



VIDAS^(R) UP *Listeria* method (VIDAS^(R) LPT) – NF BIO 12/33 – 05/12

SCOPE

This method is applicable to:

- Meat & meat products, dairy products, seafood, vegetable and environmental samples.

PRINCIPLES

Detection of *Listeria* antigens is based on an enzyme-linked fluorescent assay (ELFA) using a novel recombinant phage protein based technology performed in an automated VIDAS^(R) instrument following enrichment in LPT broth. Each test has 2 components: 1) The SPR is used as solid phase as well as pipetting device. The interior of the SPR is coated with proteins specific for *Listeria* receptors. 2) All assay steps are performed by the VIDAS^(R) instrument utilizing the ready-to-use reagents contained in the strip. Fluorescence is measured by the instrument automatically and a positive or negative report printed for each sample.

▪ Enrichment

Meat samples (25 g) are enriched in 225 mL of LPT broth. Samples are mixed using a stomacher and incubated at 30 ± 1 °C for 26-30 h. Environmental samples can be enriched in 60-100 mL of LPT broth or in 10 mL if using small swabs and incubated at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 22-30 h. A positive reference culture must be run through the enrichment daily or when testing is carried out..

▪ Immunoassay

A portion of the manually mixed broth is heated at 95-100 °C for 5 ± 1 minutes using a water bath or VIDAS^(R) Heat and Go at its default setting of 131°C and then cooled for 10 minutes. *Listeria* is detected using the automated VIDAS^(R) instrument (following the manufacturer's instructions). The enriched reference culture must be run through the immunoassay.

Note: The non-boiled enrichment broth can be stored for 72 hours at 2-8 °C before the VIDAS^(R) assay is performed.

▪ Cultural confirmation

If confirmation is not initiated immediately then enrichment broth must be stored at 2-8°C and confirmation must be conducted within 72 hours following end of incubation.

All *Listeria* positive samples must be confirmed using AS 5013.24.1. Confirmation must be carried out using the enrichment at the appropriate stage in AS 5013.24.1 i.e. plating out and identification.

CHECKLIST

Enrichment	Is the appropriate volume of LPT broth used for the sample being analysed?	_____
	Are meat samples and environment samples incubated at 30 ± 1 °C for the appropriate time i.e. 26-30 and 22-30 h respectively?	_____
	Are samples and broth mixed using a stomacher?	_____
	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?	_____
Immunoassay	Is the VIDAS ^(R) UP <i>Listeria</i> assay performed on boiled broth (95-100 °C for 5 min) or the Heat and Go and cooled to room temperature?	_____
	How are enrichment broths boiled?	_____
	Are the manufacturer's instructions available and are they reproduced in the laboratory manual?	_____
	Are kits stored at 2-8 °C?	_____
	Are kits/reagents warmed to room temperature for at least 30 min before use?	_____
Confirmation	Is <i>Listeria</i> confirmed carried out from the enrichment culture?	_____
	Is isolation carried out at a Department of Agriculture approved laboratory using a Department approved method?	_____