# Rapid 🗸 for *E. coli* O157 Lateral Flow Assay – AOAC 070201

## SCOPE

This method is applicable for the detection of *E. coli* O157:H7 in raw boneless beef and raw ground beef after 15-22h enrichment in mTSB+N. The 8-hour enrichment protocol has not been approved.

## PRINCIPLES

Samples are enriched selectively for *E. coli* O157. A portion of the enrichment is then added to the lateral flow device. The sample flows through a zone containing specific antibodies for *E. coli* O157:H7 bound to colloidal gold. *E. coli* O157 antigens bind with the gold conjugated antibodies forming a complex which migrates through a nitrocellulose matrix. It then passes a zone of anti- *E. coli* O157 antibody where it is bound and visualised by the formation of a red line. Non-bound antibody-gold complex flows to a second zone where it is also captured forming a red line. As a result, when *E. coli* O157 antigen is present, the formation of 2-red lines is observed, when no *E. coli* O157 antigen is present only one-line forms.

* Enrichment

Place meat sample in 9 x sample weight of modified TSB plus novobiocin (mTSB+n[[1]](#footnote-1)). Incubate for 15-22 h at 42 ± 1°C. This method has been validated for use with 375g samples enriched in one litre of broth.

* Immunoassay

Carry out the assay following the manufacturer’s instructions.

* Confirmation

Confirmation of *E. coli* O157 is to be carried out from enrichment using a DAFF approved confirmatory method. Confirmation must be undertaken at a DAFF approved laboratory.

## CHECKLIST

|  |  |  |
| --- | --- | --- |
| **Enrichment** | Is mTSB+N used for enrichment of samples? |  |
|  | Is novobiocin added on the day of use? |  |
|  | Is enrichment carried out at 42 ± 1°C for 15-22 h? |  |
|  | What weight of sample and volume of enrichment broth are used? |  |
|  | Is a positive control run with each batch of samples analysed? |  |
|  | Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells? |  |
| **Immunoassay** | Are the manufacturer’s instructions available and are they reproduced in the laboratory manual? |  |
|  | Are technicians familiar with positive and negative reactions? |  |
| **Cultural confirmation** | Is *E. coli* O157:H7 confirmed from initial enrichment broth at a DAFF approved laboratory? |  |
|  | Is a DAFF approved method used for confirmation? |  |
|  |  |  |

1. Modified TSB broth with novobiocin plus casaminoacids Oxoid or equivalent [↑](#footnote-ref-1)