Australian Government
Department of Agriculture and Water Resources

Solus *Listeria* ELISA – NF SOL 37/02 – 06/13

SCOPE

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

PRINCIPLES

Detection of *Listeria* is based on enzyme-linked immunosorbent assay (ELISA). *Listeria* specific antibodies are coated on the wall of microplate wells that attach with *Listeria* antigens when present in the enrichment. After washing an enzyme-labelled antibody is added to the well which then binds with the antigen-antibody complex. After a second wash, a substrate is added which in the presence of bound enzyme-antigen-antibody complex produces a blue colour. The substrate reaction is stopped after 30 seconds by acid and the intensity of the colour is measured. The detection protocol can be broken down as follows:

- **Pre-enrichment**
  Sample (25 g) is enriched in 225 mL Half Fraser broth. Samples are homogenised and at incubated at 30 ± 1°C for 24 ± 2h. A positive reference culture must be run through the enrichment daily or when testing is carried out.

- **Selective enrichment**
  Subculture of 0.2 mL of the pre-enrichment is inoculated in 10 mL of RELM broth and incubated for 24 ± 2 h at 30 ± 1°C.

- **Enzyme immunoassay**
  Solus ELISA is performed using a portion of boiled selective enrichment broth. The assay is carried out in the Solus instrument following the manufacturer's instructions.

- **Cultural confirmation**
  All *Listeria* positive samples should be confirmed using selective enrichment broth following AS 5013.24.1. Confirmation carried out at an 'off-site' laboratory must be from retained selective enrichment.

CHECKLIST

Enrichment	Is correct amount of Half Fraser broth used for the weight of sample analysed?	_____
	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?	_____
	Are pre-enrichment and selective enrichment done at 30 ± 1°C for 24 ± 2h?	_____
Enzyme immunoassay	Are the manufacturer's instructions available?	_____
	Are ELISA kits allowed one hour to warm up at room temperature before use?	_____
	Are positive & negative kit controls run with each batch of samples?	_____
	Are plates incubated at 37°C for 30 min after adding samples and again after adding conjugate?	_____
	Are ELISA plates washed using a microplate washer?	_____
	Are OD readings taken within 10 min of adding stop solution?	_____
	Are samples with OD_{450} ≥0.200 considered presumptive positive?	_____
	Are ELISA kits stored as per manufacturer's guidelines (2 -8°C)?	_____
Cultural confirmation	Is *Listeria* confirmed from selective enrichment broth?	_____
	Are *Listeria* confirmed at a department approved laboratory using AS 5013.24.1?	_____