



Thermo Scientific™ SureTect™ *Salmonella* spp PCR Assay - AOAC 2021.02

SCOPE

This method is applicable to raw beef and other foods and environmental samples.

PRINCIPLES

The Thermo Scientific SureTect *Salmonella* species PCR assay is a real-time PCR assay designed for rapid detection of *Salmonella* species. This method is used in conjunction with the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder Analysis Software; or the Applied Biosystems 7500 Fast Real-Time PCR Instrument and RapidFinder Express Software or the Thermo Scientific PikoReal Real-Time PCR Instrument and Software. The assay utilizes dye-labelled probes that target genes unique to *Salmonella* and an internal positive control. Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. The IPC template, primers, and probe provide an internal control with each reaction to show that the PCR process has occurred.

The detection of *Salmonella* spp is broken down into the following stages:

▪ **Enrichment**

Raw meat sample (25 g) is enriched in 225 mL (1:10) pre-warmed (temp $41.5 \pm 1^\circ\text{C}$) ISO Buffered Peptone Water (ISO BPW). For carcass sponges, BPW (ISO) is added to the moistened sponge to bring the total volume of 225ml. Raw meat sample (375g) is enriched in 1.5 L (1:5) pre-warmed Oxoid Modified Tryptone Soya Broth (mTSB). The sample is homogenized for 30 - 60 seconds using a stomacher and incubate at $41.5 \pm 1^\circ\text{C}$ for 9 - 24 h.

A positive control culture must be run through all procedures daily or when testing is carried out. Store enriched samples at $2 - 8^\circ\text{C}$.

▪ **Sample lysis and PCR screening**

Sample preparation for bacterial DNA extraction is carried out by using the SureTect lysis reagents (Ready-to-use SureTect Lysis Reagent 1 and Proteinase K) following the manufacturer's recommended protocol. The extracted DNA lysate (20 μl) is added into SureTect PCR Tube containing the PCR pellet and run in the Thermo Scientific™ Real-Time PCR Instrument.

▪ **Confirmation**

In all cases of PCR positive, PCR failed or a PCR signal-error the ISO BPW must be tested using AS 5013.10 (starting at the selective enrichment stage of the analysis).

Confirmation must be carried out within 72 h following the end of incubation at a DAFF approved laboratory.

CHECKLIST

Enrichment	Is the ISO BPW or mTSB pre-warmed to temp $41.5 \pm 1^\circ\text{C}$?	_____
	Is the correct amount of enrichment broth used for the weight of sample analysed?	_____
	Is a positive control run with each batch of samples analysed?	_____
	Are reference cultures inoculated into enrichment broth at a level of 10 to 100 cells?	_____
	Is enrichment carried out at $41.5 \pm 1^\circ\text{C}$ for 9-24?	_____
	Is enriched sample stored at 2-8°C for confirmation if necessary?	_____
SureTect PCR	Are the manufacturer's instructions available?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is confirmation carried out within 72 h following the end of incubation?	_____
	BPW/Oxoid mTSB should be supplied to off-site laboratories for confirmation following AS 5013.10	_____
	If <i>Salmonella</i> confirmation is done in-house refer to AS 5013.10.	_____
	Are all suspect <i>Salmonella</i> sent to a reference Laboratory (see AS 5013.10) to be serotyped?	_____