



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. All samples identified as potentially positive for *E. coli* O157:H7 using this test must be confirmed using ISO 16654:2001, MLG 5 or FDA BAM Chapter 4A using the IMS option.

▪ Sample enrichment

Sample (375 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. Cultural analysis shall continue as per ISO 16654:2001, FSIS MLG 5 or FDA BAM Chapter 4A (isolation must include immunomagnetic separation) for sample enrichments that test positive, 'warning', or have an invalid result. Or, 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 Department of Agriculture approved rapid test kit for *E. coli* O157:H7

Confirmation must be carried out at a Department of Agriculture approved laboratory.

CHECKLIST

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 Department of Agriculture approved laboratory using an Approved method (including IMS)?	_____