

Australian Government

Department of Agriculture, Water and the Environment

DuPont Qualicon BAX^(R) System PCR Assay for Real-Time *E. coli* O157:H7 - AOAC 031002

SCOPE

This method is applicable to the analysis of ground beef and beef trim for *E. coli* 0157:H7.

PRINCIPLES

The BAX System rapidly amplifies specific DNA fragments unique to *E coli* 0157:H7 using the power of the polymerase chain reaction (PCR). DuPont Qualicon BAX System PCR Assay for Real-Time *E. coli* 0157:H7 test kits must be used. All samples identified as potentially positive for *E. coli* 0157:H7 using this test must be confirmed using ISO 16654:2001, MLG 5 or FDA BAM Chapter 4A using the IMS option.

Pre-enrichment

Samples are diluted 1:5 in pre-warmed (45°C) BAX® System *E. coli* 0157:H7 MP enrichment broth. This method is validated for 375 g beef trim samples in 1.5 L of BAX® System *E. coli* 0157:H7 MP enrichment broth. Incubate samples at 42°C for 10-24 hours. A positive control culture must be run through all enrichment and testing procedures daily or when testing is carried out.

BAX system for screening *E. coli* 0157:H7

The BAX® System Real-Time *E. coli* O157:H7 assay is an automated method that uses polymerase chain reaction (PCR) technology for the detection of *E. coli* O157:H7 in meat. The system identifies specific DNA fragments, unique to *E. coli* O157:H7. *BAX*® System method uses lysis reagent which is prepared by adding 150 μ L protease to 12 mL of lysis buffer. For each sample, 20 μ L of the enrichment broth is added to 200 μ L of prepared lysis reagent in cluster tubes. Tubes are heated for 20 minutes at 37±2°C and 10 minutes at 95±3°C and then cooled for at least 5 minutes in a cooling block. PCR tablets in tubes are hydrated with 30 μ L of lysate and run in a BAX® System Q7 machine.

Confirmation

Samples that test BAX 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 pre-enrichments that test BAX positive, indeterminate, or have an invalid result. Or, the laboratory may review the cause of the indeterminate or invalid result and based on the findings re-analyse the sample by:

- Repeating the BAX analysis from the rack loading step
- Preparing new BAX tubes and repeating the analysis or
- Screen testing with a department approved rapid test kit for *E. coli* 0157
- Confirmation must be carried out at a department approved laboratory.

CHECKLIST

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Pre- enrichment	Is BAX [®] System <i>E. coli</i> 0157:H7 MP broth pre- warmed at 45°C before use?	
	Is enrichment carried out at 42°C for 10-24 h?	
	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?	
BAX screening	Are manufacturer's instructions available for reference?	
	Is 20 μl of enrichment added to the lysis reagent?	
	Is the correct cycle run for the analysis of <i>E. coli</i> 0157:H7 samples?	
Isolation	Is isolation carried out at a department approved laboratory using an Approved method (including IMS)?	
Cultural Confirmation	Are colonies confirmed using appropriate biochemical and serological tests?	
	Are tests for toxin or toxin genes carried out?	