



Assurance GDS^{TD} *Salmonella* method for foods – AOAC 2009.03 and Assurance GDS^{TD} *Salmonella* Tq method

SCOPE

These methods are applicable for detection of *Salmonella* in most foods and environmental samples.

PRINCIPLES

GDS^{TD} is an automated gene-based assay that incorporates multiple levels of specificity to ensure highly accurate results. Initially the organisms are allowed to grow in BPW. *Salmonella* that present in the samples are then concentrated, followed by amplification of a highly conserved DNA sequence. Assurance GDS^{TD} *Salmonella* Tq is a modified version of AOAC 2009.03 whereby the polymerase enzyme, instead of being added separately by the analyst, is lyophilized with the other PCR reagents inside the amplification tube at the time of manufacturing.

Detection of *Salmonella* involves the follow steps:

▪ Enrichment

Samples are diluted 1:10 in BPW¹. Incubation is carried out for 18-24 h at 35 – 37°C. A positive control culture must be run through all procedures daily or when testing is carried out. Carcass swabs should be enriched in 100 mL of BPW at 35-37°C for 18-24h.

▪ Immuno-concentration and PCR Assay

AOAC 2009.03

Enriched samples shall continue for immuno-concentration procedure in which 1mL of sample is transferred in a sample block well containing 20 µL sample concentrating reagent and the sample block is vortex at approx 900 rpm for 10 – 20 min. For meat samples (including carcass swabs), sample reagent mixture is then transferred into 0.5 mL brain heart infusion (BHI) broth and incubated for 2 – 4 hours at 35 - 37°C. Incubated broth is then transferred into 35 µL of resuspension buffer using the PickPen, this is then followed by a washing step prior to amplification.

The washed bead-bacteria complex (20 µL) is transferred into amplification tubes containing 10 µL of polymerase buffer and loaded in an Assurance Rotor-Gene™ (follow the manufacturer's recommended protocol).

Assurance GDS^{TD} *Salmonella* Tq

The Tq format will involve the following procedural changes:

- the volume of resuspension buffer has changed from 35 µL to 45 µL;
- the addition of 10 µL of polymerase buffer to the Amplification Tubes has been eliminated; and
- the volume of sample transferred into the Amplification Tubes has changed from 20 µL to 30 µL.

¹ Formula as per AS 5013.10

▪ **Interpretation**

Upon completion of the assay the Rotor-Gene program will provide a test result. Each test sample will be identified as positive, indicating that the test sample is positive for *Salmonella*, negative indicating that the test sample is negative for *Salmonella*, or “no amp” indicating that amplification did not occur.

A “no amp” reading may be due to reagent or test failure or operator error. In this event the test must be repeated using the same enrichment cultures. If the result continues to show “no amp” the equipment supplier must be contacted for technical services. In this case, the enrichment broth must be analysed using an alternate method or the sample deemed positive for *Salmonella*.

CHECKLIST

Enrichment	Is the sample enriched in buffered peptone water at 35-38°C for 18-24 h?	_____
	Is the correct amount of enrichment broth used?	_____
	Is a positive control run with each batch of samples/daily?	_____
	Are reference cultures inoculated into enrichment media at a level of 10-100 cells per sample?	_____
	Are salmonellae separated and concentrated using immuno-concentration procedure?	_____
	Is enriched sample + concentrating reagent mixture further grown in BHI broth at 35 - 38°C for 2-4 h?	_____
Immuno-concentration & PCR Assay	Are manufacturer’s instructions available for reference?	_____
	Are internal controls run with each batch of samples?	_____
	What volume of resuspension buffer is used during sample preparation?	_____
	What volume of sample wash is added to Amplification tubes?	_____
	Is polymerase buffer added to the Amplification tubes on the day of use?	_____
	Are technicians familiar with and trained in the operation of the GDS Automated System and the Rotor-Gene Program?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is confirmation carried out from the enrichment culture (BPW)?	_____
	Is confirmation carried out using AS 5013.10 at a department approved laboratory?	_____