



FSIS Procedure for the use of the BAX System PCR Assay for Screening *Salmonella* in foods - MLG 4C.07

SCOPE

This method is applicable to:

- Raw meats and meat products and carcass and environmental sponges

PRINCIPLES

This method uses a commercial PCR screening procedure. All samples identified as potentially positive for *Salmonella* using this test must be confirmed using AS 5013.10.

The detection of *Salmonella* spp. is broken down into four stages:

■ Pre-enrichment

Sample (25 g) is enriched in pre-warmed (42°C) 75 ± 1.5 mL mTSB at 42 ± 1°C for 15-24 h.

Where a single raw beef product sample is tested for *Salmonella*, *E. coli* O157 and top six STEC, a 325 ± 32.5 g sample is enriched in pre-warmed (42°C) 975 ± 19.5 mL of mTSB at 42 ± 1°C for 15-24 h. For carcass sponges, mTSB is added to the moistened sponge to bring the total volume to 60 mL and the sample incubated at 42 ± 1°C for 15-24 h.

For Ready-to-Eat foods, 325 ± 6.5 g sample is enriched in 975 ± 19.5 mL of pre-warmed Buffered Peptone Water at 35 ± 2°C for 18-24 hours.

A positive control culture must be run through all procedures daily or when testing is carried out.

■ BAX system for screening *Salmonella*

Salmonella is screened in the sample following the manufacturer's recommended protocol. The BAX *Salmonella* test is an automated method that uses polymerase chain reaction (PCR) technology for the detection of *Salmonella* in foods. The system identifies a specific DNA fragment, unique to *Salmonella*. The DNA is combined with DNA polymerase, nucleotides, and primers. The mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognise and bind to the targeted DNA sequences. The DNA polymerase then uses the nucleotides to extend the primers, thus creating 2 copies of the targeted DNA fragment; repeating the cycle results in an exponential increase in the number of target DNA fragments. A fluorescent dye then binds with double-strand DNA and emits a fluorescent signal in response to light. After amplification, the BAX System begins a detection phase in which the fluorescent signal is measured.

■ Confirmation

In cases of BAX-positive, BAX-indeterminate or a BAX-signal-error the mTSB should be tested using AS 5013.10 (starting at the appropriate stage of analysis i.e. selective enrichment). Or based on the findings of a cause analysis, the laboratory may choose to analyse the indeterminate or signal error result sample by:

- Repeating the BAX analysis from the rack loading step or
- Preparing new BAX tubes and repeating the analysis.

Confirmation must be carried out at a Department approved laboratory

CHECKLIST

Pre-enrichment	Is the mTSB pre-warmed to 42°C?	_____
	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?	_____
	What are the enrichment conditions, (42 ± 1°C for 15-24 h)?	_____
	Is the correct amount of enrichment broth used for the weight of sample analysed?	_____
BAX screening	Are the manufacturer's instructions available?	_____
Confirmation	If <i>Salmonella</i> confirmation is done in-house refer to AS 5013.10.	_____
	mTSB should be supplied to off-site laboratories for confirmation following AS 5013.10	_____
	Are all suspect <i>Salmonella</i> sent to a reference laboratory (see AS 5013.10) to be serotyped?	_____