Testing meat for *E. coli* O157:H7 or H-

February 2002

Results for today; Ideas for tomorrow

Escherichia coli (*E. coli*) are part of the normal intestinal flora of many animals, including humans. Most strains of *E. coli* have no detrimental effects on the animal host, however some strains can cause serious human illness. We need to be able to detect and minimise the presence of these harmful *E. coli* strains in the food production chain.

E. coli O157 (H7 or H-) is a particular type of *E. coli* that can cause gastroenteritis, which in some cases progresses to life threatening complications such a haemolytic uraemic syndrome (HUS). They do this by attaching and effacing the human gut and producing toxins. Sheep and cattle are known to a reservoir of these bacteria and meat has been implicated as a source of infection.

This pamphlet describes the approaches that can be taken for the microbiological testing of meat for *E. coli* O157 and explains the terminology, the tests used and their interpretation.

Identifying *E. coli* O157

E. coli is present in the faecal material of all cattle and sheep and some contamination is unavoidable during slaughter and carcase dressing. *E. coli* O157 can be identified among the harmless *E. coli* by a combination of virulence markers and by serotype.

Virulence markers

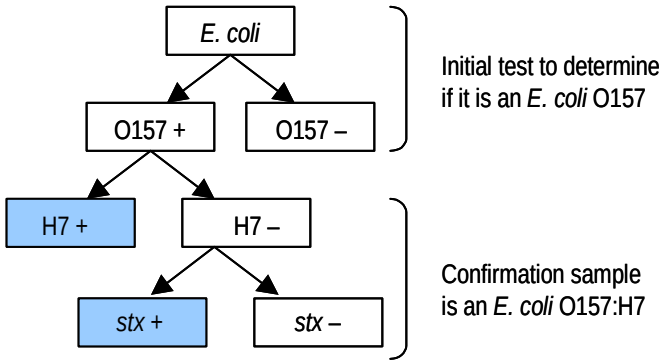
E. coli such as O157 have the ability to produce a group of toxins, known as Shiga toxins. There are two main types of Shiga toxin, Stx1 and Stx2, and these are essential virulence markers for this group. There are laboratory tests available to test for the Shiga toxins or the gene (*stx*).that encodes production of these Shiga toxins

Serotype

E. coli have specific structures or antigens in their cell wall and these can be detected by reaction with their complimentary antibodies. The test is called serotyping and the cell wall antigens are known as 'O' serotypes. Currently, there are 174 O serotypes that are referred to numerically starting at 1, hence the term O157. If the bacterium produces appendages called flagella, which allows it to move around, these can also be serotyped and are known as 'H' serotypes, ie. H7. Some O157 strains are not motile and therefore cannot be given a H serotype, these are referred to as O157:H–. *E. coli* O157:H– that produces the shiga toxin has caused HUS in Australia. Therefore, detection of this H- type is as important as detecting *E. coli* O157:H7. *E. coli* O157 with H types other than H7 have been isolated from cattle; however, these are generally not toxigenic (ie. do not have the shiga toxin) and are not a safety concern.

Most *E. coli* O157:H7 or H- have the *stx* gene and produce shiga toxin; however, other strains of O157 may not. The latter strains do not appear to present a threat to public health.

The USA Food Safety and Inspection Service (FSIS) Directive 10,010.1 defines a positive sample as one which is "positive for *E. coli* O157 and (1) the H7 antigen test is positive OR (2) the H test is non-specific or the culture is non-motile and either toxin or one or more toxin genes are present". From this definition a positive sample is confirmed positive for O157 as indicated by the shaded boxes below.



Microbiological analyses

The number of *E. coli* O157 in meat is likely to be small and the frequency of isolation low. Therefore, using tests that screen for the presence of the O157 antigen in the primary enrichment broth will reduce the number of samples that are submitted to further culture. There are a variety of screening tests available, some of which give a result in a few hours and require limited technical expertise.

A positive O157 antigen-based screen test indicates that there is evidence that the O157 cell wall antigen is present in the

FOOD SCIENCE AUSTRALIA
A JOINT VENTURE OF CSIRO AND AF
CSIRO AFISC
MEAT & LIVESTOCK AUSTRALIA
AMPC

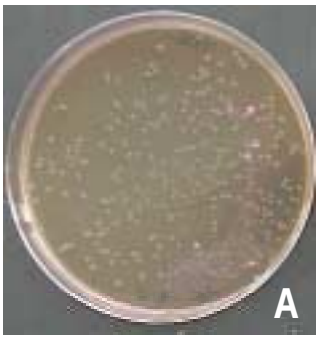
Results for today; Ideas for tomorrow

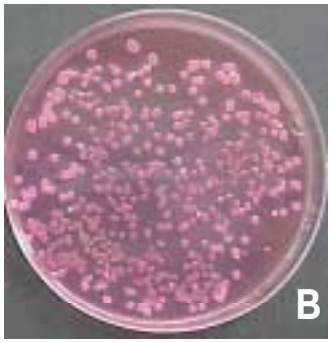
enrichment broth. It doesn't provide information about the H7 serotype or whether the bacterium has the toxin gene. Other bacterial species are known to give false positive reactions and some of these may be present in animal specimens. Commercial kits vary in their rate of false positives.

Once a positive screening test has been detected, *E. coli* O157 can be isolated from the enrichment broth on selective agar plates eg. Sorbitol MacConkey Agar (SMAC). Typical toxin producing *E. coli* O157 do not ferment sorbitol and have a distinct appearance on SMAC. The numbers of O157 bacteria in the enrichment broth is often small and the isolation rate may be poor. Isolation can be increased up to 1,000 fold by the use of immuno-capture techniques such as Dynabeads® anit-*E. coli* O157. Immuno-capture is the most sensitive method for recovering *E. coli* O157 and confirming a positive screening test.

It is recommended that all presumptive positives (ie. a positive screening test) need to be confirmed in-house or through commercial testing laboratories. This is important due to the presence of non-toxigenic *E. coli* O157 and possible false positives from the screening tests. This may influence the decision on how to handle a consignment or lot or meat with presumptive evidence of the presence of *E. coli* O157.

The following pictures both show colonies of *E. coli* O157 after immunocapture with Dynabeads and overnight incubation on SMAC.

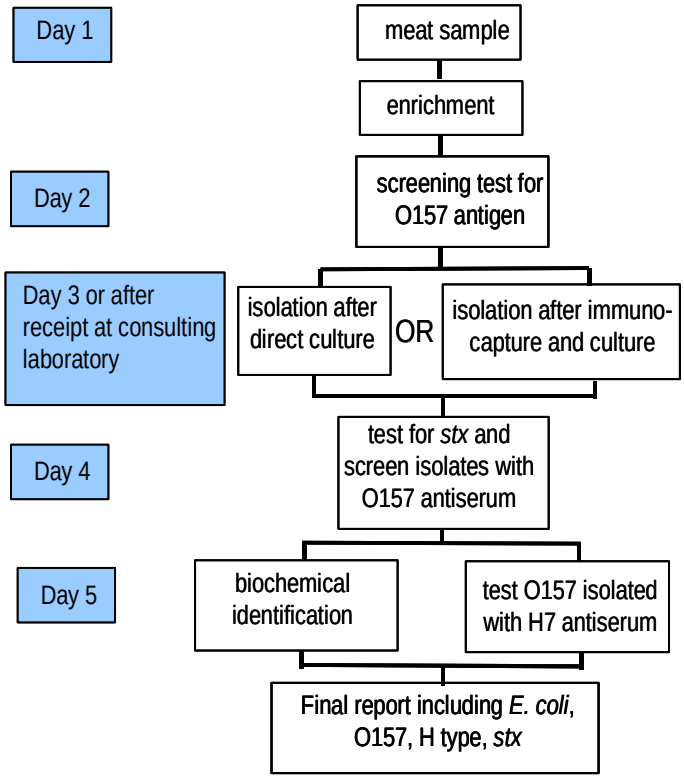




Picture A is a non-sorbitol fermenting (NSF) *E. coli* O157 that contains the Shiga toxin genes eg. Like *E. coli* O157:H7.

Picture B is a sorbitol fermenting (SF) *E. coli* O157 that does not contain the Shiga toxin genes. The immunocapture technique has resulted in almost pure growth of the particular O157 isolate as there are few SF colonies in picture A (which contains the NSF toxigenic *E. coli* O157 isolate), and few NSF colonies in picture B (which contains the SF non-toxigenic *E. coli* O157).

Typical isolates of *E. coli* are selected from SMAC agar and are serotyped using O157 specific antiserum. Motile isolates are tested for the H7 anitgen. Non-motile isolates may take up to a week to confirm lack of motility. Isolates are confirmed as *E. coli* using biochemical tests. The presence of Stx toxins can be confirmed using immunological assays eg. ELISA based tests, or *stx* genes are tested for using genetic tests, eg. Polymerase Chain Reaction (PCR). A flow diagram of the overall microbiological analyses is shown below.



Transport of samples

If a commercial testing laboratory is used to confirm positive samples, it is the responsibility of the referring organization to ensure that the sample is packed according to the 'Dangerous Goods Regulations'. For further information see the information sheet titled 'Dangerous Goods' from www.meatupdate.csiro.au

Further Information

The Food Safety and Quality Group of Food Science Australia at their Brisbane Laboratory are able to assist in the confirmation of toxigenic *E. coli* O157:H7 or H- . For further information and costs, please contact Dr Trish Desmarchelier or Dr Narelle Fegan.

Food Science Australia
PO Box 3312
TINGALPA DC Qld 4173

Ph. 07 3214 2000
Fax. 07 3214 2062

FOOD SCIENCE AUSTRALIA
A JOINT VENTURE OF CSIRO AND AF

CSIRO AFISC

MEAT & LIVESTOCK AUSTRALIA

AMPC