# The MicroSEQ(R) Real-Time PCR System for Detection of *E. coli* O157:H7 in raw ground beef and beef trim – AOAC 071001

## SCOPE

This method is applicable to the analysis of raw ground beef and beef trim for *E. coli* O157:H7.

## PRINCIPLES

The MicroSEQ(R) Real-Time PCR System is rapidly amplifies specific DNA fragments unique to *E coli* O157:H7 followed by signal detection in a single reaction. MicroSEQ(R) Real-Time PCR Kit for *E. coli* O157 must be used.

### Sample enrichment

Sample (375 ±37.5 g) is diluted 1:5 in pre-warmed (42°C) buffered peptone water (BPW). The sample is homogenised by hand-mixing in a stomacher bag or by stomaching for 1 min, and incubated at 42°C for 16 hours. A positive control culture at an inoculum level of 10-100 cells must be run through all enrichment and testing procedures daily or when testing is carried out. The sample and enrichment broth must be at the enrichment temperature for a minimum of 16 hours.

### Sample preparation and PCR screening

Sample preparation for bacterial DNA extraction is carried out by using the PrepSEQ(R) Rapid Spin Sample preparation kit following the manufacturer’s recommended protocol. The extracted DNA sample is run in the Real-Time PCR System – Applied Biosystems (Foster City, CA) 7500 Fast Real-Time PCR System.

### Confirmation

Samples that test MicroSEQ(R) PCR negative shall be reported as negative. For samples that test positive, ‘warning’, or have an invalid result, enriched media must be analysed using a DAFF approved confirmatory method. Alternatively, the laboratory may review the cause of the ‘warning’ or invalid result and based on the findings re-analyse the sample by:

* Repeating the DNA extraction and PCR analysis
* Screen testing with a DAFF approved rapid test kit for *E. coli* O157:H7

Confirmation must be carried out at a DAFF approved laboratory.

## CHECKLIST

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| **Pre-enrichment** | Is BPW pre-warmed at 42°C before use? |  |
|  | Are positive control cultures run with each batch of samples? |  |
|  | Are control cultures inoculated into the primary enrichment broth at a level of 10 to 100 cells? |  |
|  | Is enrichment carried out at 42°C and is the enrichment broth and the sample at 42°C for a minimum of 16 hours? |  |
|  | Is PrepSEQ Rapid Spin Sample preparation kit used for DNA extraction? |  |
| **PCR screening** | Are manufacturer’s instructions available for reference? |  |
|  | Are the technicians familiar with and trained in the operation of Applied Biosystems Real-Time PCR System? |  |
|  | Is the shelf-life of media and kits controlled? |  |
| **Confirmation** | Is confirmation carried out at a DAFF approved laboratory using a DAFF approved confirmatory method? |  |