Australian Government
Department of Agriculture and Water Resources

IEH PCR assay for detection of *Salmonella* in carcass and environmental sponges or swabs - AOAC 100701

SCOPE

This method is the *Salmonella* detection component of the method "IEH *E. coli* O157, *stx*-Producing *E. coli* (STEC) with Intimin and *Salmonella* Test System (AOAC 100701)" for detection of *Salmonella* spp. on carcasses.

PRINCIPLES

The IEH Test System rapidly amplifies specific DNA fragments unique to the target organism using Taq DNA polymerase. Initially the organisms are allowed to grow in Buffered peptone water (BPW) followed by PCR assays for the presence of target genes. Samples identified as positive (initial reactive) must be confirmed using the AS 5013.10 method.

- **Pre-enrichment**
  BPW is added to the sponge to bring the total volume to between 60 and 100 mL. Samples are enriched at 35 ± 2°C for 18 ± 3 h. BPW need not be warmed to room temperature before being used to re-hydrate the sponge, for all subsequent additions BPW should be warmed to room temperature. A positive control culture must be run through all enrichment and testing procedures daily or when testing is carried out.

- **Initial Screening**
  Enriched samples are screened for the presence of *Salmonella* using the IEH *E. coli* O157, *Stx*-Producing *E. coli* (STEC) with Intimin and *Salmonella* Test System using PCR buffers ES6. Initial Reactive (IR) samples undergo further testing using PCR buffers ES6 and S7 with and without immune-magnetic bead separation.

- **Confirmation**
  Confirmation must be carried out as per AS 5013.10 from sample pre-enrichments that test IEH presumptive 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 IEH Test System analysis using the primary enrichment broth, or
  - Screen the primary enrichment broth using a department approved method

  Confirmation must be carried out at a department approved laboratory.

CHECKLIST

Pre-enrichment	Is Buffered peptone water warmed to room temperature (for large quantities)?	
	Is enrichment carried out at 35 ± 2°C for 18 ± 3 h?	
	Is the correct amount of enrichment medium used?	
	Is a positive control culture run with each batch of samples?	
	Is the control culture inoculated into the primary enrichment broth at a level of 10 to 100 cells?	
Screening	Are internal controls run with each batch of samples?	
	Are technicians familiar with and trained in the operation of the IEH Automated System?	
	Is the shelf-life of media and kits controlled?	
Confirmation	Is confirmation carried out from the enrichment culture?	
	Is isolation carried out at a department approved laboratory using a department approved method?	