



DuPont Qualicon BAX® System Real-Time PCR Assays for detection of selected STEC in beef trim – AOAC 091301

SCOPE

This method is applicable for detection of selected Shiga toxin-producing *E. coli* (STEC) in beef trim. The method can be used in conjunction with the BAX RT method for the detection of *E. coli* O157:H7 in beef (AOAC 031002).

PRINCIPLES

The BAX System amplifies specific DNA fragments unique to pathogenic STEC serogroups using polymerase chain reaction (PCR). Initially the organisms are allowed to grow in BAX system MP enrichment medium followed by PCR analysis for the presence of the *eae* and *stx* genes. Samples positive for *eae* and *stx* genes then undergo further PCR screening using STEC Panel 1, 2 and BAX RT PCR (AOAC 031002) for O157:H7 (if this PCR was not run in conjunction with initial PCR for detection of *stx* and *eae* genes).

▪ Pre-enrichment

Samples (375 g) are diluted in pre-warmed (45-46°C) BAX® System MP enrichment broth (1.5L) and hand massaged or stomached for 2 minutes to mix. Samples are incubated at 39-42°C for 12-24 hours. The temperature of both the broth and sample must be at 39-42°C for a minimum of 12 h. A positive control must be run through all enrichment and testing procedures daily or when testing is carried out.

▪ BAX system for screening STEC

The BAX® System PCR Assay suite for STEC is an automated method that uses polymerase chain reaction (PCR) technology for the detection of pathogenic STEC serogroups in beef trim. The BAX® method uses lysis reagent which is prepared by adding 150 µL protease to 12 mL of lysis buffer. For each sample, 20 µL of the enrichment broth is added to 200 µL of prepared lysis reagent in cluster tubes. Tubes are heated for 20 minutes at 37°C and 10 minutes at 95°C and then cooled for at least 5 minutes in a cooling block. PCR tablets are hydrated with 30 µL of lysate and run in a BAX® System Q7 machine.

▪ PCR Confirmation

Screened samples, identified as positive, must be analysed for top 6 serogroups using BAX Panel 1 (O26, O111, O121), Panel 2 (O45, O103, O145) and BAX RT for *E. coli* O157:H7 (AOAC 031002, if applicable). The Panels and the *E. coli* O157:H7 test will screen down to serogroup level. PCR positive samples will be considered as potential positives.

▪ Confirmation

Samples that test BAX negative are reported as negative. Cultural analysis shall continue as per USDA-FSIS or equivalent methods 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 another department approved method.

Confirmation must be carried out at a department approved laboratory using a department approved method.

CHECKLIST

Pre-enrichment	Is BAX® System MP broth pre-warmed at 45-46°C before use?	_____
	Is enrichment carried out 39-42°C for 12-24 h and is the enrichment broth and sample at 39-42°C for a minimum of 12 h?	_____
	Is the correct amount of enrichment broth used i.e. 1.5L?	_____
	Is a positive control culture run with each batch of samples or daily?	_____
	Is the control culture inoculated into the primary enrichment broth at a level of 10 to 100 cells?	_____
	Are the enriched samples also analysed for <i>E. coli</i> O157:H7 using the BAX® System Real-Time Assay (AOAC 031002)?	_____
BAX screening	Are manufacturer's instructions available for reference?	_____
	Is 20 µL of enrichment added to the lysis reagent?	_____
	Are internal controls run with each batch of samples?	_____
	Are technicians familiar with and trained in the operation of the BAX® Automated System?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is isolation carried out at a department approved laboratory using a department approved method?	_____