can be used to demonstrate the lack of β-glucuronidase,

some strains of *E. coli* O157:H7 may produce atypical biochemical profiles and results should be interpreted with caution.

F10 Calculations

The test indicates the presence or absence of *E. coli* O157.

F11 Expression of results

E. coli O157 are reported as being detected or not detected in the volume of sample examined.

F12 Quality assurance

New batches of media and reagents should be tested with appropriate reference strains of target bacteria (for example, *E. coli* O157) and non-target bacteria (for example, other *E. coli*). Further details are given elsewhere[2] in this series.

F13 References

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8. Concentration technique for demonstrating small amounts of bacteria in tap water. *Acta Pathologica et Microbiologica Scandinavica*, Hammarstrom, E. & Ljutov, V., 1954, **35**, 365 – 369.

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10. Detection of toxin producing strains of *E. coli*, Report to the Department of the Environment. London, Drinking Water Inspectorate, DWI0674, 1996

11. Growth of starved *Escherichia coli* O157 cells in selective and non-selective media. *Microbiology and Immunology*, Sata, S., Osawa, R., Asai, Y. & Yamai, S., 1999, **43**, 217-227.

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However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

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