



Thermo Scientific™ SureTect™ *Listeria* species PCR Assay - AOAC 071304 (AFNOR UNI 03/09 - 11/13)

SCOPE

This method is applicable to raw ground beef, other foods and environmental samples. This method is not validated on food samples weighing more than 25 g.

PRINCIPLES

The Thermo Scientific SureTect *Listeria* species PCR assay is a real-time PCR assay designed for rapid detection of *Listeria* 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 *Listeria* 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 *Listeria* spp is broken down into the following stages:

▪ **Enrichment**

Raw meat sample (25 g) is enriched in 225 mL pre-warmed (room temperature) 24 LEB¹ supplemented with 24 LEB Buffer Supplement² and 24 LEB Selective Supplement³. For carcass sponges, supplemented 24 LEB is added to the moistened sponge to bring the total volume to 225 mL.

The sample is homogenized for 30 seconds by a stomacher and incubated at 37 ± 1°C for 22 - 26 h. A positive control culture must be run through all procedures daily or when testing is carried out. Store enriched samples at 2 - 8°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, SureTect Lysis Reagent 2 and Proteinase K) following the manufacturer's recommended protocol. The extracted DNA lysate (20µl) 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 enrichment sample must be tested using AS 5013.24.1 (starting at the appropriate stage of the analysis). Confirmation must be carried out within 72 h following the end of incubation at a DAFF approved laboratory.

¹ Oxoid 24 LEB Complete Base (CM1154B or CM1154R). 24 LEB Complete Base (CM1154) is pre-supplemented with 24 LEB Selective Supplement and does not therefore require the addition of SR0243E.

² Oxoid 24 LEB Buffer Supplement (BO1204M), 10 mL for each 225 mL of broth must be used. This product may crystallise during storage. Where crystals are present, the tube should be placed in a 37°C water bath for 5-10 minutes.

³ Oxoid 24 LEB Selective Supplement (SR0243E, 10 vials each for 500ml) must be used

CHECKLIST

Enrichment	Is the 24 LEB warmed to room temperature?	_____
	Is the correct amount of Buffer Supplement and Selective Supplement added into 24 LEB broth?	_____
	Is the correct amount of enrichment broth used for the weight of sample analysed i.e diluted 1-in-10; 25 g and 225 mL??	_____
	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?	_____
	Is enrichment carried out at 37±1°C for 22-26 hours?	_____
	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?	_____
	Enriched broth should be supplied to off-site laboratories for confirmation following AS 5013.24.1.	_____
	If <i>Listeria</i> confirmation is done in-house refer to AS 5013.24.1.	_____